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GENETIC RESOURCES OF FORAGE LEGUMES AND GRASSES IN REPUBLIC OF SRPSKA

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Original scientific paper

Abstract: The paper presents results of the Working group for fodder crops which is part of the SEEDNet Project. Inventory and storage of 22 plant species from 13 genera with 157 accessions was made. In PGBRS (Plant Gene Bank of the Republic of Srpska) 137 accessions are stored and for 20 accessions multiplication is in process. The importance of work on SEEDNet project within the group for fodder crops is reflected in the storing of accessions which are very important for the preservation of genetic resources, breeding work for creation of new varieties and other scientific purposes. In the researched area, species that are not included in previous research are diagnosed. In the following period work on compiling of the inventory and multiplication should be continued and for accessions that are stored in PGBRS characterization, evaluation, regeneration and other activities planed in the program should be performed.

Key words: resources, fodder crops, PGBRS

Introduction

Social and scientific importance of plant genetic resources for the whole humanity, community and every individual is priceless. Genetic resources are also of special economic and breeding importance in regard to breeding activities for a certain plant species. There are numerous international conventions and recommendations which state that plant genetic resources must be saved. The importance of determination of the origin and diversity of cultivated plant species first was established by Russian scientist *N. I. Vavilov* during period from 1923 till 1931. In that period, all around the world were gathered and collected about 300.000 species *Borojevic (1992)* and first Institute for storing of collection of plant material (VIR) was founded in Saint Petersburg. Today, there are more than 1.300 such institutions in the world *(FAO, 1996)*.

The rapid progress of science and technological achievements in the last 60 years contribute to the overall development in all areas of human activities as well as globally. Technological development in different parts of the world is uneven and this reflects on agriculture. This process had a significant impact on reduction of biodiversity throughout the world. The "green revolution", from the fifties to the eighties, destroyed and in some areas, more than halved the biodiversity. People understood that with the destruction of the environment they will destroy themselves, so knowledge about the necessity of organized protection of biodiversity was spread all around the world.

In our region, this process began in the former Yugoslavia through organizing of the PGBY (Plant Gene Bank of Yugoslavia). The first organized meetings related to this activity were in 1980, and after initial studies, network of scientists and experts was organized, as well as, workers in the field for the protection of biodiversity, and collecting of plant genetic material was defined. Richness of biodiversity in Bosnia and Herzegovina was noticed more than 100 years ago *Beck (1906-1923)*, but significant organized and long-term measures to protect this wealth had not been taken.

Bosnia and Herzegovina, and subsequently Republic of Srpska, has favorable agro ecological conditions, characterized by the abundance of forage legumes and grasses. In the plant world there are about 18.000 species that may be considered as fodder plants *Prodanovic et al. (2006)*. Also, the floristic composition of grasslands of Serbia state 200 forage plants. Many authors state importance of the richness of diversity in the Republic of Srpska and in Bosnia and Herzegovina *(Nedovic, et al. 2003)*. At the Agricultural Institute of Republic of Srpska from Banja Luka, breeding processes on cereals, corn and fodder crops take place since 1950. For the purposes of selection, plant genetic material was collected; primarily indigenous and new varieties of fodder crops were selected. Numerous varieties of *Lotus corniculatus* L., *Medicago sativa* L., *Dactylis glomerata* L., *Phleum pratense* L., *Festuca pratensis* Huds. and *Pisum sativum* L. were created and there is a significant breeding material of *Lolium perene* L. and *Lolium multiflorum* Lam. as well. A part of written evidence is included in the Project YF 875-11 and feasibility study: Plant Gene Bank of Yugoslavia. Very extensive and significant research was interrupted in period from 1991 till 2004.

Through the project South Eastern European Development Network on Plant Genetic Resource (2004-2014), where University of Banja Luka is one of the partners, group for fodder crops continued the work on compiling of the inventory and collection of legumes and grasses.

The aim of the work is, for current diversity of forage legumes and grasses, to make inventory and collection of accessions and store those accessions in base and active collections, as seeds, vegetative or partly "in situ". Although in the previous period, in some area inventory of legumes and grasses was performed, it was not done in detail and did not include complete and relevant areas of the Republic of Srpska, which was necessary to be completed with this research, to get knowledge of what exists in the grassland, how big was, in the meantime, anthropogenic impact and what was potentially lost. In parallel with these operations, plan for the protection of biodiversity was made in a way that was possible at this moment.

Materials and Methods

Collecting of the accessions was done on natural meadows on the whole territory of Republic of Srpska. In the field, members of the working group for fodder crops followed phenotypic characteristics of plants in certain locations during the vegetation period. The locations are positioned with coordinates and altitude (GPS).

When located populations were in generative stage of development, gathering of plant material (seeds and clones) was accomplished. Determination of individual species was based on publication Flora of SR Serbia *(Josifović, 1970-1977)* and taxonomy and nomenclature were adjusted.

Collection forms that were fulfilled in the field during collection, contain the standard parameters for the description of habitats and accessions. In the Seed Testing Laboratory at Faculty of Agriculture Banja Luka from collected accessions seeds were separated. For accessions which had 10.000 and more seeds (plants with small seed mass), or 5000 or more seeds (plants with a large mass of seeds) seeds were packed in paper bags, MCPDs (Multi Crops Passport Descriptors) were completed and saved in the Gene Bank. For accessions where it was not possible to collect enough seeds for storage, multiplication was performed, after which sufficient quantities of seeds will be saved in the Gene Bank.

Perennial cross pollinated accessions, that are less represented, were transplanted clonally at the experimental field of Center for Development and Education Manjaca, where reproduction in order to obtain sufficient quantities of seeds for storage, was conducted.

Results and Discussion

Explored area covers the whole territory of the Republic of Srpska, where the expeditions were done between 2004 and 2009. Natural grasslands of Republic of Srpska in relation to total agricultural areas cover 47.50% (700.000 ha). Within the project inventory of 122 legumes and grasses species (Mandate list) was planned. Based on conducted expedition, inventory and storage of 22 plant species from 13 genera, in total 157 accessions, was done (Table 1). Of total number of accessions, 137 were stored in PGBRS (Plant Gene Bank of the Republic of Srpska) with passport descriptors and collection forms, and for 20 accessions, multiplication is in progress. For 12 accessions multiplication is ongoing from seeds, and for 8 accessions clone multiplication at the trial field of Center for Development and Education Manjaca (Center for Development and Improvement of the village, Banja Luka) is in progress.

Table 1. Accessions for which inventory was compiled in the period 2004-2009

Genus	Species	Accessions in PGBRS[1]	Multiplication	
			Seed	Clone
Agrostis	*alba* L.	3		
Dactylis	*glomerata* L.	19		
Festuca	*ovina* L.	1		
	pratensis Huds.	23	2	
	rubra Huds.	5	2	
Latyrus	*silvestris* L.	1		
Lolium	*italicum* L.	2		
	perenne L.	11		
Medicago	*lupulina* L.	6		
Lotus	*corniculatus* L.	13		3
Nardus	*stricta* L.	1		
Phleum	*pratense* L.	10		
Poa	*pratensis* L.	3		
	trivialis L.	1		
Trifolium	*alpestre* L.	-	4	1
	montanum L.	-	1	1
	pratense L.	27		2
	repens L.	6	1	1
Trisetum	*flavescens* L.	3		
Vicia	*cracca* L.	1		
	faba L.	1		
	sativa L.	-	2	

[1]PGBRS - Plant Gene Bank of the Republic of Srpska

The feasibility study of Plant Gene Bank of Yugoslavia planned inventory of 33 most important genus of forage plants. In relation to our research, only genus *Nardus* was not included and that genus is very frequently represented in our area in the associations of mountain grasslands. Also, at researched area species included in the feasibility study of Plant Gene Bank of Yugoslavia were diagnosed and those species would be included in the inventory and stored in the Plant Gene Bank of the Republic of Srpska as important genetic fund.

Based on previous researches *Nedović, et al. (2003),* the dominance of associations that are classified by syntaxonomy in followed classes is confirmed: *Molinio-Arrhenatheretea* Tx., *Festuco- Brometea*-No. Bl. et Tx., *Brachipodio-Chrysopognetea* Ht., *Elyno-Seslerietea* Br.Bl., *Nardo-Callunetea* Preis. and

Caricetea-Curvulae Br.Bl. Plant species that were surveyed are numerous in associations of mentioned classes.

Conclusions

In the research period from 2004 until 2009 the inventory was conducted and in Plant Gene Bank in Banja Luka, 157 accessions of fodder crops were stored, which were represented by 22 species from 13 genera.

Collected and stored accessions are only part of the genetic resources of this area, it would be necessary to expand research on higher number of species and habitats and especially pay attention to the types of genera: *Trifolium, Medicago, Lotus, Lolium, Festuca*, etc.

The importance of previous work on SEEDNet project within the group for fodder crops is reflected in the storing of accessions which are very important for the preservation of genetic resources, breeding work and creating new cultivars and other scientific purposes.

In the future it would be necessary to continue with the inventory, multiplication and to initiate activities on characterization, evaluation and elaboration of descriptors of stored accessions.

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Genetički resursi krmnih leguminoza i trava u Republici Srpskoj

Đ. Gatarić, Z. Kovačević, B. Đurić, V. Radić, Ž. Lakić

Rezime

U radu su predstavljeni rezultati grupe za krmno bilje u okviru Projekta SEEDNet. Izvršena je inventarizacija i pohranjivanje 22 biljne vrste iz 13 rodova sa 157 prinova. U BBGRS (Banku biljnih gena Republike Srpske) je pohranjeno

137 prinova, a za 20 prinova se vrši multiplikacija. Značaj dosadašnjeg rada na SEEDNet Projektu u okviru grupe za krmno bilje se ogleda u pohranjivanju prinova koje su veoma važne za očuvanje genetičkih resursa, selekcijeki rad na stvaranju novih sorata i u druge naučne svrhe. Na istraživanom području konstatovane su vrste koje nisu obuhvaćene u toku dosadašnjeg istraživačkog perioda. U nastavku rada potrebno je nastaviti inventarizaciju, multiplikaciju, a za prinove koje su pohranjene u BBGRS izvršiti karakterizaciju, evaluaciju, regeneraciju i druge aktivnosti po programu.

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DIALLEL ANALYSIS OF THE INHERITANCE OF DRY MATTER QUALITY TRAITS IN ALFALFA (*Medicago sativa* L.)

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Original scientific paper

Abstract: The objectives of this paper were to use diallel analysis to determine types of gene action and the mode of inheritance of alfalfa quality and to assess the potential value of parental components for the development of new cultivars with a higher nutritive value (improved dry matter quality). Significant GCA and SCA effects were found for the proportion of leaves in yield and protein content. Significant reciprocal effects (maternal and non-maternal) were also observed for the former trait. Diallel analysis revealed highly significant GCA and SCA effects for leaf proportion and crude protein content alike, which indicates that additive as well as non-additive effects were significant in determining dry matter quality.

Key words: diallel, GCA, SCA, protein, proportion of leaves

Introduction

Along with selection for higher yields, breeding for improved forage quality is one of the most important directions and objectives in the development of new alfalfa varieties. Alfalfa forage quality has two main components: protein digestibility and protein content. Both these traits are positively correlated with the proportion of leaves in the forage *(Julier et al., 2001)*. Besides protein digestibility, protein content is also of great importance in assessing the nutritional value of alfalfa, which on average contains 200 g kg^{-1} of protein *(Hill et al., 1998; Riday and Brummer, 2002)*.

Alfalfa breeding for increased nutritional value is performed by developing cultivars with a higher proportion of leaves in dry matter yield, because leaves are richer in protein and vitamins and forage quality is thus improved in an indirect manner *(Katic et al., 2005a)*. Leaf contribution to dry matter yield depends on genetic factors, i.e. the variety *(Katic et al., 2005a; Katić et al., 2005b)*, as well as on plant density and the stage of alfalfa growth and development *(Lamb et al., 2003)*. The proportion of leaves in alfalfa yields is an indirect indicator of quality, as it is positively correlated with the crude protein content *(Julier et al., 2001)*, while at the same time being negatively correlated with green forage and hay yields of the crop *(Julier et al., 1997)*.

It has been determined that there is great genetic variation in the parameters of quality among and within alfalfa populations *(Julier et al., 2000)* and that the inheritance of the nutritional value of forage is for the most part additive in nature *(Guines et al., 2002; Veronesi et al., 2006)*. *Annicchiarico (2007)* reported significant variations in the proportion of leaves in Italian alfalfa cultivars.

The genetic variation of protein and cellulose contents is one of the main reasons for the variability of forage quality in alfalfa *(Riday and Brummer 2002)*. The crude protein content varies depending environmental factors but is also dependent on genetic factors, as it has been established that varieties with a higher percentage of genes originating from yellow alfalfa (*M. falcata*) have higher protein contents *(Riday and Brummer 2002; Katić et al., 2005b)*. Alfalfa quality also depends on the phenological stage and declines with plant age. As the plant matures the cellulose content increases, while the protein content decreases *(Rotili et al., 1999; Katic et al., 2005a)*. *Julier et al. (1996)* noted that the protein content of the more recently developed alfalfa cultivars has been increased by about 1% using certain breeding procedures.

Diallel analysis is one of the progeny tests that are used to evaluate parental components in alfalfa breeding. It provides information about the general and specific combining abilities of the parents, F_1 progenies, and reciprocal effects, i.e. full-sib populations. *Guines et al., (2002)* used diallel analysis to determine the mode of inheritance of alfalfa nutritive value and obtained significant GCA and SCA values among seven genotypes as well as a significantly higher GCA than SCA for leaf contribution to yield and crude protein content.

The objectives of our study were to determine the effects of gene action and the mode of inheritance of direct and indirect quality indicators and to assess the potential value of parental components for the development of alfalfa populations with increased nutritious value.

Materials and Methods

The study was conducted at the Rimski Šančevi, experiment field of the Institute of Field and Vegetable Crops in Novi Sad during 2006-2008.

Complete diallel manual crosses were made in 2003 and 2004 and the F_1 progeny and parents were planted in a comparative trial in 2006 using a randomized block design with three replications. Twenty plants were analyzed per replicate, that is, 60 per genotype.

For each genotype, we determined the proportion of leaves in dry matter yield (%) by separating the leaves from the stems and then drying and measuring them. This parameter was monitored during 2007 (2nd year of plant life, five cuttings) and 2008 (3rd year, five cuttings). The crude protein content (g kg^{-1} DM) was determined in the first cut of 2008 using the standard method proposed by Kjeldahl.

The GCA and SCA in the diallel crosses were calculated according to *Griffing (1956)*, Method 1, Mathematical Model 2 (parents, F_1s, and reciprocal crosses). The mode of inheritance was determined using the DILALLEL-SAS05 program according to *Zhang, et al.* (2005). The experiment tested 25 alfalfa genotypes. This included five varieties with different geographic origins (parents), namely NS Banat ZMS II (Serbia), Ghareh Yon Geh (Iran), Zuzana (Czech Republic), Pecy (France), and RSI 20 (Spain), and the progeny of diallel crosses made with these varieties (20 hybrids).

Results and Discussion

The results of the diallel analysis showed that there was significant variation among the parents and the F_1 hybrids for leaf proportion in dry matter yield and protein content (Table 1 and 4).

Table 1. Analysis of variance of diallel cross for proportion of leaves (GLM)

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Model	53	809.366667	15.271069	7.32**
Year	1	280.1666667	280.1666667	134.28**
Rep. (year)	4	34.3733333	8.5933333	4.12**
Genotype	24	430.8266667	17.9511111	8.60**
Year x Genotype	24	64.0000000	2.6666667	1.28
Error	96	200.293333	2.086389	
Total	149	1009.660000		

** Mean squares significant at $\alpha = 0.01$

Leaf contribution to yield indicated significant GCA x year interaction (Table 2). The interaction was indicative of different responses of the hybrids in 2007 and 2008, which may have been a result of biological factors acting in the second and third years of alfalfa plant life. The differences could also have been caused by the materials responding differently to environmental conditions present in 2007 and 2008.

Table 2. Mean squares for leaf proportion (%) from diallel analysis performed over two years (2007-2008) according to *Griffing* (1956), Method 1, Model 2 (1956)

Source of variation	Degree of freedom	2007-2008
GCA	4	53.0800**
SCA	10	13.9090**
REC	10	7.9417**
MAT	4	9.6167**
NMAT	6	6.8250**
GCA x yYear	4	7.3167**
SCA x Year	10	1.4317
REC x Year	10	2.0417
MAT x Year	4	2.9000
NMAT x Year	6	1.4694
** Mean squares significant at α = 0.01		

The effects of GCA, SCA, reciprocal crosses as well as the maternal and non-maternal effects were significant for leaf proportion in dry matter yield (Table 2). The GCA effects were significant and negative in the varieties NS Banat ZMS II and Ghareh Yon Geh and significant and positive in the French variety Pecy and the Spanish cultivar RSI 20 (Table 3.). Significant positive SCA effects were found in the combinations ♀ Banat x ♂ Pecy and ♀ Zuzana x ♂ Pecy, indicating desirable gene recombination. In ♀ Zuzana x ♂ RSI 20, SCA effects were negative, which suggests that recombining genes of these parents is undesirable when attempting to increase the proportion of leaves in yield.

The presence of significant reciprocal effects originates from maternal and non-maternal effects, which is of importance when deciding how an alfalfa cross will be made, i.e. which parent will be used as the female and which one as the male *(Guines et al., 2002)*. *Zhang and Kang (1997)* consider maternal effects to be connected with the cytoplasm of the female and non-maternal ones to reflect interaction between female's cytoplasm and the nucleus of the male. In the present study, non-maternal effects were dominant in the cultivar Ghareh Yon Geh, while significant non-maternal effects were found in ♀ Banat x ♂ RSI 20 and ♀ Ghareh Yon Geh x ♂ RSI 20 (Table 3). Significant general reciprocal effects obtained in ♀ Ghareh Yon Geh x ♂ Banat also indicate that the Iranian variety Ghareh Yon Geh should be used as the female in indirect breeding for quality (leaf proportion), while the Spanish cultivar RSI 20 should be used as the male in hybridizing alfalfa to increase the proportion of leave in yield.

Diallel analysis was indicative of additive gene action for leaf contribution to dry matter yields, as the GCA effects were significantly higher than the SCA ones. However, the significant SCA values for leaf proportion indicate that non-additive gene action was also present for this trait to a certain extent.

Table 3. Estimate of diallel analysis effects for general combining ability (GCA), specific combining ability (SCA), effects of reciprocal crosses (R), maternal (M) and non-maternal (NM) effects and their standard errors (in brackets) for leaf proportion (%) during 2007- 2008

Estimated effects							
GCA_{11}	-1.110**	SCA_{12}	-0.406	SCA_{15}	-1.550	M_{11}	-0.383
GCA_{22}	-0.726**	SCA_{13}	-0.556	SCA_{25}	-0.166	M_{22}	0.533*
GCA_{33}	-0.090	SCA_{14}	1.060*	SCA_{35}	-1.933*	M_{33}	-0.016
GCA_{44}	1.223**	SCA_{23}	0.143	SCA_{45}	0.283	M_{44}	0.250
GCA_{55}	0.523*	SCA_{24}	-0.823	(1.00)		M_{55}	
	(0.248)	SCA_{34}	1.360**			(0.248)	
		(0.51)					
Estimated effects							
R_{23}	0.666	R_{12}	-1.583**	NM_{12}	-0.666	NM_{15}	0.916*
R_{24}	0.500	R_{13}	-1.083	NM_{13}	-0.716	NM_{25}	-1.00*
R_{25}	-0.083	R_{14}	-0.166	NM_{14}	0.466	NM_{35}	-0.033
R_{34}	-0.833	R_{15}	0.916	NM_{23}	0.116	NM_{45}	0.116
R_{35}	0.333			NM_{24}	0.216		
R_{45}	0.750			NM_{34}	-0.566		
	(0.621)				(0.481)		

*, ** Estimated effects significantly different from 0 at = 0.05 and 0.01

Guines et al. (2002) obtained significant GCA and SCA values for the proportion of leaves and crude protein content but also point to the importance of maternal and non-maternal effects when crossing divergent alfalfa genotypes. Our results are in agreement with the findings of *Guines et al. (2002)* concerning leaf proportion and crude protein content in alfalfa. The results of our study indicate that the proportion of leaves varies significantly among the parents and their progenies. This is in agreement with the findings of *Annicchiarico (2007)*, which also point to the influence of genetic factors (variety) in the variation of leaf contribution to alfalfa yields.

The obtained results indicate that the protein contents of the parental populations and their hybrids varied significantly (Table 4).

Table 4. Analysis of variance of diallel cross for protein content (g kg^{-1} DM) (GLM)

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Model	26	8377.09333	322.19590	2.43**
Replicate	2	1352.106667	676.053333	5.10**
Genotype	24	7024.986667	292.707778	2.21**
Error	48	6361.89333	132.53944	
Total	74	14738.98667		

** Mean squares significant at $\alpha = 0.01$

The diallel analysis of crude protein content was indicative of additive gene action for this trait (Table 5).

Table 5. Mean squares for protein content (g kg^{-1}) from diallel analysis (2008) according to Griffing (1956), Method 1, Model 2 (1956)

Source of variation	Degree of freedom	2007-2008
GCA	4	601.627 **
SCA	10	286.965*
REC	10	174.883
MAT	4	297.267
NMAT	6	93.294

*, ** Mean squares significant at α = 0.05 and 0.01

Significant negative GCA values were found in the Iranian cultivar Ghareh Yon Geh, while the French cultivar Pecy had significant positive GCA values (Table 6). Significant negative SCA values were observed in the hybrids ♀ Banat x ♂ Ghareh Yon Geh and ♀ Zuzana x ♂ RSI 20. None of the hybrids had significant positive SCA values. The lack of significant positive SCA effects for protein content and the presence of significant negative ones suggest indirectly that negative heterosis manifested itself in the case of this trait. Of all the parental populations, only the cultivar Pecy had significant GCA values, which indicates that it would combine well in crosses aimed at increasing crude protein content in alfalfa.

Table 6. Estimate of diallel analysis effects for general combining ability (GCA) and specific combining ability (SCA) and their standard errors (in brackets) for protein content (g kg^{-1} DM) during 2008

Estimated effects					
GCA_{11}	-1.513	SCA_{12}	-5.386	SCA_{15}	-13.633
GCA_{22}	-6.446**	SCA_{13}	1.680	SCA_{25}	-9.233
GCA_{33}	1.986	SCA_{14}	-8.020*	SCA_{35}	-18.133*
GCA_{44}	5.686**	SCA_{23}	0.780	SCA_{45}	-4.266
GCA_{55}	0.286	SCA_{24}	5.413	(8.176)	
	(2.028)	SCA_{34}	-3.686		
		(4.181)			

*, ** Estimated effects significantly different from 0 at = 0.05 and 0.01

Our findings show that crossing for increased protein content (quality) makes sense but that the use of hybridization in breeding for quality should be viewed in a broader context.

The nutritional value of alfalfa (forage quality) is a complex trait that is influenced not only by protein content but by fibre content as well. The structural carbohydrates (fibres) are negatively correlated with protein content. When looking at the results of the present study, we must take into account the fact that the crude protein data originated from a single cut (first cutting of 2008), that the samples were taken from the replicates over a period of five days, and that the protein content of alfalfa decreases by about 3g/kg per day *(Katić et al., 2005a)*, resulting in variations among the replicates (Table 4.). Also, unpublished data from the experiment discussed in this paper that concern yield and yield components showed that the yields of some of the hybrids increased significantly and that heterosis for yield was manifested, which agrees with results indicating that there is a negative correlation between yield levels and yield quality in alfalfa *(Julier et al., 1997; Julier et al., 2001; Katić et al., 2005b)*.

The present findings show that significant SCA effects were present for both the proportion of leaves in yield and protein content. Since alfalfa quality is a complex, polygenic trait that is controlled by a number of additive genes, non-additive gene action is not expected. The presence of the non-additive mode of inheritance can be explained by the cumulative effects of many different dominant alleles that act together and cause the appearance of non-additive gene expression (dominant inheritance), which manifests itself when individual crosses are made among geographically and genetically divergent populations of alfalfa *(Riday and Brummer, 2002)*.

Despite the fact that nutritive value is a complex character that incorporates a number of traits, alfalfa breeding for increased nutritional value can be effective if crosses are made among genetically divergent populations and the newly developed hybrids (populations) are used as donors of genes for quality in the development of new synthetic varieties, all the while making sure to maintain high yield levels, which are a key element of any alfalfa breeding program.

Conclusion

The results of the study showed that there was significant variability in leaf contribution to yield and crude protein content among the parents used in the experiment and their progenies.

Diallel crossing produced genotypes with significant positive and negative GCA values for both of the quality traits. Significant positive and negative SCA effects were also found for both traits. Significant reciprocal effects (both maternal and non-maternal) were recorded for leaf proportion. A pronounced maternal effect was observed in the Iranian cultivar Ghareh Yon Geh, so this genotype should be used in hybridization as the female parent. Diallel analysis revealed highly significant GCA and SCA effects for leaf proportion and crude protein content alike, which indicates that additive as well as non-additive effects were significant in determining dry matter quality.

The proportion of leaves in yield should be used as an indirect indicator of quality in alfalfa breeding, and some hybrid combinations can be used as parental components in the synthesis of new alfalfa cultivars that will have a higher nutritive value and good yield levels.

Dialelna analiza nasleđivanja nekih osobina kvaliteta suve materije lucerke (*Medicago sativa* L.)

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Rezime

Cilj rada je bio da se dialelnom analizom odrede tipovi genskih akcija, način nasleđivanja direktnih i indirektnih pokazatelja kvaliteta lucerke i oceni potencijalna vrednost roditeljskih komponenti za stvaranje sorti veće hranljive vrednosti u cilju povećanja kvaliteta suve materije lucerke. Utvrđeni su značajni efekti opštih (OKS) i posebnih (PKS) kombinacionih sposobnosti za udeo lista u prinosu i sadržaj proteina. Registrovani su značajni recipročni efekti (majčinski i nemajčinski efekti) za udeo lista u prinosu suve materije. Dialelna analiza je pokazala visoku značajnost OKS i PKS za udeo lišća i sadržaj sirovih proteina, što ukazuje da su aditivni ali i neaditivni efekti gena bili značajni za određivanje kvaliteta suve materije lucerke.

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VETCHES (*Vicia* spp.) ADOPTION AND UTILIZATION IN AUSTRALIA

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Invited review paper

Abstract: The concept of improved adoption of vetches in Australia is based on germplasm diversity and the recombination of germplasm to breed new varieties. Since the breeding of vetches began in Australia in 1992 there has been rapid progress, providing improved vetch varieties that have been well adopted into Australian field crop rotations. Vetches are well adapted to most Australian farming soil types and the Mediterranean type of climate in southern and western Australian cropping zones. *V. sativa* (common vetch) and *V. villosa* (woolly pod vetch) are the main species grown in Australia. Vetches are a significant component of cereal farming rotations in Australia's low and medium rainfall cropping zones. Its versatility has allowed it to spread into areas where no other legume crops are grown. The main limiting factors in Australian vetch production are abiotic and biotic stresses; like drought, soil acidity, boron and magnesium toxicity, frost, disease and insect resistance/tolerance. The original genetic introductions sourced for the breeding program required minimal selection for some specific targeted trait(s), but no one line has the all positive characteristics required by new vetch varieties for Australian conditions. In Australia common vetch varieties are used for hay, silage, grain, grazing and green manuring, and woolly pod vetch varieties are grown for hay, silage, grazing and green manuring. Only a small proportion of the vetch hay and seeds produced are exported from Australia because the domestic demand for vetch seeds and hay is significantly higher that supply.

Key words: Adoption, vetches, drought, abiotic, biotic

Introduction

Vetch breeding and cultivation in Australian agriculture is currently going through a renaissance that could well lead to a revolution in current farming practices. Vetches have an advantage over most other field legumes in that they are better suited to both grain and hay production than most other species and are thus genuine dual purpose crops. Vetches can grow in most Australian soil types in low/medium rainfall areas (280-400mm/yr) because of their broad adaptability they have the potential to grow where other legumes and cereal crops are not suited. These growing conditions can include extremes of winter cold/wet and frosted days from seeding to pod setting. In many years growing conditions change from wet/cold very sharply to high temperatures at or around the flowering stage (second part of September and early October) and continue as very dry conditions up to harvest. Most vetch production is in the southern part of Australia that is characterised by a hot dry summer and short winter rainfall season (growing season) of 4-6 months that allows the growth of short season crops. There is a large variation in annual rainfall across the cropping areas, and droughts are common in most Australian cropping zones.

Vetch has been adopted by Australian farmers as a pulse rotation crop where drought is the major environmental stress. Farmers perceive vetch as a reliable, versatile legume, which can be used to manage cereal diseases and grass weeds, improve soil fertility and contribute to increased yield and protein content in subsequent cereal crops. Also, vetch hay as a crop in farm rotations is one of the best methods to reduce herbicide resistant weeds and to avoid chemical contamination of paddocks. Vetch is versatile in terms of its potential end use - grain, pasture, silage, hay or green manure. Vetch's versatility allows farmers to decide the end use between seeding and harvesting. In Australia, over the last 5-7 years farmers have based these and use decisions mainly on the basis of the relative prices of grain and hay during the season.

Since 1992 the Australian National Vetch Breeding Program (ANVBP) has tested over 10 *Vicia* species for use as grain, hay/silage, pasture plant or green manure crops. Several *Vicia* species, like *V. sativa, V. narbonensis, V. ervilia, V. pannonica* and *V. benghalensis* are classified as genuine dual purpose crops for grain and hay/silage productions in Australia. *V. villosa* is predominantly used as a hay/silage, pasture and green manuring crop.

Germplasm and breeding for vetch adoption in Australian cropping

Traditionally, Australian farmers planted vetches mostly as pasture plants and later used them as grain, hay/silage and green manuring crops. But in the early 1990s, comprehensive collecting, selection and breeding took place, resulting in the increased inclusion of vetch in crop rotations. Since 1995, a large amount of germplasm was imported into the country from the Vavilov Institute Collections *(VIR)*, St Petersburg, Russia, and from the International Centre for Agricultural Research in the Dry Areas *(ICARDA),* Aleppo, Syria. The Australian gene pool is based on collections from many countries around the world and contains approximately 5,000 accessions and more than 4,000 inbreed lines, this is located at the Australian Temperature Field Crop Collection *(ATFCC)* at Horsham, and the South Australian Research and Development Institute *(SARDI)* at Adelaide. Several species of *Vicia* are important as forage and grain crops *(Robertson et al., 2000)*. The Australian National Vetch Breeding Program concentrates on two species; common vetch (*V. sativa*) and woolly pod vetch (*V. villosa*). Other species like, *V. pannonica* and *V. palestina* are included in selection for germplasm that can be grown in Australia. Valuable diversity has been observed in these introductions for recombination of genes for better vetch varieties. Interaction between maturity of vetch germplasm and rainfall, explained in Table 1.

Table 1. Five years results for grain and dry matter yield (2004-08) of common vetches - maturity Vs low, medium and high rainfall areas**

Maturity*	Low[1]		Mid[1]		High[1]	
	Grain[2]	Dry matter[2]	Grain[2]	Dry matter[2]	Grain[2]	Dry matter[2]
Early (<95days)	125	98	102	91	97	87
Mid (95-110days)	102	105	117	111	114	109
Late (>110days)	97	101	109	124	121	135
Average yield (t/ha)	0.87	3.32	1.43	4.15	1.82	6.25
No of accessions (mean of 5yrs)	126	34	126	34	126	34

* Days from seeding to >50% flowering, ** 2006, 07 and 08 were noticeably lower in rainfall than long term averages, [1] Low <330; Mid 330-400; High >450, [2] % relative to experiment average.

In selecting for grain production in Australian farming conditions ANVBP relies on parental germplasm from landrace/varieties that grow in Mediterranean conditions. Geographic origin was the most important trait for grouping the genotypes on the basis of total mutual similarity *(Mikic, 2008)*. Most of the germplasm/plants selected for grain production are early/mid maturity, but they are smaller plants than the later maturing germplasm. Germplasm/lines of big plants with mid/late maturity with longer flowering duration are selected for dry matter production. Green forage yield has a highly significant positive correlation with number of days from sowing until cutting and plant height at cutting *(Mikic, 2008)*.

Several programs around the world, including *ANVBP* focus on breeding common vetch *(V. sativa)*. Common vetch is the most widely distributed of the vetches due to its high and stable yields and reliable seed production *(Mihailovic et al., 2005)*. *ANVBP* is breeding/selecting common vetch germplasm for multipurpose end uses for; forage/hay and grain for ruminant production or as a pasture or green manure crop. But for hay/silage production in Australia, woolly pod (*V. villosa*) is the more predominant species. For these two main species, *V. sativa* spp and *V. villosa spp.* the ANVBP is focused on the recombination of diverse germplasm for superior varieties. The main/key breeding objectives are: yield, adaption to drought stress, resistance to the main vetch diseases (rust and *ascochyta*), and hay and grain quality. Of not much less importance in breeding objectives are; early root and plant establishment/vigour, plant morphology, shattering resistance, seed softness and harvestability. The program has 14 sites across Australia taking in all environments including extremes like <150 mm to >400 mm from seeding to harvesting, on acid soils pH-5.2 to alkaline soils with pH-8.5. Understandably selecting germplasm for specific stress/traits is difficult unless the selections are exposed to the specific stresses and conditions in the field.

Common vetch (*V. sativa* spp. *sativa*) is a self-pollinated species and the program conducts pedigree, single and mass plant selection and backcrossing methods in combination with recombined-derived family (RDF) methods. These methods speed up elimination of off-type plants in $F_{2,3}$ and were used to identify superior progeny for further backcrossing, testing and selection *(Matic et al., 2007).* Elimination of low yielding crosses in $F_{2,3}$ progenies is limiting diversity in the populations, but has resulted in quicker delivery of new varieties. RDF takes place with early generation testing (EGT) to identify F_2 plants with superior progenies, this has resulted in release of improved variety(s) one to a few yrs earlier than standard breeding methods *(Slinkard et al., 2000)*. However, individual plant selection for yield in early generations generally has not been successful. From ANVBP experiences assessing single plants/plots for yield, at one site for one year does not provide satisfactory information, testing has to be conducted across multiple sites in replicated plots for a minimum of 3 years, this extends the time from cross to new variety release to 7-9 years.

Many of the targeted traits; yield, drought tolerance, disease resistance, and grain quality; are controlled by multiple genes. In vetch, yield especially is a trait of low heritability, but at the same time yield is also the output from the integration of many other components; drought tolerance, disease resistance, maturity, root/plant vigour. The program is selecting genotypes to maximise the range of genetic diversity. In low rainfall areas and areas with unreliable rainfall, drought is the major limiting factor in vetch crop yields. For these areas genotypes have to possess; good early establishment, strong roots and a shorter period between seeding and full (harvest) maturity that can avoid sharp finishes to a season. The most useful gene pool for these breeding/selection criteria is taken from countries/areas where drought occurs nearly every year, like, Syria, Morocco, Ethiopia, Spain, Cyprus and other areas/countries where drought is the main limiting factor in plant production. For the southern Australian cropping belt, study and analysis of agronomy data from many years suggests that seeding in May compared with late June or early July showed a positive effect in increasing yield.

For areas >450 mm/yr the ANVBP focuses on breeding mid/later maturing varieties. Later maturing genotypes, those taking more days to mature are usually bigger plants, with more developed shoots and leaves than early maturing varieties, resulting in higher production of biomass/dry matter and grain in higher rainfall areas. For hay production varieties that can be cut for hay in 110 to130 days from seeding; and for grain to be harvest ready/mature in 130 to160 days from seeding. In these areas rust and *ascochyta* are more common than in lower rainfall areas. In early 1992, 93 & 94 these diseases reduced grain yield by 50-90% in many vetch production areas *(Matic, 1995)*. Germplasm for later maturity is selected from land race and varieties that are grown in many parts of Russia, Serbia, Bulgaria, Romania, Hungary, Germany, France, Spain and other areas/countries that have >350mm of rain during the season.

This program has released two common vetch varieties that are rust and *ascochyta* resistant; Morava in 1998 and Rasina in 2006. Both varieties are, soft-seeded (<2% hard seeds), rust and *ascochyta* tolerant, have good initial growth, purple flowering, non-shattering and have grain with beige cotyledons. Morava is the most widely grown variety for hay in areas >300mm/rain/yr and grain in areas with >400mm/rain/yr. Rasina is more of a grain variety, it is a smaller plant, earlier than Morava for 15-20 days and excepted by farmers in low/medium rainfall areas mostly for grain production. Significant increases in yield of both grain and dry matter and the area sown to vetch were realised with the release of these two vetch varieties.

From parental inheritance expressions of genes are recorded for: maturity, plant size and morphology, flower colour, coat and cotyledons colour, and anti-nutritional components in the grain. Other more important traits; like yield, disease resistance, drought tolerance, pod shattering and seed-softness are achievable by recombination of germplasm, backcrossing and inclusion of more diverse parental germplasm with strong expression of the targeted trait(s). But, these traits are a combination of germplasm expression and adaption to the biotic and abiotic stresses. To overcome any or all of these problems in Australian vetch production, there is need to identify the right variety and variety use for a particular area. No one vetch variety is appropriate in all of the very different and diverse cropping areas in Australia.

Utilization of common vetches in Australian farming

The desired characteristics for utilization of a vetch crop, for:

Green manuring - strong roots, good cold season growth, early nodulation, big biomass;

Green grazing – good initial/winter growth, the leaf and stem to be palatable from early stage to the end of growth;

Hay/silage – robust plants, disease resistant, good leaf retention, and leaf Vs stem ratio, high feeding value of hay/silage and to be able to grow well in a mixture with cereals for hay/silage.

Grain – tolerant to biotic and abiotic stresses, non-shattering, uniform maturing, high harvest ability.

Domestic and export seeds – varietal purity, soft seeds/high germination % (>95%), and non contamination with prohibited weeds, diseases or pests.

Benefits of the inclusion of vetches in crop rotations and use on farm:

Vetches can fix up to 200kg of nitrogen in the soil *(Rochester, 2008)*, a dry land vetch crop used for grain or hay or green manure leaves/returns to the soil 56, 94 and 136 kg of nitrogen, respectively *(Matic et al, 2005)*.

Vetches are a very important crop in crop rotations. They significantly reduce soil diseases like *rhizoctonia*, and take all, as well as reducing grass weeds that are hard to control in following cereal crops *(Matic et al, 2008)*.

Cereal crop yields following a crop of vetch are usually at least 30-50% higher than those derived from continues cropping with cereals *(Evans et al., 2003; Unkovich et al., 1997)*.

Inclusion of 50% to 75% of vetch grain with cereal grains in diet for lambs, significantly increased lamb growth *(Matic et al., 2006)*.

Vetch hay and grain have excellent nutritive feeding values and are a very satisfactory feed source for ruminants, see following data:

Table 2. Mean values for tested vetch hay and grain samples

Years	No of tested samples	Crude Protein (%)	Dry Matter Digestibility (%)	Metabolically Energy (MJ/kg DM)	Water Soluble Carbohydrates (%)	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)
2007 & 08	317	21.6	75.8	11.3	11.4	39.5	29.3
	32	29.4	85.7	12.6	14.3	41.2	32.5

Vetch as forage can be cut depending on the quality requirements of the forage. As the plant matures, dry matter digestibility, leafiness and crude protein decrease, and neutral detergent fibre and acid detergent fibre increase *(El Moneim et al, 1998)*.

Grain from common vetch varieties can be used for all ruminants in a feed diet as pure grain or mixed with cereal grain, to create a more balanced diet. Vetch grain can be a valuable source of protein for pigs between 66 and 101 days of age. *Collins et al. (2002)* reported that *V. sativa* grain could completely replace soya-beans in diets for growing pigs without adversely affecting growth performance or meat quality.

Woolly pod vetch plants in early growth stages contain toxins that prevent farmers from using it for grazing before it reaches 10 nodes *(Harper et al., 1993)*. It can only be grazed from the 10 node stage to flowering; it makes excellent hay/silage at this stage as well. Also the grain cannot to be used to feed any livestock (*Enneking, 1995)*.

Woolly pod vetches are a very good hay/silage and green manuring crop, because in areas that receive >400mm/yr rainfall they produce very good green/dry biomass yields, that is very useful in Australian farming.

Conclusion

ANVBP has been the focus for national interest in vetch improvement and development. Vetch production in Australia is limited by biotic (vetch diseases), and abiotic stresses (drought, frost, soil acidity, boron and magnesium toxicity). New vetch variety acceptance/adoption and adaptability is based on germplasm that can overcome the biotic and abiotic limitations and the profitability of producing vetches in particular areas. A vetch crop is beneficial to growers and end-users, because they increased total farm profitability through the reliability, versatility and productivity of the new varieties. Breeding of new vetch varieties is based on targets and traits that are required by, and dictated by growers and end users.

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Mogućnost gajenja i iskorišćavanja grahorica (*Vicia* spp.) u Australiji

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Rezime

Koncept poboljšanja adaptacije grahorica u Australiji je zasnovan na različitosti germplazme i rekombinaciji germplazme sa ciljem stvaranja novih sorti. Otkako je počelo gajenje grahorice u Australiji 1992. godine, došlo je do brzog napretka, dobijene su nove sorte koje su uključene u plodored. Grahorice su dobro prilagođene većini australijskih tipova poljoprivrednog zemljišta i mediteranskom tipu klime u južnoj i zapadnoj zoni uzgajanja u Australiji. *Vicia faba* (bob) i *V. villosa* (maljava grahorica) predstavljaju glavne vrsta koje se gaje u Australiji. Grahorice su i značajna komponenta plodoreda u zonama Australje sa niskim i srednjim količinama padavina. Njihova prilagodljivost je omogućila širenje u oblastima u kojima nema gajenja drugih leguminoza. Glavni limitirajući faktori u proizvodnji grahorica u Australiji su abiotički (suša, kiselost zemljišta, toksičnost bora i magnezijuma i mraz) i biotički (otpornost/tolerancija na bolesti i insekte). Originalni genetski materijal introdukovan za program oplemenjivanja je zahtevao minimalnu selekciju na neku specifičnu ciljanu osobinu, ali nijedna linija nema sve pozitivne karakteristike za dobijanje novih varijeteta prilagođenih australijskim uslovima. U Australiji varijeteti bobova se koriste za seno, silažu, za seme, ispašu i kao zelenišno đubrivo, sorte krmne grahorice se gaje za seno, silaža, ispašu, kao i za zeleno đubrivo. Samo mali deo sena i semena grahorice koji se proizvedu se izvoze iz Australije, jer je domaća potražnja za semenom i senom grahorice znatno veća od ponude.

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DEPENDENCE OF ALFALFA YIELD ON DENSE AND SPACED PLANTING

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Abstract: Commercial alfalfa is planted in dense stands, but spaced planting is applied for breeding purposes, to be able to analyze experimental plants easily. Numerous studies have indicated that alfalfa yield performance varies with the method of planting. The objective of this study was to establish correlations between yields of green forage and dry matter, plant height and growth rate of seven synthetic varieties of alfalfa grown in a spaced stand in a nursery and in a dense stand. An appropriate number of plants (60-100) is needed to obtain reliable information on yield, height and regrowth rate. Significant rank correlations in green forage yield and regrowth rate existed between spaced and densely planted plants. Significant differences were also obtained (Pearson's coefficients) in dry matter yield, plant height and regrowth rate between spaced and densely planted plants.

Key words: genotype, alfalfa, planting method, yield

Introduction

In commercial production, alfalfa is grown in dense stands. However, for the purpose of breeding, aflalfa plants are grown in a nursery, where value of germplasm is assessed by observing individual plants. Mass selection, i.e., selection of plants within a population, based on phenotypic characteristics, is often used in the breeding of forage crops because they are variable and the uniformity is not required *(Rumbaugh et al., 1988; Burton, 1990)*. Many forage crops are perennials, and their most important traits such as the yield of the whole plant are inherited polygenically *(Burton, 1990)*. Mass selection is not targeted at yield increase because the heritability of individual plants is low. The degree of heritability can be increased by maintaining of a uniform environment for all plants in the population from the moment of planting until individual plants are visually checked. This is the most important objective to be achieved in order to increase the efficiency of mass selection for improved forage yield.

Spaced planting is applied if a limited amount of seed is available *(Mackie et al., 2005)*, or if some morphological and biological traits are difficult to assess in a dense stand. *Rotili et al. (1999)* claimed that, in some cases, individual plants may be more effective for assessing genotype value under stress conditions, but not when stress conditions do not exist. Characteristics typically determined on individual plants are: dry matter yield, height of the first raceme on the main stem, number of stems and phenological stages.

However, some researches indicated that biomass yield of alfalfa genotypes varied between spaced plants and a thick stand. Low correlation values were obtained by *Pearson and Elling (1961)* and by *Rotili et al. (1999)* (r = 0.62 and r = 0.4 - 0.6, respectively) for dry matter yield, plant height and number of shoots between individual plants and dense planting. These authors indicated that heritability varied depending on the density of plants.

Riday and Brummer (2004) obtained a higher manifestation of heterosis in spaced plants, probably because of lower competition between individual plants. Differences in yield and morphological characteristics between densely planted and spaced plants are explained by interplant competition and the decline of weaker plants, so that only the most powerful (best) plants take part in yield formation *(Veronesi and Lorenzetti, 1983; Viand et al., 1988; Riday and Brummer, 2004)*.

The aim of this study was to determine correlations between the yields of green forage and dry matter, plant height and regrowth rate in seven alfalfa synthetics grown in dense and spaced stands.

Materials and Methods

In this study we used seven alfalfa cultivars varying in geographic origin: two from Serbia (NS Banat ZMS II; NS Mediana ZMS V), three from Greece (Pella, Dolichi, Hyliki), one from Estonia (Jogeva 118) and one from Bolivia *(UMSS 2001)*.

The experiment was established in 2006 on a slightly calcareous chernozem soil at the experiment fields of Institute of Field and Vegetable Crops, in a randomized block layout with three replications.

The nursery (spaced plants): the elementary unit was a row 10 meters long, with row spacing of 80 cm and the spacing in the row of 50 cm, which makes 20 plants per row. The distance between blocks was 1 m, the three replications analyzed a total of 60 plants per cultivar. The cultivars were planted in a random arrangement. To make sure there will be a plant in each hill, 4-5 seeds were planted per hill at a depth of 2 cm. After emergence, the stage of 3-5 true leaves, the plants were thinned to a single plant per hill.

Yield, plant height and regrowth rate were monitored in the 2nd, 3rd and 4th cuts of 2007 and the 1st and 2nd cuts of 2008. Green forage yield was measured immediately after cutting. For dry matter determination, average samples (200 g) were taken and dried at 60°C for 5 days and after that determined the yield of dry matter expressed in g per plant. Plant height (cm) was determined shortly before cutting, measuring the highest shoot, regeneration rate 15 days after each cut.

Dense stand: The part of the experiment with the dense stand was conducted at the same site, the same time and using the same alfalfa cultivars. The experimental unit size was 5 m^2 (5 x 1 m), with the spacings between rows, experimental units and blocks of 20 cm, 40 cm and 1 m respectively. The planting rate used was 15 kg/ha of seed. Planting was done by hand, at the depth of 2 cm. The experiment was cut 5 times in 2007 and four times in 2008. The analyzed material was from the 2nd, 3rd and 4th cuts of 2007 and the 1^{st} and 2^{nd} cuts of 2008. Green forage yield was measured on the site. Dry matter content was determined after drying of samples (0.5 kg) which were then used for dry matter yield calculation. Plant height (cm) was determined in the laboratory, measuring 20 randomly selected shoots of each cultivar, from the cutting point to shoot tip. Regeneration rate was measured 15 days after cutting at 3 places in each experimental unit.

Analysis of results: Data were analyzed by the two-way analysis of variance for randomized block design experiment. Cultivars were the independent factor, the cuttings the dependent one. Significance of differences among the mean values of the cultivars was evaluated by the LSD test.

Coefficients of Pearson's correlations and Spearman's rank correlation coefficients *(Hadživuković, 1991)* were calculated between the spaced plants and the dense stand.

Results and Discussion

The order of cultivars regarding the yield of green forage was fairly similar between the spaced plants and the dense stand (Tables 1 and 2).

Table 1. Yield, height and regrowth rate of alfalfa cultivars, 2007-2208 nursery

Cultivar	Green forage g per plant	Dry matter g per plant	Height cm	Regeneration rate cm
NS Banat ZMS II	537	131	89.3	41.1
NS Mediana ZMS V	564	143	88.4	40.7
Pella	669	159	90.2	42.2
Dolichi	599	137	94.6	41.4
Hyliki	589	138	89.7	38.6
Jogeva 118	523	114	49.2	20.7
UMSS 2001	517	127	75.1	35.8
Average	571	136	82.4	37.2
0.05	90.2	27.3	3.7	3.2
LSD 0.01	118.8	36.0	4.9	4.2

The Greek cultivars Pella, Dolichi and Hyliki had highest yields, and Estonian cultivar Jogeva 118 and the Bolivian cultivar UMSS 2001 the smallest A similar ranking of cultivars was regarding the dry matter yield. The cultivar Pella had the highest dry matter yield in the nursery, the cultivar NS Banat ZMS II in the dense stand, however, the difference between these two values was not significant (Table 2). Jogeva 118 and UMSS 2001 had lowest dry matter yield in both experiments. The ranking of cultivars according to height was similar to that for green forage (Table 1 and 2). Most were of the Greek cultivars were tallest, Jogeva 118 and UMSS 2001 the shortest. Regarding the regrowth rate, the cultivar Pella was fastest in both experiments. Jogeva 118 and UMSS 2001 had slowest regrowth rates both in the experiment with spaced plants and dense crop. The analysis of these results showed that the yield and regrowth rate are correlated in dense stands.

Table 2. Yield, height and regrowth rate of alfalfa cultivars, 2007-2008 dense stand

Cultivar	Green forage t ha^{-1}	Dry matter t ha^{-1}	Height cm	Regrowth rate cm
NS Banat ZMS II	74.8	20.9	67.4	33.4
NS Mediana ZMS V	68.0	17.6	68.9	31.9
Pella	76.0	20.6	66.4	34.0
Dolichi	78.4	20.0	71.2	33.2
Hyliki	76.4	20.4	68.9	33.7
Jogeva 118	40.0	9.2	41.4	16.9
UMSS 2001	55.2	14.8	62.4	31.2
Average	67.0	17.4	63.8	30.6
0.05	3.6	1.0	2.5	1.6
LSD 0.01	4.7	1.2	3.3	2.1

Calculate Spearman's rank correlation coefficients have established a solid link between the yield of green forage in a dense crop and spaced plants, r = 0.82 (Table 3).

Table 3. Averages, ranking and rank correlations of alfalfa cultivars

Trait	Spaced plants		Dense stand		r_s	r^z
	Average	Rank	Average	Rank		
Green forage	571 g/plant	517-669	67.0 t ha^{-1}	40.0-78.4	0.82*	0.66
Dry matter	136 g/plant	114-159	17.4 t ha^{-1}	9.2-20.9	0.54	0.75*
Height	82.4 cm	49.2-94.6	63.8 cm	41.4-71.2	0.72	0.98**
Regrowth rate	37.2 cm	20.7-42.2	30.6 cm	16.9-34.0	0.75*	0.98**

Also, a significant rank correlation coefficient was obtained for the regrowth rate, r = 0.75. The rank correlation coefficient for plant height was close to being significant, r = 0.72 (Table 3). Dry matter yield had a modest rank correlation (r = 0.54) between the two experiments.

Pearson's correlation coefficients between spaced plants and dense stand showed significant values for dry matter yield (r = 0.75), height (r = 0.98) and the regrowth rate (r = 0.98). Regarding the yield of green forage, Pearson's correlation coefficients between spaced plants and dense stand were modest (r = 0.66). The high correlation coefficients for yield and regrowth rate between spaced plants and dense stand indicate that reliable information can be provided on the value of alfalfa germplasm from spaced plants. *Guines et al. (2002)* and *Julier (1997)* indicating the presence of high correlations between quality traits of alfalfa plants planted as spaced plants and in a dense stand. Previous studies have rendered significant differences between experiments with spaced plants and those planted in a dense stand *(Veronesi and Lorenzetti, 1983; Viand et al., 1988)*. Such results are explained by the fact that, in a thin stand, a low competition between plants enables their maximum development, while in a dense stand the weak plants tend to perish.

Another source of variation may be a relatively small amount of seed used for planting spaced plants. The planted seeds may not include all combinations (genotypes) contained in the initial sample. Therefore, number of plants to be used in the analysis should be taken into account. For the analysis of ecotypes, *Rumbaugh et al. (1988)* advises at least 50 plants, while *Rotili et al. (1999)* accept that in some cases spaced plants may provide more reliable data than those obtained in a dense stand and propose 70-100 plants to be used per genotype. In our experiment we analyzed 60 plants per cultivar.

Rotili et al. (1999) consider a dense alfalfa crop as a system, and for a better understanding of the system we must have some information about the units that constitute it. When alfalfa plants are grown at a space, it is easier to understand and analyze their morphological and biological features and gain impression on advantages and disadvantages of an analyzed germplasm. Individual plants produce preliminary results in the evaluation of alfalfa breeding material *(Mackie et al., 2005)*.

In our experiments plants were grown in field conditions, which could cause the weak plants to perish in both experiments. By thinning the plants per hill and leaving best plants, we may have reduced the differences between spaced and dense alfalfa stands.

Conclusion

Significant rank correlations were observed in the of green forage yield and regrowth rate between spaced and dense planting. Significant differences were also obtained (Pearson's coefficients) in dry matter yield, height and regrowth rate between spaced and dense planting. An appropriate number of plants (60-100) should be planted in a thin stand to obtain reliable information on yield performance, plant height and regrowth rate. Plants grown in a thin stand can provide information on their morphological and biological characteristics, which can not be obtained from plants growing in a dense stand. For breeding alfalfa, it is necessary to know advantages and disadvantages of experiments with spaced and dense planting.

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Međuzavisnost prinosa lucerke gajene u gustoredoj i kućičnoj setvi

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Rezime

Lucerka se gaji u proizvodnji gustoredo, ali za potrebe oplemenjivanja se primenjuje kućična setva da bi se biljke mogle lakše analizirati. Postoje brojna istraživanja koja ukazuju na različite prinose zavisno od načina setve. Cilj rada je bio da se odrede korelacione veze za prinos zelene krme i suve materije, visinu biljaka i brzinu porasta sedam sintetičkih sorti lucerke gajenih u matičnjaku i gustom sklopu. Analizom odgovarajućeg broja (60-100) pojedinačnih biljaka mogu se dobiti prve informacije o prinosu, visini i brzini regeneracije. Postoje značajne korelacije ranga u prinosu zelene krme i brzine regeneracije između pojedinačnih biljaka i gustog useva. Dobijene su i značajne razlike (Pirsonovi koeficijenti) u prinosu suve materije, visini i brzini odrastanja između pojedinačnih biljaka i biljaka u gustom sklopu.

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VARIABILITY OF AGRONOMIC TRAITS OF RED CLOVER GENOTYPES (*Trifolium pratense* L.)

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Abstract: In this paper, the most important agronomic traits of the two experimental red clover genotypes (*Trifolium pratense* L.) in relation to the variety Kolubara and Viola (standard) were emphasized. The tests were conducted on the experimental field of the Agricultural Institute in Banja Luka, Republic of Srpska during the period 2006-2008. During the three-year study, significant differences between red clover genotypes, in regard to yield and quality of biomass, as well as seed yield were established. The highest three-year average yield of dry matter (DM) was achieved with genotype CD-TR/2004 (12.4 t ha^{-1}). Selected genotypes of red clover realized good average seed yields, and differences compared to the standard (Kolubara and Viola) were highly significant. The genotype CD-BL/2001 achieved the largest two-year average seed yield (373.1 kg ha^{-1}).

Key words: red clover, genotype, dry matter, quality, seed yield

Introduction

Red clover is a short-lived plant species which lives two to three years. This species has a good tolerance to shallower and acid soils, which are not suitable for alfalfa, and also gives high yields of good quality forage *(C. Mousset-Deklas, 1993)*. It is cultivated in different manners, namely: as a pure crop or in mixtures with different grass species *(Đukic et al., 2009)*. It is characterized by highly significant biological properties, which enables it to grow in different agroecological conditions. *Gatarić (1999)* reported that it is the very important feeding culture due to its nutritious value, which slightly falls behind in terms of alfalfa yield and quality of forage. Domestic populations of red clover are characterized by good resistance to drought and cold. Collection and examination of morphological and biological properties, and productivity of local populations of red clover, at the Agricultural Institute of Banja Luka, started in 1965. The objectives of breeding red clover are often different, and according to *Đukic et al. (2007)* they usually include forage and seed yield, seed longevity and resistance to wilt. The aim of this study was to examine the most important quantitative properties of local genotypes of red clover *(Trifolium pratense* L.), such as yield and quality of biomass and seed yield, in relation to the foreign varieties in the agro ecological conditions of the Banja Luka region.

Materials and Methods

The trials were conducted on the experimental field of the Agricultural Institute Banja Luka, Republic of Srpska in the period 2006-2008. The study included two red clover genotypes which occurred as a result of breeding by using the introduced genotypes and indigenous populations, as well as the two foreign varieties Kolubara and Viola (standards).

On well-prepared brown-valley soil, the two-factorial trial was set up (factor A - genotype, factor B - year) by randomized block design in four repetitions. Sowing was done manually with 20 kg ha^{-1} of seed.

During the three-year testing, the most important quantitative properties of red clover were monitored, as follows: dry matter yield (t ha^{-1}) and seed yield (kg ha^{-1}). Quality of dry matter was determined using standard methods of chemical analysis, as follows: content of crude protein (CP) by *Kjeldahl,* and crude fiber content (CC), by *Honneberg-Stohman.*

The results of dry matter yield and seed were processed by the analysis of variance (ANOVA), and significant differences between mean values were determined with LSD test. Also, dry matter and seed yield of red clover, genotype x environment interaction was also calculated.

Result and Discussion

Dry matter yield of. In the first year of testing (B1) of red clover genotypes the highest total dry matter yield was achieved with genotype CD-TR/2004 (9.4 t ha^{-1}). In relation to the Kolubara (standard), with this genotype higher yields of DM were achieved by 2.2 t ha^{-1}, and in comparison with Viola (Standard) the achieved yields are higher by 2.5 t ha^{-1}. During the second year of testing (B_2) according to the total DM yield genotype CD-TR/2004 gave the best results (18.1 t ha^{-1}), and the differences in relation to Kolubara (11.1 t ha^{-1}) and Viola (7.1 t ha^{-1}) were statistically highly significant. During this year, high dry matter yield was also achieved with red clover genotype CD-BL/2001 (16.6 t ha^{-1}). In 2008 (B_3), third year of testing, the average yield of red clover genotypes was 7.7 t ha^{-1} DM. The lowest dry matter yield gave the red clover genotype Viola (4.2 t ha^{-1}), and the highest CD- BL/2001 (10.1 t ha^{-1}), therefore the differences were highly significant. High dry matter yield in this year was also achieved with the genotype CD-TR/2004 (9.7 t ha^{-1}, which is by 2.7 t ha^{-1} more comparing to

Kolubara and by 5.5 t ha^{-1} higher yield in comparison with Viola. *Vasiljević et al. (1999)* reported that during the three-year trial with the red clover variety, Viola gave the average dry matter yield of 10.4 tha^{-1}, while the average yield of Kolubara variety was 12.1 tha^{-1}. According to *Lugić et al.(2004)*, the new red clover variety Kruševačka K-38 gave the average dry matter yield of 15.8 tha^{-1} during the three-year research, namely yields were in the range from 10.2 t ha^{-1} to 19.5 tha^{-1}.

Table 1. Dry matter yield of red clover genotypes per cuttings and total yields (t ha^{-1}), 2006-2008

Genotype(A)	B_1: 2006/Year				B_2: 2007/Year				B_3: 2008/Year				Average(A) (t ha^{-1})
	Cutting (C) (t ha^{-1})			Σ	Cutting (C) (t ha^{-1})			Σ	Cutting (C) (t ha^{-1})			Σ	
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3		
CD-TR/2004	5.1	2.3	2.0	9.4	9.8	6.4	2.0	18.1	5.4	3.1	1.1	9.7	12.4
Kolubara	3.8	2.0	1.4	7.2	5.8	3.9	1.4	11.1	5.0	1.4	0.6	7.0	8.4
CD-BL/2001	4.7	2.1	1.7	8.5	8.3	6.4	1.9	16.6	6.4	2.6	1.2	10.1	11.7
Viola	3.6	1.9	1.4	6.9	4.1	2.1	0.9	7.1	3.0	0.9	0.3	4.2	6.1
Average(B)	4.3	2.1	1.6	8.0	7.0	4.7	1.5	13.2	4.9	2.0	0.8	7.7	9.7
Cutting percentage (%)	54.1	26.0	19.9	100	53.0	35.5	11.5	100	63.7	26.1	10.2	100	-

LSD	A	B	AB
0.05	1.25	1.09	2.16
0.01	1.67	1.44	2.89

Based on the results of these studies, a three-year average yield of dry matter genotypes red clover was 9.7 tha^{-1}. The highest dry matter yield was achieved with genotype CD-TR/2004 (12.4 t ha^{-1}) which was 4.0 t ha^{-1} more than Kolubara and 6.3 t ha^{-1} SM more than Viola, and the differences were highly significant (Table 1). For understanding the quantitative properties of red clover genotypes, in addition to the total yield of dry matter, a special significance also had distribution of yield per cuttings. During these researches, in the first year of the cutting percentage was 54.1%, while the second and third 45.9%. In the second year (2007) in the first cutting 53.0% was achieved, and with second and third mowing 47.0%, while in the third year of the first cutting the percentage was estimated to 63.7%, while the second and third 36.3%.

Dry matter quality. During the three-year of study (2006-2008), in the first cutting of red clover utilization, a good quality of dry matter was achieved, on average, crude protein was 153.2 g kg^{-1} DM and crude cellulose was 254.2 gkg^{-1} DM (Table 2). In the first year of study the average of crude protein content was 168.3 g kg^{-1} DM, in the second year 148,9 g kg^{-1} DM and in the third year 142,4 gkg^{-1} DM. The highest crude protein content was achieved with the red clover variety Kolubara (163,3 gkg^{-1} DM), and the lowest content gave CD-BL/2001 (134,2 gkg^{-1} DM. The highest content of crude fibre was established in dry matter of CD-BL/2001 (276.5 g kg^{-1}). Contrary to that, the lowest crude fibre content gave the variety Viola (236.6 g kg^{-1}DM), therefore it can be concluded that the differences were significant.

Table 2. Crude protein content (CP) and crude cellulose (CC) of red clover genotypes in the first cutting (C_1), 2006-2008 (g kg^{-1} DM)

Genotype (A)	Year(B)						Average(A)	
	B_1: 2006		B_2: 2007		B_3: 2008			
	CP (g kg^{-1})	CC (g kg^{-1})	CP (g kg^{-1})	CC (g kg^{-1})	CP (g kg^{-1})	CC (g kg^{-1})	CP (g kg^{-1})	CC (g kg^{-1})
CD-TR/2004	173.1	235.2	139.4	290.6	149.4	259.0	154.0	261.6
Kolubara	176.9	224.0	165.6	237.9	147.5	264.5	163.3	242.1
CD-BL/2001	151.9	230.0	126.3	322.8	124.4	276.7	134.2	276.5
Viola	171.3	197.9	164.4	262.9	148.1	248.9	161.3	236.6
Average (B)	168.3	221.8	148.9	278.6	142.4	262.3	153.2	254.2

Beković (1997) reported that during the three-year study in dry matter of red clover, the average content of crude protein was 176.3 g kg^{-1} and crude cellulose 228.1 g kg^{-1}. According to *Kašper (1988)*, dry matter of red clover had an average content of crude protein 168.0 g kg^{-1} and crude cellulose 245.0 g kg^{-1}.

Seed yield. Yield of red clover seed depends on many factors, of which particularly is genotype, growing conditions and genotype x environment interactions, thus the variability of this trait is strongly expressed. During the two-year study of red clover genotypes an average seed yield of 265.0 kg ha^{-1} was achieved (Table 3).

Table 3. Seed yield of red clover genotypes (kg ha^{-1}), 2006-2007

Genotype(A)	Seed yield of red clover genotypes (kg ha^{-1}),		Average (A)
	B_1: 2006.	B_2: 2007.	
CD-TR/2004	277.5	401.3	339.4
Kolubara	212.3	222.5	217.4
CD-BL/2001	321.3	425.0	373.1
Viola	132.5	127.5	130.0
Average(B)	235.9	294.1	265.0

LSD	A	B	AB
0.05	50,3	35,6	71,2
0.01	68,2	48,2	96,5

In the first year of study an average of red clover seed yield of 235.9 kgha^{-1} was achieved, or 132.5 kg ha^{-1} (Viola) to 321.3 kg ha^{-1} (genotype CD-BL/2001) respectively. In the second year, the average of 294.1 kg ha^{-1} seed was realized or from 127.5 kgha^{-1} (Viola) to 425.0 kg ha^{-1} (genotype CD-BL/2001). According to *Boisson (1967)* in the sowing year an average red clover seed yields were 220-330 kgha^{-1}, while in the second year of life yield was about 600 kg ha^{-1}. *Vojin (2000)* reported that in the average of 349 kg ha^{-1} seed was achieved with more genotypes of red clover in agro ecological conditions of the Banja Luka region, during the two-year study. During the two-year of testing, by the amount of seed yield CD-BL/2001 genotype gave the best results (an average of 373.1 kg ha^{-1}), while the lowest yield was achieved with the variety Viola (130.0 kg ha^{-1}). Also, relative to the amount of seed yield the genotype CD-TR/2004 gave the best results (an average of 339.4 kg ha^{-1}).

Conclusion

Based on the three-year research of yield and quality of dry matter and seed yield of red clover genotypes, the following conclusions can be given:

The highest dry matter yield was achieved with genotype CD-TR/2004 (12.41 t ha^{-1}, and the lowest with variety Viola (6.06 t ha^{-1}), therefore the differences were highly significant.

During the three-year study of the dry matter quality, the highest content of crude protein content had the variety Kolubara, (an average of 163.3 g kg^{-1}). At the same time, the lowest content of crude cellulose was found in dry matter of Viola variety (an average of 236.6 g kg^{-1}).

According to the achieved seed yield of red clover the genotype CD-BL/2001 gave the best results (an average 373.1 kg ha^{-1}) and the lowest yield was achieved with Viola variety (an average 130.0 kg ha^{-1}).

Varijabilnost agronomskih svojstava genotipova crvene deteline (*Trifolium pratense* L.)

Ž. Lakić, S. Vojin

Rezime

U radu se ističu najvažnija agronomska svojstva dva eksperimentalna genotipa crvene deteline (*Trifolium pratense* L.) u odnosu na sorte Kolubara i Viola (standard). Ispitivanja su obavljena na oglednom polju Poljoprivrednog Instituta Republike Srpske u Banja Luci u periodu 2006-2008. godine. Tokom trogodišnjeg istraživanja, utvrđene su značajne razlike između genotipova crvene deteline, kako za prinos i kvalitet biomase, tako i za prinos semena. Najveći trogodišnji prosečan prinos suve materije (SM) ostvaren je sa genotipom CD-TR/2004 (12,4 t ha^{-1} SM). Odabrani genotipovi crvene deteline ostvarili su zavidne prosečne prinose semena, a razlike u odnosu na standard (Kolubara i Viola) su visoko signifikantne. Sa genotipom CD-BL/2001 ostvaren je najveći dvogodišnji prosečan prinos semena (373,1 kg ha^{-1}).

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BREEDING OF SORGHUM-SUDANGRASS HYBRIDS FOR GREEN MASS PRODUCTIVITY

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Original scientific paper

Abstract: The results of tests of selected sorghum-Sudangrass hybrids in Agricultural Institute-Shumen, Bulgaria, conducted in 2005-2008, have been presented. The significant differences in the agro-climatic factors of the years of our study allow reliable assessment of the productive potential and the adaptability of the sorghum-Sudangrass hybrids in conditions of extreme deviations from the norm. The results confirm the high productive potential of the hybrids in conditions of more often extreme deviations from the agro-climatic norms. The total dry matter yield of tow swaths in flower stage is from 20 to 30 t ha^{-1}. The green yield in late milk stage varies depending on the soil's water reserve – from 40 to 80 t ha^{-1}, with dry matter of 30-50%. Effective breeding of segregating populations of F_2-F_3 progenies for isolation of genotypes with improved productivity indices, and for enrichment of the gene-pool of pollinators for creation of new sorghum-Sudangrass hybrids, was realized.

Key words: sorghum, Sudangrass, hybrids, breeding, productivity

Introduction

Sudangrass forages are grown extensively to provide supplementary forage for animals as pasture, green chop, silage and hay *(Lenobles, 1983; Moyer et al., 2004)*. They are known for their better tolerance to drought than other annual summer grasses and are more yielding than corn in areas with higher temperatures and lower and uneven precipitation *(Fribourg, 1995)*.

The Sudangrass Sorghum sudanense (Piper) Stapf is a natural interspecies hybrid of *S. bicolor* and *S. arundinaceum (Harlan and Wet, 1972)*. It is introduced in 1900's in the USA from Ethiopia and Sudan, and in 1930's its introduction in Russia and Eastern Europe began *(Haecker, 1992)*. A survey of breeders of Sudan type Sorghum ssp. by *Kalton (1988)* showed that selections made were primarily for total yield, leafness, digestibility, re-growth capacity, diseases resistance and low prussic acid content.

Since 1950's Sudangrass has been hybridized with other Sorghum ssp. to increase forage productivity. The development of the CMS-system in Sorghum widens dramatically the possibilities of use of MS-lines as maternal component and lines and varieties of Sudangrass as pollinators for obtaining of F_1 hybrids *(House, 1985)*. The study of the combining ability and the correlations of yield components with the concrete agro-climatic conditions multiplies the selection potential of great genetic diversity of Sorghum hybrids *(Sotomayor- Rios and Torres-Cardona, 1984; Shon Yun et al., 1999; Paknejad et al., 2001)*.

Two types of Sudangrass hybrids are currently grown in the world. The true Sudangrass hybrids of Sudangrass MS-lines and restorers resemble the common Sudangrass in growth and quality characteristics however they tend to be taller, have an intermediate/medium stem diameter and are higher yielding than Sudangrass. These hybrids recover rapidly after harvest and are very productive *(Beurlein et al., 1968)*. Sorghum-Sudangrass hybrids, *S. bicolor* (L) Moench x *S. sudanense* (Piper) Stapf, are more vigorous and taller than Sudangrass, have larger stems and coarser leaves, and give higher forage yield when harvested two or more times at the flower stage for green chop, or one time at the late milk stage for silage production *(Snyman and Youbert, 1996; Paknejad et al., 2001)*. The hybrids of Sudangrass show their high productivity potential in optimum conditions of cultivation, but owe their wide spreading to their high adaptability and resistance to extreme droughts, high temperatures and salt resistance, that's why it attains actuality in South-Western Europe *(Antoche, 2007; Kertikov, 2007; Uzun et al., 2009)*.

In the article are given results of Sorghum-Sudangrass hybrids breeding in the Agricultural Institute-Shumen during the last ten years.

Materials and Methods

This study was conducted at the Agricultural Institute-Shumen, located in North-Eastern Bulgaria, during the period 2003-2008. The soil type of the experimental fields was a carbonate black-earth with good mechanical structure and weakly alkaline reaction of the soil solution.

In 2004 were obtained hybrids of the stabilized populations Sudan Sooner, Sudan Sweet, and Sudan Elit with 2 MS-lines of grain sorghum of the French company Euralis Semences : RS28/A – indicated with SAF_1, ZAF_1 and VAF_1, and RS22/A- indicated with SBF_1, ZBF_1 and VBF_1. In 2005 and 2006 were harvested seeds from selected plants in F2 and F3 progenies of the hybrids SAF, ZAF and VAF. After their assessment in 2006 and 2007 through uniting of the best of them by dry matter content, green yield and dry matter yield, the synthetic populations S-SP, Z-SP and V-SP have been created.

The used experimental design for the tests of the varieties and hybrids during 2005 and 2008 was a random complete block in 4 repetitions, and the long plots method with 2 repetitions for the tests of the selected individual progenies in 2006 and 2007. The experimental plot in 2005 and 2008 was 10.8 m^2, in three rows with 8m length, row spacing was 45 cm. The experimental plot in 2006 and 2008 was 3.6 m^2, in 1 row. Seeds were sown at 20 kg ha^{-1} seed rate, at 4-5 cm depth, in the period 25.04 – 05.05. The tested origins were harvested two times at flower stage and once at late milk stage.

Green yield was measured by reaping and weighing the fresh herbage in the plots. Afterwards, the dry matter content and dry matter yields were determined by drying (at 70 ^oC for 48^h).

In Table 1 information about precipitation and average monthly temperatures during 2005-2008, and the 50 years norm is given. 2005 is characterized with extreme high amount of precipitation during the vegetation (445 mm) and comparatively low average monthly temperatures. The next 2006 appears as near to the norm regarding the precipitation and temperatures. 2007 and 2008 are extremely dry, with high temperatures in July and August.

Table 1. Agro-climatic values for Shumen region, Bulgaria.

Month	Years				
	2005	2006	2007	2008	1955-2009
	Precipitation				
IV	44	49	8	40	41
V	94	9	34	38	64
VI	127	85	24	101	75
VII	146	68	4	16	60
VIII	78	44	51	5	42
IX	178	76	84	88	28
Vegetation sum	445	226	113	150	-
Mean temperature (^0C)					
IV	10.8	11.9	11.1	13.9	12.0
V	15.9	16.2	17.9	16.0	16.8
VI	18.0	19.5	22.9	20.9	22.9
VII	21.8	21.4	25.3	22.3	20.1
VIII	22.8	23.2	23.5	24.1	16.6
IX	17.0	17.6	16.7	16.6	14.5
Vegetation sum	2355	2605	2809	2625	-

Group Standard was used, formed by the Sudangrass hybrid variety Verkor and sorghum-Sudangrass hybrid varieties Susu and Super Sweet. The results were treated statistically using dispersion analysis.

Results and Discussion

The vegetation rainfalls have the highest effect on the productivity of the Sudangrass and its hybrids. The development of sorghum and Sudangrass is strongly affected by the vegetation temperature sum. The significant differences in the agro-climatic factors of the years of our study allow reliable assessment of the productive potential and the adaptability of the sorghum-Sudangrass hybrids in conditions of extreme deviations from the norm.

The results for the productivity of the tested origins Sudangrass and sorghum-Sudangrass hybrids in 2005 (Table 2) are indicative for their high productive potential in conditions of heavy rainfalls during the vegetation.

Table 2. Productivity of sorghum-Sudangrass hybrids for green yield at flower and late milk stage. Group standard – Verkor, Susu, Super Sweet, 2005.

Variant	Flower stage(harvested twice)				Late milk stage		
	Green yield		Dry yield		Dry matter %	Green yield	
	tha^{-1}	Rel. %	tha^{-1}	Rel. %		tha^{-1}	tha^{-1}
Gr. Standard	97.1	100.0	21.2	100.0	37.6	73.6	100.0
Verkor	91.4	91.4	25.1	117.9	37.7	67.0	91.0
Susu	113.6	117.1	24.1	113.4	40.5	73.8	100.2
Super Sweet	86.3	88.8	14.6	68.6	34.7	80.1	108.9
Sudan Sooner	58.9	60.6	11.9	55.8	42.7	49.2	66.8
Sudan sweet	74.0	76.2	17.4	82.1	26.7	59.3	80.6
Sudan Elite	69.3	71.3	16.5	77.6	37.5	50.1	68.1
SAF_1	77.5	79.8	23.9	112.7	42.7	58.9	80.0
SBF_1	98.1	100.1	20.7	97.6	31.5	75.4	102.4
ZAF_1	92.2	94.9	23.5	110.8	31.7	60.2	81.7
ZBF_1	114.0	117.4	26.9	126.6	28.2	75.6	102.6
VAF_1	120.8	124.4	29.3	138.1	40.0	81.1	110.2
VBF_1	102.7	105.7	25.8	121.6	32.0	60.4	82.1
GD 1%	14.7	15.1	4.26	20.1		5.45	7.40

GD is the minimum value of the proved differences between the variants

The green yield from two harvests in flower stage reaches 97 t ha^{-1} for the Group Standard. Two of the hybrids – ZBF_1 and VAF_1 exceed the standard with proven differences. The higher dry matter content of the hybrids reflects in the high values of the dry yield. Only one of them falls back the Standard. The highest is the yield of dry mass of VAF_1 – 29 t ha^{-1}. The hybrids combine high green mass yield and high dry matter content.

The green yield in late milk stage varies from 49 to 81 t ha^{-1}, and three of the hybrids exceed the Standard. The comparatively low values of dry matter content – from 26.7% to 42.7% are as result of the heavy rainfall. The high productivity and dry matter content of the hybrid VAF_1 for both variants of hay-making are impressive.

100 F_2 progenies from the tested hybrids have been selected, which were tested in 2006 for productivity and dry matter content from a single swath in flower stage. For check are included the Standard varieties Verkor, Susu and Super Sweet, as well as the hybrids SAF, ZAF and VAF (Figure 1). In the years are registered above the norm quantities of rain. The average productivity of the three standard varieties is 44 t $ha^{-1}$with 24.6% dry matter content. With average yield of 56 t ha^{-1} the variation for the F_2 progenies is significant – from 29 to 104 t ha^{-1}, with variation coefficient 35.4%. The dry matter varies from 19.4% to 39.5%, variation coefficient 19.1%.

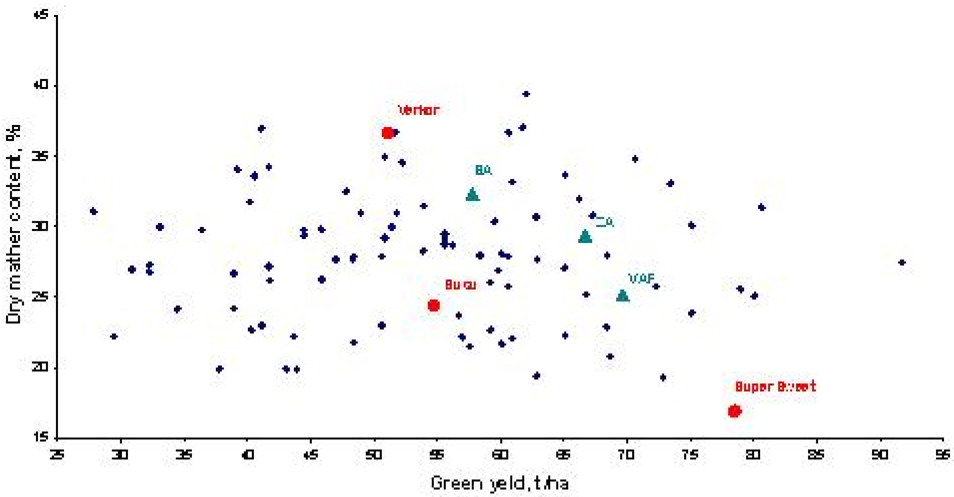

Figure 1. Distribution of selected individual F2 progenies of sorghum-sudan grass hybrids SAF, ZAF, VAF1, group standard – Verkor, Susu, Super Sweet - 2006

From 5 progenies, exceeding the most yielding Standard Super Sweet, and from 3 progenies, exceeding in dry matter content the Standard Verkor 64 F_3 progenies have been selected. They have been tested in 2007, which is with above the normal rainfalls (Figure 2). The variation in these conditions is in comparatively narrow limits.

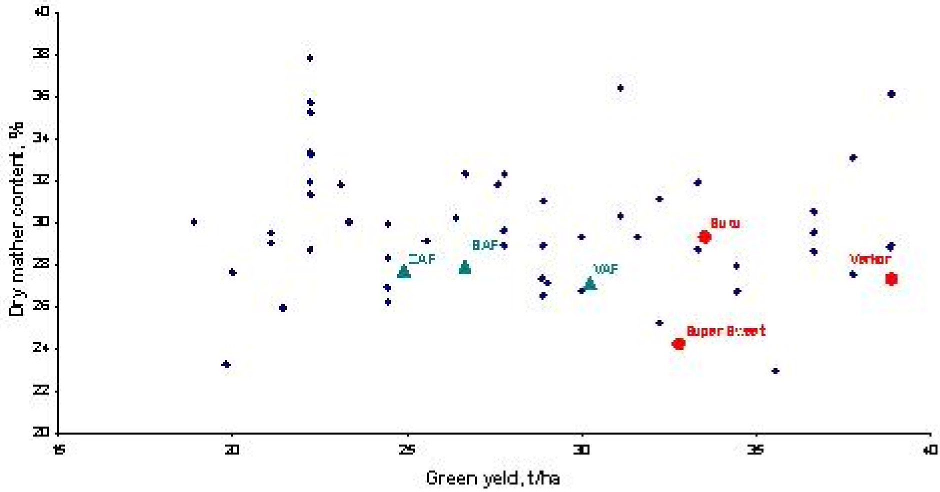

Figure 2. Distribution of selected individual F2 progenies of sorghum-sudan grass hybrids SAF, ZAF, VAF1, group standard – Verkor, Susu, Super Sweet - 2007

The average productivity of the F3 progenies – 29.4 t ha^{-1}, is below that of the Group Standard - 35 t ha^{-1} and varies from 20 to 46 t ha^{-1} , with variation coefficient Cv% = 25.8%. The dry matter content varies from 22.9% to 86.4%, and the mean value of the F_3 progenies – 29.4%, exceeds the Standard value by 26.9%. From the three F_3 progenies with higher than the most productive standard variety Verkor's productivity was formed a synthetic population Z-SP. The F_3 progenies with higher dry matter content than that of Susu were separated in two populations. In S-SP were included the progenies with the biggest dry matter content, and in V-SP – those with the highest productivity.

In 2008, in conditions of extreme drought and high temperatures, the Standard varieties, the pollinators, the sorghum – Sudangrass hybrids and the newly formed synthetic populations were tested (Table 3). The productivity of the hybrids varies close to the Group Standard's values of 67 t ha^{-1} green yield and 22 t ha^{-1} dry yield, formed from two swaths in flower stage. Two of the hybrids – VAF_1 and VBF_1 exceed the Standard with proven differences. In late milk stage three of the hybrids significantly exceed the Standard's value of 49 t ha^{-1}. The dry matter content values vary from 44.0 to 52.0%.

Table 3. Productivity of sorghum-Sudangrass hybrids for green yield at flower and late milk stage. Group standard – Verkor, Susu, Super Sweet, 2008.

Variant	Flower stage(harvested twice)				Late milk stage		
	Green yield		Dry yield		Dry matter %	Green yield	
	tha^{-1}	Rel. %	tha^{-1}	Rel. %		tha^{-1}	Rel. %
Gr. Standard	67.3	100.0	22.0	100.0	48.7	51.4	100.0
Verkor	75.1	111.7	23.5	106.8	45.8	41.3	80.3
Susu	69.3	103.0	24.1	109.5	47.9	53.0	103.0
Super Sweet	57.4	85.4	18.4	83.7	52.4	60.0	116.7
Sudan Sooner	40.4	60.0	16.9	76.8	46.8	33.9	65.9
Sudan sweet	50.4	74.9	17.2	78.4	50.0	44.5	86.4
Sudan Elite	59.6	88.7	18.7	84.9	46.8	42.3	82.2
SAF_1	68.7	102.2	22.7	103.1	45.2	46.7	90.8
SBF_1	68.5	101.9	23.0	104.6	44.0	47.4	92.2
ZAF_1	65.0	96.7	22.6	102.7	49.3	60.8	118.2
ZBF_1	64.1	95.3	22.3	101.3	44.0	61.1	118.8
VAF_1	74.8	111.2	24.8	112.7	50.6	45.1	87.7
VBF_1	88.9	132.2	29.6	134.6	49.3	58.8	112.7
S-SP	55.1	81.8	25.1	114.1	48.9	39.3	76.4
Z-SP	69.6	103.5	18.7	85.1	44.0	48.5	94.4
V-SP	67.0	99.7	20.0	91.1	48.1	48.2	93.6
GD 1%	11.0	16.4	3.72	17.3		5.29	10.3

After two cycles of individual selection have been differentiated three perspective synthetic populations with improved own productivity and dry matter content, which have a good potential for use as paternal components for the creation of new sorghum-Sudangrass hybrids.

Conclusion

The results of the tests confirm the high productivity potential of the selected sorghum-Sudangrass hybrids in conditions of optimum soil's water reserve and temperatures during the vegetation. The sorghum-Sudangrass hybrids show excellent adaptive potential in conditions of more often going extreme deviations from the agro-climatic norms.

It has been realized an effective breeding of segregating population of F_2-F_3 progenies for isolation of genotypes with improved productivity indices and for enrichment of the gene pool of pollinators for creation of new sorghum-Sudangrass hybrids.

Oplemenjivanje hibrida sirka i sudanske trave na produktivnost zelene mase

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Rezime

Prikazani su rezultati testiranja hibrida sirka i sudanske trave selekcionisanih u Poljoprivrednom institutu-Shumen, Bugarska, u periodu 2005.-2008. godine. Značajne razlike u agro-klimatskim faktorima u godinama ispitivanja omogućavaju pouzdano određivanje produktivnog potencijala i adaptabilnosti hibrida sirka i sudanske trave na uslove ekstremne klime. Rezultati potvrđuju visok produktivni potencijal ovih hibrida u uslovima čestih klimatskih promena. Ukupan prinos suve mase u fazi cvetanja je bio od 20 do 30 t ha^{-1}. Prinos zelene mase u kasnoj mlečnoj fazi varira u zavisnosti od prisustva rezerve vode u zemljištu i iznosi od 40 do 80 t ha^{-1}, sa udelom suve materije od 30-50%. Odabrana su potomstava u F_2 i F_3 generaciji, koja se karakterišu povećanom produkcijom i obogaćen je genski pul u cilju kreiranja novih hibrida sirka i sudanske trave.

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THE IMPACT OF *Colletotrichum trifolii* ISOLATES ON RESISTANCE IN DIFFERENT RED CLOVER CULTIVARS

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Abstract: Red clover is attacked by a number of phytopathogenic fungi which cause serious damage. One of the most significant pathogen is *Colletotrichum trifolii*, which causes anthracnoses of alfalfa and red clover. Anthracnose can be very destructive to red clover stems and causes reduced forage yields, losses in plant vigor and stem depletion. Pathogenicity assessment of two selected *C. trifolii* isolates was tested by the agar-plate methods in laboratory conditions. Two isolates of *C. trifolii*, Luc-17 and Luc-27 were studied and the susceptibility of different red clover cultivars to *C. trifolii* was investigated on four cultivars: Manuela, Margot, K-32 i K-39 and one breed population L-50. Investigated cultivars showed various resistances to different *C. trifolii* isolates. Population L-50 had higher tolerance to Luc-17 (36.28% healthy plants), but that same population showed lowest resistance to Luc-27 isolates – there were no healthy plants.

Key words: anthracnoses, red clover, pathogenicity, isolates

Introduction

Red clover (*Trifolium pratense* L. var. *sativum* Sherb.) is one of the most important forage crops in Serbia. The most important fungal pathogens on red clover are *Colletotrichum trifolii*, *Cercospora medicaginis*, *Pseudopeziza trifoli*, *Peronospora trifoliorum*, *Stemphylium botryosum*, *Leptotrichia medicaginis*, *Fusarium* spp., *Rhizoctonia* spp., *Sclerotinia* spp. and *Phoma* spp. *(Ivanović, 2005; Krnjaja et al., 2005)*. Anthracnose of alfalfa and red clover, caused by *C. trifolii* was reported early in 1906 by Bain and Essary *(Stuteville and Erwin, 1990)*. Plants may become infected at any growth stage during the growing season. However, symptom development is most common after the second cutting. The most characteristic symptom of red clover anthracnose is a diamond-shaped stem lesion, which occurs usually in the base of the stem. Stem infection results in wilting and death of the upper portion of the stem and thus making the characteristic "shepherd's crook" symptom (Figure 1).

Figure 1. "Shepherd's crook" symptom on red clover

Damage caused by presence and development of pathogen agent causes the reduction of quantity and quality of fresh mass of 10% up to 30% depending on cultivar of alfalfa, species of pathogen, climatic and edaphic factors *(Stuteville and Erwin, 1990; Vasić, 2007)*. *C. trifolii* spreads rapidly during warm, wet weather. Conidia form in the acervuli on stem lesions. Rain splash or wind carries the conidia to the growing petioles and stems. Infection hyphen proliferate within susceptible host tissue and form oval-shaped lesions. In hot, dry weather, infected stems may wilt and die. The fungus grows down infected stems into the crown and taproot, causing killing of tissue, predisposition to winter injury, wilting or plants death *(Schubiger et al., 2003).* Anthracnose of alfalfa, causal agent *C. trifolii*, is prevalent in wide area in Serbia and the induced damages are of great economic importance *(Robotić and Klokočar-Šmit, 1983)*.

Aim of this study was to determine resistance of the four tested cultivars and one breed population of red clover to *C. trifolii* isolates.

Materials and Methods

Pathogenicity assessment of two selected *C. trifolii* isolates (Luc-17, Luc-27), were tested by using the agar-plate methods according to *Graham et al. (1976)*. Susceptibility of different red clover cultivars to *Colletotrichum trifolii* was investigated on four cultivars: Manuela, Margot, K-39, K-32 and breed population L-50. Spores of *C. trifolii* were streaked onto cornmeal agar in Petri dishes and are allowed to grow at 25°C. After five days, 75 red clover seeds in each plate were placed in five repetitions per treatment. The seeds surface were sterilized by immersion in 0.26% sodium hypochlorite for five minutes and then dried. The agar plates were placed in the dark at room temperature for two days, until most of the seeds had germinated. They were then kept at room temperature. After 10 to 14 days, hard seeds, infected seedlings, deformed seedlings, and seedlings growing in areas free of inoculate were removed with tweezers.

Plants were categorized in 5 score classes, based on the anthracnose reaction using the 1 to 5 scale (1 = no stem lesions or only few small water-soaked or black spots; 2 = stems with elongated black lesions but without acervuli; 3 = stems with long, wide, but non-gridling lesions, with acervuli present; 4 = large, coalescing and sporulating lesions which kill upper part of seedling; 5 = seedling dead) *(Mc Kinny, 1923 loc. cit. Ivanović and Ivanović, 2005).*

Based on the results, severity index was calculated according to the following formula:

$$I=\Sigma\frac{(n\times k)}{N\times K}\times 100$$

I = severity index	N = total number of seedling
n = number of seedlings in class	K = number of categories
k = number of individual categories	∑ = sum of products

According *Ostazeski et al. (1969),* where 1 and 2 are assigned to resistant plants. Plants were scored individually, two weeks after inoculation. Disease intensity or severity of infected plants was calculated using the severity index.

Results and Discussion

Investigated cultivars showed various resistances to different *C. trifolii* isolates (Table 1). According to *Ostazeski et al. (1969)*, sensitive cultivars have 10% of healthy plants, while the cultivar with over 65% can be deemed resistant. Cultivar Arc, which is resistant to race 1 of *C. trifolii,* has the severity index 2.72 to 2.25 *(Ostazeski et al., 1979)*. The isolate Luc-27 showed higher pathogenicity and number of resistant plant were very low in all tested varieties inoculated with this isolate. Breed population L-50 had the highest ratio of healthy plants (36.26%) for isolate Luc-17, but it had the lowest resistance for isolates Luc-27, meaning that there were no healthy plants.

By the severity index (2.64), it can be concluded that the breed population L-50 (inoculated with isolate Luc-17) is resistant to the studied isolate. Also cultivars K-39 showed a different resistance to the tested isolates Luc-17 and Luc-27. Based on the severity index as a criterion for resistance according to *Ostazeski et al. (1969)* it can be seen that the cultivar K-39 is moderate resistant to isolate Luc-17, with severity index 2.98.

Table 1. The resistance of different red clover cultivars to *C. trifolii*

		Percentage of plants in score classes						
Isolates of *C. trifolii*	Red clover cultivars	1	2	3	4	5	Severity index (%)	Resistant plants (1+2%)
Luc-17	K-39	18.62	15.96	18.09	10.64	11.70	2.9893	34.58
	Manuela	16.57	12.21	12.21	13.08	19.19	3.0753	28.78
	K-32	17.09	11.71	18.99	10.44	17.09	3.3154	28.80
	L-50	16.71	19.55	9.95	11.37	17.42	2.6445	36.26
	Margot	8.91	17.82	11.80	11.14	16.34	2.7542	26.73
Luc-27	K-39	1.61	2.57	22.53	17.38	30.90	3.9785	4.18
	Manuela	0	2.73	15.00	19.10	38.19	4.7090	2.73
	K-32	0	3.00	13.55	10.65	43.55	4.2064	3.00
	L-50	0	0	16.92	22.18	35.92	4.3098	0
	Margot	0.71	1.42	14.15	21.23	30.43	3.4811	2.13

The cultivars K-32 showed a different resistance to the tested isolates. The lowest resistance of this cultivar was for isolates Luc-27 (3.0%), while when inoculated with isolates Luc-17 the ratio of healthy plants was 28.80%. Based on the severity index (3.41), this cultivar is moderate resistant to tested isolates. The cultivars Manuela and Margot showed similar results for sensitivity on the tested isolates Lus-17 and Luc-27. Both cultivars responded similarly to isolate Luc-17, and they showed moderate resistance, but tolerance to isolate Luc-27 was low in both cultivars (Table 1).

The resistance degree depends on both the isolates of *Colletotrichum trifolii* and red clover cultivars. Three races of *Colletotrichum trifolii* (1. 2 and 4) have been identified and characterized according to the responses of reference alfalfa cultivars. In these host plants, resistance to race 1 and race 2 was found to be conferred by two single dominant genes, An_1 and An_2, respectively *(Elgin and Ostazeski 1985)*. Race 4 was identified more recently in Australia *(Mackie et al. 2003)*. The genes for resistance to any *Colletotrichum* spp. have not been cloned so far.

Despite the large number of studies, which indicated that resistance to anthracnose is controlled by major dominant or recessive genes and other resistance mechanisms, resistance to *C. trifolii* is not clear enough *(Melotto et al., 2000; Schubiger et al., 2004)*. In the studies by *Schubiger et al. (2003),* only two Mattenklee cultivars (Pavo and Merula) exhibited 50% resistance or more to *C. trifolii*. The other Swiss cultivars of the persistent Mattenklee type showed an intermediate level of resistance.

Conclusion

Investigated cultivars and breed populations showed various resistances to different *C. trifolii* isolates. The resistance degree depends on both the isolates of *Colletotrichum trifolii* and red clover cultivars. The higher resistance was determined in breed population L-50 inoculated with Luc-17, with the severity index 2.62. The same breed population was very sensitive to second tested isolates, Luc-27. Other tested red clovers cultivars showed similar results. These results are considered only as preliminary, because they are obtained from experiments with two isolates and four red clover cultivars and one breed population. It is known that isolates differ in pathogenicity and that the cultivars can react differently to them, which can be seen from our results. Therefore, this study will be continued, with the aim to select red clover cultivars, tolerant to *C. trifolii*.

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Uticaj izolata *Colletotrichum trifolii* na osetljivost nekih sorti crvene deteline

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Rezime

Crvenu detelinu napada veći broj fitopatogenih gljiva, izazivajući ozbiljna oštećenja. Jedan od najznačajnijih patogena crvene deteline je prouzrokovač antraknoze, *Colletotrichum trifolii*. Antraknoza kod osetljivih sorti crvene deteline prouzrokuje gubitak zelene mase, a takođe smanjuje vigor i vek iskorišćavanja useva. Patogenost izolata *C. trifolii* poreklom sa lucerke ispitivana je veštačkim inokulacijama klijanaca crvene deteline u laboratorijskim uslovima. Ispitivana je osetljivost četiri sorte crvene deteline, Manuela, Margot, K-39, K-32 i oplemenjivačke populacije L-50 na dva izolata *C. trifolii*, Luc-17 i Luc-27. Proučavane sorte i oplemenjivačke populacije crvene deteline pokazale su različite nivoe osetljivosti prema ispitivanim izolatima *C. trifolii*. Oplemenjivačka

populacija L-50 je pokazala visoku tolerantnost na izolat Luc-17, sa 36.26% zdravih biljaka, ali i visoku osetljivost na izolat Luc-27.

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APPLICATION OF BIOLOGICAL MARKERS IN PERENNIAL FORAGE LEGUMES BREEDING

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Abstract: Combining biological markers application with strong population improvement programs will enable the maximum genetic potential of perennial forage legumes to be realized. Isozymes and molecular markers - MMs (RAPD, AFLP, SSR, SSR, RFLP etc.) are powerful tools which can be used for germplasm genetic diversity characterization. That means that protein and molecular markers could help to identify genetic differences and relationships among and within germplasm accession of perennial forage legumes. Molecular markers can be used to develop detailed genetic linkage maps and to conduct marker-assisted selection. Among the forage legumes, alfalfa genome mapping is most advanced.

Key words: perennial forage legumes**,** biological markers, germplasm characterization, genome mapping, marker-assisted selection

Introduction

Globally, the selection of perennial forage legumes based on the scientific application of genetic methods began at the beginning of the 20th century. In Serbia, this happened later, in the mid-20th century. The earliest objective of the domestic breeding programs was to develop perennial forage legumes with high yields and good quality that were suitable for a wide range of growing conditions *(Katic et al., 2008)*.

Identification of crop cultivars is fundamental to commercial seed production and certification programs. Traditionally, cultivars have been differentiated on the basis of morphological and agronomic characteristics. Although such characters are genetically well characterized, they are limited in number and may be subject to environmental and ontogenetic influences *(Kongkiatngam et al., 1996)*. By combining morphological, biochemical and molecular methods in identification and description of agronomically important genotypes, it is possible to investigate genetic variability and to reveal their unique genetic profiles e.g. fingerprints. An estimate of the extent of variation within and between populations of forage legumes is useful for analyzing genetic structure of crop germplasm *(Hayward and Breese, 1993)*, monitoring germplasm during maintenance phase *(Moore and Collins, 1983)*, and predicting potential genetic gain in a breeding program *(Moreno-Gonzales and Cubero, 1993)*.

Combining molecular genetic innovations with strong population improvement programs will enable the maximum genetic potential of forage legumes to be realised. Application of molecular marker will advance our understanding of the genetic control underpinning phenotypic traits.

Germplasm characterization

Several studies have reported that genomic heterozygosity among intra-populations was higher than those of inter-populations in outcrossing forage species, such as red clover *(Milligan, 1991; Kongkiatngam et al., 1995; Campos-de-Quiroz et al., 2001)*, white clover *(Gustine et al., 2002)*, alfalfa *(Yu et al., 1993)*. Because these forages have self-incompatibility systems and hence are cross-pollinated breeding systems, their varieties are a heterogeneous population of individual genotypes. The red clover populations are heterogeneous and showed high levels of genetic variation within and among populations *(Vasiljević et al., 2000)*. The populations are represented by various biotypes that differ a lot in terms of productivity and the type of development, which is indicative of their great potential if used as starting materials for breeding *(Vasiljević et al.,* 2001*)*. Based on morphological characters alone, it is difficult to distinguish accessories of red clover from each other because they have overlapping variations in terms of the major delimiting morphological and biological characters such as stem length, stem thickness, number of internodes, length and width of central lamina of medial leaflet, shape of medial leaflet, colour of leaf in the year of sowing, intensity of white marks on the leaftime of flowering, growth habit, yield of green mass per plant, yield of dry matter *(Vasiljevic et al.*, 2007*)*.

Isozymes and molecular markers - MMs (RAPD, AFLP, SSR, cpSSR, RFLP etc.) are powerful tools which can be used for germplasm genetic diversity characterization. That means that MM could help to identify genetic differences and relationships among and within germplasm accession *(Miftahudin et al., 2004)*. Isozymes have also been used extensively as codominant genetic markers for cultivar identification in crop species *(Yan et al., 1999, Weeden, 1989)*. Isozymes have been found to be independent of environmental influence *(McMilin, 1983)*. However, the use of isozymes to distinguish cultivars of crossed-pollinated, heterogeneous crops like red clover (*Trifolium pratense L.*) is not as straightforward as in homogeneous cultivars of selfing or clonally propagated crops or hybrid cultivars since allele frequencies of isozymes from a large number of individuals (> 100) are required *(Adam et al., 1987)*. Protein data used to obtain electrophoretic or chromatographic profiles are mostly expressions of genes that are unevenly distributed in the genome *(Galovic, 2002)*.

As specific gene products, proteins could indicate the genetic specificity of tested plant material, and therefore could be used as markers for characterization of varieties, for seed purity testing, or to resolve taxonomic relationships *(Nikolić et al., 2007; Drinić-Mladenović and Konstantinov, 2001; Nikolić et al., 2008)*. For some crops, however, biochemical methods have not proven sufficiently discriminative. Due to their low polymorphism levels seed storage proteins and isozymes cannot be used for genetic identification of sunflower hybrids *(Nikolić et al., 2008)*. In the plant with a narrow genetic base in their pool, such as soybean, protein markers may not be sufficient for characterisation and study of genetic diversity *(Nikolić et al., 2005)*.

According to *Marshall and Brown (1975)* more than 75% of nucleotide substitutions have no effect on the characteristics of the coded proteins and will therefore remain undetected by protein analysis techniques. *Stuber et al. (1988)* stated that genome coverage by biochemical markers is 30% at the most.

Cultivars of red clover in use today are composed of heterogeneous individuals obtained through mass selection *(Vasiljevic et al, 2003)*. Within the genus *Trifolium*, isozymes have been used to study genetic variation of the colonizing species *Trifolium hirtum* All. (rose clover) in California *(Molina-Freaner and Jain, 1992)*. *Hickey et al. (1991)* analyzed two natural populations of red clover from Ohio with introduced alsike clover (*Trifolium hibridum* L.) and with two endangered native species: running buffalo clover (*Trifolium stoloniferum* Muhl.) and buffalo clover (*Trifolium reflexum* L.) by assaying 12 enzyme systems.

Semerikov et al. (2002) assessed the genetic variation at 17 allozyme loci in seven Russian cultivars, bearing the names of localities of the Urals, two American ones that have been used in Russia for scientific experiments and seven wild populations of the Urals and Western Siberia. Variation at the 17 protein loci supports western European origin of the cultivars and also indicates that gene flow between cultivars and wild populations was limited or has not acted sufficiently long to affect the genetic composition of the red clover wild populations of the Urals. *Semerikov and Belyaev (1995)* investigated the genetic relationships between three natural populations and two cultivars using 17 isozyme loci, and found that genetic differences among groups of cultivars and native populations was rather large and significantly exceeded the variation within groups.

Isozyme loci that show cultivar specific alleles could be used to differentiate between cultivars but only if they were present at a reasonably high frequency *(Kongkiatngam et al., 1995)* and large sample sizes would be essential to obtain accurate estimates of allele frequencies *(Booy et al., 1993)*.

Genetic diversity among 32 red clover cultivars were investigated based on seed protein profiles produced by SDS-PAGE electrophoresis *(Nikolic et al., 2010)* The proteins in the area of higher molecular high weight, 55-95 kDa, were identified as polymorphic. All the cultivars were placed into three clusters on the basis of Ward's distance range, and each cluster was further divided into 2 sub-clusters. Almost all Serbian cultivars were collected in one cluster, Kolubara, Avala and Una were grouped in sub-cluster with Lutea at a Ward's distance of 1.49.

Previous research based on morphological-biological characteristics according to UPOV protocol has been shown similarities of Serbian cultivars *(Vasiljevic et al., 2006)*.

Estimates based on isozyme polymorphism may underestimate overall levels of genetic variation because they are sampling only coding regions that may be conserved to maintain the function of the enzymes *(Gottlieb et al., 1981)*.

The complete coverage of a genome can be achieved only by the use of molecular variability indicators (DNA polymorphism), i.e. molecular markers *(Galovic et al, 2006)*. Molecular techniques have been rapidly developing and there is diversity of different methods that, if applied properly, can be used as an absolute indicator of distance, stability and similarity among different genotypes *(Zlokolica et al., 1999)*. The most commonly used methods for DNA profiling and genotype characterization by determining their distance and uniformity are the RFLP and the PCR-based (RAPD, AFLP and SSR).

Restriction Fragment Length Polymorphisms (RFLPs) are codominant markers and can be visualized as variability in the size of restriction DNA fragments that is detected by electrophoretic separation on agarose gel. The selected fragments are transferred from the agarose gel onto a nylon or nitrocellulose membrane filter in a procedure called Southern blotting. The filter with radioactively labelled probe is exposed to conditions that promote hybridization and subsequently photographed using X-rays. The shortcomings of this method when used for identification purposes are duration, technical difficulties, increased costs and poor availability of the specific probes.

High variation within cultivars of red clover has also been observed using chloroplast DNA RFLP markers *(Milligan, 1991)*. RFLPs detected a loss of genetic diversity associated with selection for resistance to sorghum midge in Australian sorghum materials *(Jordan et al., 1999)*.

The RAPDs (Random Amplified Polymorphic DNA) are dominant markers and are based on the detection of polymorphism of multiplied random sequences that are distributed throughout the genome using short arbitrary primers. Both nuclear and organellar DNA can serve as template for RAPD amplification, but polymorphic markers are usually inherited in a Mendelian fashion in many crop species *(Kazan et al., 1993b; Yu and Pauls, 1993)*, indicating that they are of nuclear origin. RAPD markers could be very valuable in studies that require a high number of polymorphic loci, such as constructing a genetic linkage or marking a single gene *(Michelmore et al., 1992)*. They are especially suited to species with little molecular information such as red clover due to their following attributes: 1) No previous knowledge of the genome is required; 2) Rapid results can be obtained when compared with alternatives such as RFLP; and 3) A universal set of primers which can be used for genomic analysis in any species is commercially available *(Campos de Quiroz and Ortega Klose, 2001)*.

Kongkiatngam *et al.* (1996) used fourteen 10-mer primers to amplify genomic DNA from combined leaf samples of 15 red clover cultivars from European, Japanese and North American origins. Bulked samples consisting of 20 individuals of each red clover cultivar were able to generate RAPD patterns that could remove intra-cultivar variation. In comparison, the appropriate number of individuals pooled to produce RAPD markers that could represent the RAPD patterns observed in larger samples of alfalfa (*Medicago sativa L.)* was seven *(Yu and Pauls, 1993)*. In perennial ryegrass (*Lolium perenne L.)*, leaves from 30 individuals were used for bulked genomic DNA extraction to generate RAPD markers *(Sweeney and Danneberger, 1994)* which could be used for cultivar identification. A total of 79 RAPD products, of which 55 were polymorphic, were obtained. Cultivar-specific bands were observed with 13 primers. The amplification patterns obtained from two primers could distinguish all 15 red clover cultivars. Rogers' genetic distances for all 105 pair wise comparisons were calculated to evaluate relationships among cultivars under study. Cluster analysis based on these genetic distances separated 15 investigated cultivars into three groups, with two of the groups consisting of a single Japanese cultivar each, while the third group included cultivars from European, North American and Japanese origins. *Kongkiatngam et al. (1996)* recommended the use of red clover seedlings for bulked DNA extraction instead of leaf tissue because only a small amount of DNA is required for RAPD assays. RAPD markers obtained from bulked genomic DNA may also be useful for other genetic and breeding studies in red clover, for example to mark monogenic traits *(Michelmore et al., 1992)*. RAPD markers were also found to be variable within and between two cultivars of red clover *(Kongkiatngam et al., 1995)*. Bulked genomic DNA from several individuals was used to generate RAPD markers in these two crops in order to amplify only DNA sequences shared among most of individuals of a given cultivar. The pooled samples produced amplification patterns that represented the characteristics shared within heterogeneous populations. Similarly, high variation within cultivars has also been observed in both cross-pollinated alfalfa *(Yu and Pauls, 1993)* and perennial ryegrass *(Sweeney and Danneberger, 1994)* using RAPD markers.

Kongkiatngam et al. (1995) used morphological, isozyme and RAPD markers to estimate genetic variation within and between two cultivars of red clover (*Trifolim pratense* L.), Essi from Europe and Ottawa from Canada. Six monogenic morphological characters (leaf mark, stem hair, petiole hair, basal internode, stipule and flower colour) were observed for 80 plants from each of these two cultivars. Genetic variability of these two cultivars may be overestimated with the morphological trait since a small number of loci were examined. A total of 21 enzyme-coding loci with 43 alleles were detected using 12 enzyme systems. Thirteen and nine of these loci were polymorphic in Essi and Ottawa, respectively. Seventeen random 10-mer primers were screened for RAPD markers. Nine primers were used to assay 20 individuals from each cultivar. Each primer gave from 7 to 20 amplified bands with an average of 14.8 bands per primer. One hundred and eight of 116 putative loci were polymorphic in Essi and 90 of 98 loci were polymorphic in Ottawa. High within-cultivar variation was observed in both cultivars using both isozymes and RAPD markers. This high polymorphism makes these markers useful for germplasm characterization and genetic studies in red clover.

Campos de Quiroz and Ortega Klose, (2001) applied RAPDs in order to assess the genetic relationships and levels of genetic variability existing among a group of 16 elite red clover parents organised in four subsets of 4 parents each. Out of 55 primers 21 provided reproducible results. A total of 135 reliable and polymorphic RAPD bands were detected which were used to estimate genetic distances among pair-wise combinations of elite parents. On average 8.7 bands were generated per primer, a value lower than 14.8 bands per primer reported by *Kongkiatngam et al. (1995)* in other red clover genotypes. Nei and Li's similarity values ranged from 0.60 to 0.77, with a mean of 0.66, which reflected rather high genetic variability among the genotypes evaluated and which were comparable to those detected for alfalfa, whose Nei and Li's similarity values averaged 0.57 (0.46-0.62) *(Kidwell et al., 1994)*. Lower levels of genetic variability, as detected by polymorphic loci and mean heterogeneity values, were detected in a subset of parents selected for resistance to the stem nematode. An analysis of molecular variance detected substantial levels of variation within subsets of parents and is consistent with observations in the cross-pollinated turfgrass species *Buchloe dactyloides (Huff et al., 1993)* and alfalfa *(Yu and Pauls, 1993 a)*.

High level of variation within cultivars was found with RAPD markers in alfalfa (*Medicago sativa* L.), which is tetraploid outcrossing species *(Yu and Pauls, 1993)*. Little or no variation was found within each accession of the *Stylosanthes guianensis* (Aubl.) Sw. species complex, which includes several diploid pasture legumes widely used in many parts of the tropics and subtropics *(Kazan et al., 1993)*. Two factors should be taken into the consideration in using RAPD markers to estimate genetic variation of cross-pollinated species like red clover *(Kongkiatngam et al., 1995)*. The first one is the assumption that each band is a locus with two alleles. At least some RAPD markers have been shown to be codominant *(Tinker et al., 1994; Williams et al., 1990)*. If the co-dominance was not known and all bands were assumed to be dominant, each band would be scored as a different locus rather than as different alleles of the same locus. These errors in scoring would result in an overestimation of the number of polymorphic loci and an underestimation of the mean number of alleles per locus *(Kongkiatngam et al., 1995)*. The second factor is the dominant nature of most RAPD markers. The only monomorphic loci observed with dominant markers are those of dominant alleles (presence of a band), not recessive alleles (absence of a band). Thus, the percentage of polymorphic RAPD loci, mean number of alleles per locus and expected heterozygosity may be overestimated.

The AFLP (Amplified Fragment Length Polymorphism) method is based on restriction fragment length polymorphism. It is dominant method just like RAPD, but is technically more advanced in being able to distinguish between homozygous and heterozygous genotypes and also allows the detection of a large number of loci in a single assay *(Vois and Kuiper, 1998; Powell et al., 1996)*.

Kölliker et al. (2003) used AFLP analysis in order to assess genetic variability within and among 19 landraces and cultivars of red clover (*Trifolium pratense* L.). The number of polymorphic markers detected with each primer combination ranged from 36 to 58 with an average of 46. Analysis of molecular variance based on 276 polymorphic AFLP markers revealed 80% of total variability to be due to variability within populations, while 12% were attributed to variability among groups. Stepwise discriminate analysis identified a subset of 126 AFLP markers which best separated individual plants into the three respective groups. Genetic distances between populations were considerably larger among groups than among populations within the same group providing further evidence for the genetic distinction between landraces, cultivars and field clover cultivars.

Simple Sequence Repeats (SSRs) or microsatellites are based on the total length of the sequence, which is determined by a large number of repeatable units. It is co-dominant method and able to distinguish between heterozygotes for different fragments in diploid genomes. *Jones et al. (1997)* stated that results of SSR method are highly reproducible, and although this marker system is species-specific, costly to create and requires prior knowledge of the sequence, once it has been developed, it pays off financially.

Nelke et al. (1993) were able to distinguish individual genotypes in seven cultivars of red clover using DNA fingerprints generated by probing with human mini satellite DNA probes *(Jeffreys et al., 1985a, 1985b)*, which detected variable numbers of tandem repeats (VNTRs) in mini satellites.

Genetic linkage maps-QTL-MAS

Among the forage legumes, alfalfa genome mapping is most advanced *(Woodfield and Brummer, 2001)*. Cultivated alfalfa (*Medicago sativa* L.) is a tetraploid (2n = 4x = 32) open pollinated species with polysomic inheritance and is characterized by high levels of polymorphism within and among populations. It belongs to the *Medicago sativa* complex where interfertile diploid and tetraploid forms coexist *(Quiros and Bauchan, 1988)*.

Because of the tetrasomic inheritance, most genetic maps of alfalfa were constructed in diploids *(Brummer et al., 1993)*. In that way genetic complexity of the species determined largely prevalent use of diploid alfalfa in mapping process and construction of genetic linkage maps. Mapping at the diploid level in alfalfa is more convenient and easier because knowledge about the genomic constitution of most polyploids is still very limited, large segregating populations are needed and the mapping statistics are far more complicated for autotetraploids than for diploids *(Veronesi et al., 2003)*.

Several research groups *(Kiss et al., 1993; Echt et al., 1994; Tavoletti et al., 1996; Diwan et al., 1997, 2000; Kaló et al., 2000; Porceddu et al., 2002)* have been working on mapping at the diploid level using the closely related 2x species *M. falcata* and *M. coerulea*.

The linkage map that was constructed by *Kaló et al., (2000)* was one of the most completed. That map is based on an F2 population derived from a cross between *M. sativa* subsp. *falcata* and subsp. *coerulea* that includes more than 900 RFLP, RAPD, isozyme, seed protein, and morphological markers *(Kaló et al., 2000)*. Several research groups *(Yu and Pauls, 1993b; Brouwer and Osborn, 1999; Ma et al., 2002)* have been working on mapping at the tetraploid level of alfalfa.

The first linkage map of red clover was RFLP linkage map and it was constructed by using cDNA probes with a backcrossed mapping population *(Isobe et al., 2003)*. The main reason for choosing the RFLP markers from cDNA probes for map construction was the fact that they have the most stable expression in various germplasms and have the ability to detect homologous sequences in distantly related genomes. Using the first backcrossed progeny as the mapping population is the strategy that could obtain a much larger number of bi-markers than by a two way pseudo-test cross.

Herrmann et al. (2007) evaluated plant vigour and various morpho-physiological characters in a mapping population of red clover consisting of 280 F_1 individuals and QTL analysis was performed based on a genetic linkage map consisting of 42 SSR and 216 AFLP markers during four growing periods. A weighted average of vigour scores assessed over two winters and three growing seasons was identified as optimal method to phenotype persistence. For this index, one QTL explaining 12.2% of the total phenotypic variation was identified. A detailed characterisation of the genetic control and the development of molecular genetic markers linked to persistence may help to improve this complex trait and to complement traditional breeding approaches through marker assisted selection. Findings based on phenotypic observations were supported by molecular genetic analyses which revealed QTLs for seed yield and length of stem to be located close to the QTL s for persistence identified on LG 3 and LG 4 *(Herrmann et al., 2006; Herrmann et al., 2008)*.

The linkage maps provide a fundamental basis for QTL analysis and useful genetic information for analyzing the relationship between functional genes and QTLs. The identification of Quantitative Trait Loci (QTL) associated with target traits enables the development of new breeding strategies involving Marker Assisted Selection (MAS). If some QTLs are closely related to the markers on genetic map, the relation of QTLs and the function of the markers could be analyzed, and they could be developed as DNA markers for marker-assisted selection. MAS is performed at the genotypic level by using DNA markers closely linked to genes controlling qualitative or quantitative traits. MAS relies on identifying markers at or closely linked to genes of interest that can be used to indirectly select for traits .Thus MM and maps may be very useful for identifying genes of interest and breeding programs through MAS. Since the conventional breeding system means a difficult and time consuming work MAS can be very useful tool and can increase the efficiency of breeding by allowing specific genes to be targeted more precisely, especially for traits that are hard to evaluate in the field.

Although MAS is being practised in many crops *(Young, 1999)*, the true benefits of the technology may be most successfully realised in perennial forage crops, where each cycle of selection takes several years. For example, selection for winter hardiness in alfalfa takes three to five years, because winter severity is variable. A MAS approach could decrease cycle time significantly if markers linked to winter survival could be identified. Perhaps the most important requirement for using MAS is the development of transportable maps based on microsatellite and single dose restriction fragments that will facilitate moving mapping information across populations *(Woodfield and Brummer, 2001).*

Various types of molecular markers have been used in the examinations and experiments with alfalfa (RAPD, ISSR, AFLP, RFLP, SSR). Several research groups were working on targeting of alfalfa genes with MMs. Some of studied genes and traits were 2n egg trait *(Barcaccia et al., 2000)*, 2n jumbo pollen *(Tavoletti et al., 2000)*, winter hardiness and fall dormancy *(Brouwer et al., 2000)*, resistance to downy mildew *(Obert et al., 2000)*, Al tolerance *(Sledge et al., 2002)*, stem morphogenesis *(Julier et al., 2002)*, non-nodulating phenotype *(Endre et al., 2002)*.

Primena bioloških markera u oplemenjivanju višegodišnjih krmnih leguminoza

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Rezime

Primena bioloških markera predstavlja pomoć u oplemenjivanju i analizi genetičke divergentnosti roditelja i početnog selekcionog materijala višegodišnjih leguminoza. Proteinski a naročito molekularni markeri su korisni za karakterizaciju genetičke raznovrsnosti i mogu se koristiti da bi se identifikovale najreprezentativnije populacije u određenom regionu. Osim toga oni bi se mogli koristiti za zvaničnu karakterizaciju lokalnih populacija i sorti višegodišnjih leguminoza. Najveći napredak u primeni molekularnih markera kod višegodišnjih leguminoza je postignut kod lucerke. Upotreba molekularnih markera u oplemenjivanju lucerke od naročito je značaja za genetičko mapiranje vezanih gena i marker asistiranu selekciju.

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FIRST ATTEMPTS OF GROWING FORAGE SOYBEAN IN SERBIA

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Original research paper

Abstract: A small-plot trial was carried in 2009 at two locations in Serbia, Rimski Šančevi near Novi Sad and Zemun Polje near Belgrade, including four US forage soybean cultivars, Derry, Donegal, Tara and Tyrone. The highest green forage yield was determined in the cultivar Tara at Zemun Polje (52.0 t ha^{-1}) and in the cultivar Tyrone at Rimski Šančevi (51.5 t ha^{-1}), while the highest forage dry matter yield was determined in the cultivars Tyrone at Rimski Šančevi (12.9 t ha^{-1}), Tara at Zemun Polje (12.5 t ha^{-1}) and Donegal at Rimski Šančevi (12.4 t ha^{-1}). In average, the cultivar Donegal had the highest yields of both green forage (48.9 t ha^{-1}) and forage dry matter yield (12.0 t ha^{-1}).

Key words: forage dry matter yield, green forage yield, Serbia, soybean.

Introduction

The most important and the most traditional annual forage legumes in Serbia are forage pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.). Both of them are cultivated in their winter- and spring-sown forms, as well as pure stands or in mixtures with small grains. If grown for forage, pea and common vetch may be used in the form of green forage, forage dry matter and forage meal, while, due to their abundant and nitrogen-rich below- and aboveground biomass, they represent a valuable green manure in organic farming and sustainable agriculture *(Ćupina et al., 2004)*.

Soybean (*Glycine max* (L.) Merr.) is the most important annual legume crop in Serbia, with a harvested area of about 150,000 ha *(Mikić et al., 2009)*. Like in many other countries, such as USA and in EU, it is cultivated mainly for protein- and oil-rich grains. However, soybean may represent an additional source of quality forage during the summer, when the winter-sown crops such as pea or vetches are absent from the field *(Bilgili et al., 2003)*.

Recent advances in herbage preparation and ensiling methods accelerated the forage soybean breeding program in USDA-ARS. The first forage soybean cultivars in USA were introduced from Asia and were not adapted the conditions in USA. On the other hand, soybean breeding programs for grain production brought forth adapted grain cultivars with improved disease, insect, and nematode resistances. Such genotypes and a traditional hay-type cultivar were used as parental lines in the forage soybean breeding program, with conventional plant breeding used exclusively and developed lines that are exceptionally tall and lodging resistant *(Devine et al., 2007)*.

The aim of the research was to assess the possibility of the growing soybean for forage in Serbia by testing the performance of purposely developed forage soybean cultivars from USA.

Materials and Methods

A small-plot trial was carried in 2009 at two locations in Serbia, namely Rimski Šančevi near Novi Sad and Zemun Polje near Belgrade. It included four forage soybean cultivars from USA, namely Derry *(Devine et al., 1998a)*, Donegal *(Devine and Hatley, 1998)*, Tara *(Devine and McMurtrey, 2004)* and Tyrone *(Devine et al., 1998b)*.

The trial was established on April 23 at Zemun Polje and on April 28 at Rimski Šančevi. All four cultivars were sown at a density of about 75 viable seeds m^{-2} *(Acikgoz et al., 2007)*, a plot size of 5 m^2 and at a row spacing of 20 cm *(Seiter et al., 2004)*. The dominant weather and soil conditions during the trial period at Rimski Šančevi and Zemun Polje are given in Tables 1 and 2.

Table 1. Average monthly temperatures and monthly precipitation sums at Rimski Šančevi and Zemun Polje during the trial in 2009

Location	Year	April	May	June	July	Average/sum
Average monthly temperature (°C)						
Rimski Šančevi	2009	15	18	20	23	19
	Long-term	11	17	20	21	17
Zemun Polje	2009					
	Long-term	13	18	22	23	19
Total monthly precipitation (mm)						
Rimski Šančevi	2009	2	47	123	57	229
	Long-term	47	59	85	70	261
Zemun Polje	2009	7	27	72	31	137
	Long-term	43	41	76	56	216

Table 2. Chemical properties of the surface soil layers at Rimski Šančevi and Zemun Polje during the trial in 2009

Location	pH (H_2O)	N (%)	P_2O_5 (mg 100^{-1} g^{-1})	K_2O (mg 100^{-1} g^{-1})	$CaCO_3$ (%)	Humus (%)
Rimski Šančevi	7.89	0.195	51.13	40.00	8.64	2.95
Zemun Polje	7.97	0.177	26.80	24.90	1.90	2.80

Each of the four tested cultivars was cut in the stages of full bloom and first pods forming, as an optimal balance between yield and quality in all annual forage legumes *(Mihailović et al., 2009)*. At both locations, this was in mid-August. Main forage yield components, such as plant height (cm), number of internodes (plant^{-1}) and number of leaves (plant^{-1}), as well as green forage yield per plant (g), were measured on the plant samples taken before the cutting. Green forage yield (t ha^{-1}) was calculated on the basis of the green forage yield per plot, measured immediately after the cutting. Forage dry matter yield (t ha^{-1}) was calculated on the basis of the ratio between the masses of green forage samples before and after the drying at the room temperature.

The results were processed by the method of analysis of variance (ANOVA) using the LSD test.

Results and Discussion

The average plant height varied between 121 cm in the cultivar Derry and 133 cm in the cultivars Donegal and Tara (Table 3). The smallest plant height was in the cultivar Derry at Zemun Polje (115 cm), while the greatest plant height was in the cultivar Donegal at Rimski Šančevi (140 cm), what was on the same level as its result in the trial at Ames, Iowa (*Darmosarkoro et al., 2001*).

The cultivar Donegal had the highest average values of both number of internodes (27 plant^{-1}) and number of leaves (23 plant^{-1}). The cultivar Tyrone had the smallest average number of internodes (21 plant^{-1}), while the cultivar Derry had the smallest number of leaves (18 plant^{-1}). There were significant differences between the values of number of internodes and number of leaves for two locations within the same cultivar.

Table 3. Forage yield components of four soybean cultivars at Rimski Šančevi and Zemun Polje in 2009

Cultivar	Location	Plant height (cm)	Number of internodes ($plant^{-1}$)	Number of leaves ($plant^{-1}$)
Derry	Rimski Šančevi	126	16	12
	Zemun Polje	115	33	24
	Average	121	25	18
Donegal	Rimski Šančevi	140	23	20
	Zemun Polje	126	31	25
	Average	133	27	23
Tara	Rimski Šančevi	135	29	27
	Zemun Polje	131	15	12
	Average	133	22	20
Tyrone	Rimski Šančevi	125	24	22
	Zemun Polje	134	18	16
	Average	130	21	19
	$LSD_{0.05}$	8	7	5
	$LSD_{0.01}$	12	11	8

The cultivar Tara had the highest average values of forage yields per plant, namely 92.87 g $plant^{-1}$ of green forage and 22.57 g $plant^{-1}$ of forage dry matter (Table 4). At the same time, the cultivar Derry had the lowest average values of forage yields per plant, namely 42.38 g $plant^{-1}$ of green forage and 10.37 g $plant^{-1}$ of forage dry matter.

The highest green forage yield was determined in the cultivar Tara at Zemun Polje (52.0 t ha^{-1}) and in the cultivar Tyrone at Rimski Šančevi (51.5 t ha^{-1}), while the highest forage dry matter yield was determined in the cultivars Tyrone at Rimski Šančevi (12.9 t ha^{-1}), Tara at Zemun Polje (12.5 t ha^{-1}) and Donegal at Rimski Šančevi (12.4 t ha^{-1}).

In average, the cultivar Donegal had the highest yields of both green forage (48.9 t ha^{-1}) and forage dry matter yield (12.0 t ha^{-1}), almost exactly repeating its excellent results from the trial carried out by the Royal Agricultural College at Cirencester, UK *(Koivisto et al., 2003)*.

In comparison to the traditional annual forage legumes in the conditions of Serbia, the four tested forage soybean cultivars proved at least as equal in forage yields to forage pea *(Mihailović et al., 2004)* and common vetch *(Mihailović et al., 2005)*.

Table 4. Forage yields of four soybean cultivars at Rimski Šančevi and Zemun Polje in 2009

Cultivar	Location	Green forage yield (g plant^{-1})	Green forage yield (t ha^{-1})	Forage dry matter yield (g plant^{-1})	Forage dry matter yield (t ha^{-1})
Derry	Rimski Šančevi	40.50	28.4	10.13	7.1
	Zemun Polje	44.26	31.0	10.62	7.4
	Average	42.38	29.7	10.37	7.3
Donegal	Rimski Šančevi	70.89	49.6	17.72	12.4
	Zemun Polje	68.83	48.2	16.52	11.6
	Average	69.86	48.9	17.12	12.0
Tara	Rimski Šančevi	55.87	39.1	13.97	9.8
	Zemun Polje	129.88	52.0	31.17	12.5
	Average	92.87	45.5	22.57	11.1
Tyrone	Rimski Šančevi	73.64	51.5	18.41	12.9
	Zemun Polje	64.08	44.9	15.38	10.8
	Average	68.86	48.2	16.89	11.8
	$LSD_{0.05}$	18.76	13.45	4.25	2.91
	$LSD_{0.01}$	23.87	18.96	5.78	4.03

It is noteworthy that the stands of all four cultivars, despite its great height, remained erect until the moment of cutting, without a single plant lodged. Also, by some reason that still needs to be clarified, the cultivar Tara suffered from a reduced stand during its growing period, resulting in smaller number of plants per plot and a increased forage yield per plant due to more excessive growth of the aboveground parts.

Conclusion

The preliminary results of the cultivation of forage soybean in the agro-ecological conditions of Serbia support the idea that this crop may represent a valuable source of forage during the summer. The research will be carried out during the years to come with more parameters examined.

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Prvi pokušaji gajenja krmne soje u Srbiji

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Rezime

Mikroogled je izveden tokom 2009. gidne na dva lokaliteta u Srbiji, Rimskim Šančevima i Zemun Polju, uključivši četiri američke sorte krmne soje, Derry, Donegal, Tara and Tyrone. Najveći prinos zelene krme određen je kod sorte Tara u Zemun Polju (52.0 t ha^{-1}) i sorte Tyrone na Rimskim Šančevima (51.5 t ha^{-1}), dok je najveći prinos suve materije krme odrežen kod sorti Tyrone na Rimskim Šančevima (12.9 t ha^{-1}), Tara u Zemun Polju (12.5 t ha^{-1}) i Donegal na Rimskim Šančevima (12.4 t ha^{-1}). U proseku, sorta Donegal ostvarila je najveći prinos zelene krme (48.9 t ha^{-1}) i suve materije krme (12.0 t ha^{-1}).

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SCREENING ROMANIAN ALFALFA GERMPLASM FOR SALT AND WATER STRESS

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Abstract: The present paper reports the reactions of some Romanian alfalfa genotypes to salt and water stress. The aim was to establish screening criteria to facilitate the development of genotypes with enhanced tolerance to field stress conditions. The alfalfa yield for all studied genotypes was significantly reduced under hydric and salt stress and stresses combination caused a reduction on fresh biomass, too. Salt stress significantly decreased biomass by over 37 % while hydric stress by over 73%. The effects of salt and water stress on yields are additive but not equal. Alfalfa responds to drought by decrease of leaves transpiration and between biomass accumulation and leaves transpiration under hydric and salt stress there are linear relationships. Under optimal condition the proline content was very low but the high proline content under all stress treatments was obvious. The negative effect of salinity and stress combined on alfalfa growth could be attributed to an osmotic effect. Yield loss results from closing stomata (as result the transpiration decrease) and from energy and carbohydrate use in osmoregulation. The leaves transpiration and biomass accumulation were correlated that indicated to be used as screening tools for drought and saline tolerance of alfalfa genotypes.

Key words: alfalfa, salinity, water stress, proline, biomass accumulation.

Introduction

Abiotic stress conditions cause extensive losses to agricultural production worldwide *(Bray et al., 2000)*. Drought and salinity stress can significantly affect plant yield in arid and semi-arid regions and not only. Romania is expecting a gradual decline in rainfall and an increase in aridity. The more drought-prone Baragan region of Southeast Romania has experienced a gradual decline in annual rainfall during last 5 years of about 90 mm, accompanied by an increase in the number of tropical days (when maximum temperatures exceed 30°C) from 10 to over 30 during the same period *(Petcu et al., 2009)*.

Despite extensive studies of NaCl tolerance of leguminous plants, very few studies have been conducted on *M. sativa* explaining drought tolerance. Genetic improvement of drought tolerance has traditionally been a problematic topic in plant breeding for numerous reasons, among which is the lack of clearly defined selection criteria for tolerance. Moreover, plant breeding using conventional procedure is time consuming and sometimes impossible for a number of plant species *(Ehsanpour et al., 2001)*.

The aim of this study was to evaluate of new Romanian alfalfa cultivars for salt and drought tolerance.

Materials and Methods

The study was conducted in a vegetation house using Mitcherlich pots filled with a soil-sand mixture (3:1). The nine alfalfa genotypes were grown under optimal watering regimes up to beginning of the flowering. After this period in the control variant the plants were maintained in the same conditions, in the hydric stress variant the watering was reduced for 10 days, salt stress was imposed by adding of *300 mM NaCl/l* and under combined stress the plants were treated with 300 *mM NaCl/l* one week before to reducing of watering (for 10 days). The following determinations were made:

The yield was estimated by measuring biomass accumulation of aerial part (shoots and leaves), (fresh weight).

Cuticular transpiration according to *Clark (1992)* method, modified by *Petcu et al., (2006)* was measured on excised trifoliate leaves.

Proline content was determined spectrophotometrically following the ninhydrin method described by *Bates et al., (1973)* using L-proline as a standard. Approximately 0.5 g of fresh leaves was homogenized in 10 mL of 3% aqueous sulphosalicilic acid and filtered. To 2 mL of the filtrate, 2 mL of acid ninhydrin was added, followed by the addition of 2 mL glacial acetic acid and boiling for 60 min. The mixture was extracted with toluene, and free proline was quantified spectrophotometrically at 520 nm.

Results and Discussion

The analysis of variance regarding the effect of drought and salinity on biomass of alfalfa showed very significant influence of treatments, genotype and their interaction, but the variance of treatment was higher than the variance due to genotypes (Table 1).

Table 1. Analysis of variance for biomass

Source of variance	DF	Sum of squares	Mean square	F value
Treatment (stresses)	3	79572.34	26524.12	4270.64***
Error A	6	37.2648	6.2108	
Genotype	8	1164.8230	145.6029	603.027***
Interaction	24	2522.7160	105.1132	435.3357***
Error B	64	15.4530	2415	

For all genotypes, the biomasses obtained in each treatment were significantly different from the control and the general trend was more pronounced decrease under drought and combined stress. Salt stress significantly decreased biomass over 37 % while hydric stress over 73%. The effects of salt and water stress on yields are additive but not equal (Figure 1).

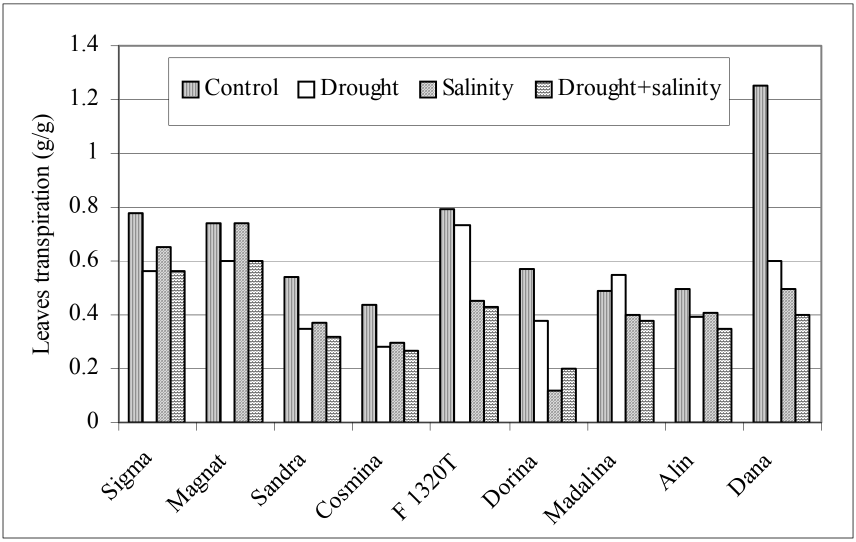

Figure 1. The effect of different stresses on cuticular transpiration for studied alfalfa genotypes

A decrease in biomass accumulation due to drought and salinity was associated with decreased transpiration. The genotypic differences in relation to water loss through transpiration are obvious. Cultivars Sandra, Cosmina and Dorina had relatively low leaves transpiration while the cultivars Dana and F 130T, had registered highest water loss, both under control and under stress conditions. The effect of salt and water stress on leaves transpiration was additive but not equal (Table 2).

Table 2.The effect of different stresses on biomass accumulation for studied alfalfa genotypes

Genotype	Fresh weight (g) per plant		
	Control	Drought	Salinity
Sigma	89.3	15.7	42.3
Magnat	93.4	16.7	58.6
Sandra	72.25	13.7	46.5
Cosmina	79.9	12.9	39.5
F 1320T	73.6	19.5	42.5
Dorina	75.8	16.4	37.2
Madalina	88.9	15.7	37
Alin	94.1	14.9	34.5
Dana	69.1	15.2	36.9

The alfalfa biomass related linearly to transpiration and the values of the correlation coefficients between biomass and transpiration under hydric and salt stress were r =0.82, r = 0.76 (P<0.01), (Table 3).

Table 3. Relationship between biomass and cuticular transpiration under stress conditions

Specification	Transpiration		
	Drought	Salt	Drought+salt
Biomass	r = 0.82* y = 0.0643x – 0.5117	r = 0.76* y = 0.0166x – 0.2402	r = 0.35 y = 0.0276x – 0,0328
	Proline content		
	r = -0.64* y = -3.3x + 103.44	r = 0.088 y = 1.1864x + 241.12	r = -0.64* y = - 29.001x + 647.79

Under optimal conditions the proline content was very low (1.7-5.4 mg Proline/g. f.w) but the high proline content under saline stress (156-441 µM praline per g of fresh weight) and hydric stress (45-68 µM praline per g of fresh weight) was obvious and the effect of those two stress appeared to be additive but not equal (120-330 µM praline per g of fresh weight) (Table 4). There is correlation between biomass and proline content only under the drought and combined stress (Table 3), that suggested that for salt stress proline accumulation is necessary but isn’t enough to provide tolerance to salinity.

The negative effect of salinity and stress combined on alfalfa growth could be attributed to an osmotic effect. Osmotic stress inhibits water uptake from the soil and requires the plant to use energy and carbohydrate in synthesizing organic solutes to adjust its internal osmotic potential.

Table 4. The effect of different stresses on proline content (µM praline per g fresh weight) for studied alfalfa genotypes

Genotype	Control	Drought	Salinity	Drought+salinity
Sigma	1.25	38	310	120
Magnat	1.74	40	250	110
Sandra	3.54	68	410	280
Cosmina	4.45	62	440	330
F 1320T	2.36	46	250	173
Dorina	5.2	51	120	230
Madalina	2.45	49	340	155
Alin	5.4	50	280	250
Dana	2.25	55	215	180

The significance of proline accumulation in osmotic adjustment is still debated and varies according to the species. *Ashraf (1989), Lutts et al. (1996), Feitosa de Lacerda et al. (2001)* and *Meloni et al. (2001)* reported that proline is not involved in the osmotic adjustment of black gram, sorghum, rice and cotton cultivars, respectively. Recently, *Heuer (2003)* reported that proline was not able to counteract salt stress effects in salt-sensitive tomato plants.

Conclusion

Our results have established the negative effect on biomass accumulation and leaves transpiration and positive effect on proline accumulation. Yield loss resulted from closing stomata (as result the transpiration decrease) and increasing of energy and carbohydrate use in osmoregulation.

The leaves transpiration and biomass accumulation were correlated which indicated that it can be used as screening criteria for drought and saline tolerance of alfalfa genotypes.

While final evaluation and selection for drought and salt tolerance in alfalfa will require field evaluations, this is a simple and efficient method of screening for tolerance to drought and saline conditions during the first year of growth.

Tolerantnost rumunske germplazme lucerke na sušu i zaslanjena zemljišta

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Rezime

U radu je ispitivana tolerantnost rumunskih sorti lucerke na sušu i zaslanjena zemljišta. Cilj rada je utvrđivanje screening agensa za pronalaženje i kreiranje genotipova sa poboljšanom poljskom tolerancijom na ova dva strsna uslova. Prinos lucerke je znatno redukovan pod uticajem stresnih faktora.

Nedostatak vode je smanjio prinos i do 73%., a povećana koncentracija Na do 37%. Lucerka reaguje na sušu i povećanu koncentraciju soli smanjenjem lisne transpiracije, ali između odgovor alucerke na ove stresne faktore nem alinearnih korelacija. Pod optimalnim uslovima, sadržaj prolina u lucerki je jako nizak, ali u stresnim uslovima sadržaj prolina raste. Negativan uticaj soli i nedostatka vode zajedno mogu uticati na osmotski pritisak. Smanjenje prinosa lucerke je rezultat zatvaranja stoma i korišćenje nergije i karbohidrata u osmoregulaciji. Lisna transpiracija i akumulacija biomase (prinos) je u korelacionoj vezi i ukazuje da transpiracija moze biti pokazatelj tolerantnosti na sušu i povećanu koncentraciju soli, pa se može koristiti u selekciji lucerke.

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INVESTIGATION OF THE YIELD COMPONENTS OF NEWLY CREATED FODDER BEET HYBRIDS

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Abstract: This paper presents the results of the investigation of two newly created fodder beet hybrids (SR 071 and SR 072) as well as a comparison with the standard fodder beet variety. The yield of root, leaves and dry matter (kg ha^{-1}) were also determined. The examined hybrids showed highly significantly higher root yield than the standard one, while the dry matter yield was at the standard level. As for the leaf yield, SR 071 hybrid had highly significantly higher yield than the standard, while the achieved yield of SR 072 hybrid was at the level of standard. In terms of biological characteristics and resistance to diseases both hybrids showed the properties and characteristic of the fodder beet.

Key words: hybrid, root yield, leaf yield, dry matter yield, fodder beet

Introduction

Fodder beet originates from the Mediterranean region, and it is widespread in many countries. As for the European countries, it is mostly grown in Germany. In our country, the growing of fodder beet began at the end of the nineteenth century, but it has not significantly spread. In 1993 fodder beet was grown on the area of only 8328 ha *(Lukić and Vasiljević, 1997)*. It is grown as fodder because of its thickened root. The root is the most interesting part of the plant for farmers. It grows partly in the soil, and partly above it, and it can grow up to 10 kilos. The significant amounts of nutrients are accumulated in it. Fresh leaves as well as silage leaves could also be used as fodder.

Fodder beet has the advantage in the production of fodder, as a plant species with the highest yield of biomass and nutritive matters *(Šibalić and Kunc, 1993)*. It is highly rated in the diet of dairy cows because of its positive impact on milk. The first domestic fodder beet varieties were bred in DP "Selekcija" in Aleksinac where the plant breeding of fodder beet began in 1983 *(Veselinović et al., 2001)*. The hybrids that have been created up to now are in the type of semi sugar monogerm diploids and triploids, which means that one of the components of sugar beet is implemented into them (MS line), *(Veselinović et al., 2002)*.

Materials and Methods

The hybrids of fodder beet were investigated during 2007 and 2008 in four localities, with the aim to determine their quantitative and qualitative properties and to make comparisons with the standard variety Binal. The investigation comprised two fodder beet hybrids, SR 071 and SR 072, which were created in AD "Agrosit" in Aleksandrovac. These two hybrids entered the process of registration by the Commission for the registration of newly created hybrids. The trials were placed in random block design in four repetitions at locations in Novi Sad, Sombor, Pancevo and Krusevac. During the investigation the common agro technique of fodder beet growing was applied.

SR 071 hybrid is a monogerm diploid hybrid variety of green to light green leaf color, and with orange root. SR 072 hybrid is a monogerm triploid hybrid variety of green to olive-green leaf color, with orange root also.

In this study we examined the yield of root and leaves (kg ha^{-1}), as well as the yield of dry matter (kg ha^{-1}). We also examined the biological characteristics (% of root in the soil and the phenomenon of boltings) and resistance to diseases (*Cercospora beticola* Sacc.).

Results and Discussion

Fodder beet and fresh sugar beet noodles are highly wet concentrates. Theoretically, they could be used as the only nutrient for cows due to their high structural values, but in practice it is better to combine these products with nutrients such as haylage grass, especially if the classic concentrates with high content of starch are added to them.

Table 1. Yield of root, leaves and dry matter in the root (kg ha^{-1})

Locality	Year	Root		Leaf		Dry Matter	
		SR 071	Binal	SR 071	Binal	SR 071	Binal
Novi Sad	2008.	113400	91240	15760	16600	10574	11350
Kruševac	2007.	50050	39050	9778	9443	7958	6442
	2008.	50720	54440	5680	7060	8114	6532
	x	50385	46745	7729	8252	8036	6487
Sombor	2007.	42700	44600	-	-	-	-
	2008.	103375	86093	28145	34467	-	-
	x	73037	65346	28145	34467	-	-
Pancevo	2008.	48920	43100	16760	15620	4809	6562
X 2007-2008.		68194++	59754	15225- -	16638	7864^{0}	7721
d 0,05		4000		981		642	
0,01		5673		1395		915	
Cv		13.93		12.77		15,84	

The main economic parameters of fodder beet are root yield, leaf yield and dry matter yield per area unit. The use of semi sugar varieties of fodder beet allows the combination of high yield of fodder beet root and of dry matter per area unit. Dry matter yield per area unit is the most important economic index for determining the value of forage crops.

In terms of root yield SR 071 hybrid showed a higher value in all localities. The achieved average root yield of 68.194 kg ha^{-1} is highly significantly higher than Binal standard variety (Table 1).

Table 2. Biological characteristics and resistance to *Cercospora beticola* Sacc.

Locality	Year	% of root in soil		Boltings (%)		*Cercospora beticola* (Score 1-9)	
		SR 071	Binal	SR 071	Binal	SR 071	Binal
Novi Sad	2008.	40	60	-	-	7.0	5.0
Kruševac	2007.	38.5	45.8	-		5.0	5.0
	2008.	41.9	58.2	-	-	5.0	5.0
	x	40.2	52.0	-	-	5.0	5.0
Sombor	2007.	95	90	-	-	0	0
	2008.	55	65	-	-	0	0
	x	75	77.5	-	-	0	0
Pancevo	2008.	60	60	-	-	7.0	7.0
X 2007-2008.		53.8	62.4	-	-	6.3	5.7

In terms of leaf yield SR 071 hybrid achieved an average yield of 15.225 kg ha^{-1}. The achieved leaf yield is highly significantly lower than the standard, so it is recommended for the production of root.

Table 3. Yield of roots, and a list of root dry matter (kg ha^{-1})

Locality	Year	Root		Leaf		Dry Matter	
		SR 072	Binal	SR 072	Binal	SR 072	Binal
Novi Sad	2008.	121840	91240	21120	16600	9842	11350
Kruševac	2007.	48540	39050	10744	9443	8512	6442
	2008.	67240	54440	8080	7060	5380	6532
	x	57940	46745	9412	8252	6946	6487
Sombor	2007.	42960	44600			-	-
	2008.	101586	86093	27949	34467	-	-
	x	72283	65346	27494	34467	-	-
Pancevo	2008.	42640	43100	15440	15620	4957	6562
X 2007-2008.		70818++	59754	16576	16638	7173^{0}	7721
d 0,05		4000		981		642	
0,01		5673		1395		915	
Cv		13.93		12.77		15,84	

In terms of dry matter yield SR 071 hybrid achieved an average yield of 7.864 kg/ha. There is no statistically significant difference between this hybrid and the standard variety concerning dry matter yield.

In terms of biological properties SR 071 hybrid showed the value (in %) of root in the soil characteristic of fodder beet plants (53.8%), while the emergence of boltings was not found either in investigated hybrids or the standard one. The average tolerance grade to *Cercospora beticola* Sacc. (6.3) is at the level of standard and points out a relatively good tolerance to the economically significant genus Beta disease (Tab.2). Hybrid SR 072 achieved an average root yield of 70.818 kg ha^{-1}. This value is highly significantly higher than Binal standard variety (Table 3). The leaf yield of SR 072 hybrid was 16.576 kg ha^{-1}. Statistical analysis shows that there is no significance in comparison with the standard. In terms of dry matter yield SR 072 hybrid achieved an average yield of 7.173 kg ha^{-1}. There is no statistically significant difference between this hybrid and the standard variety concerning dry matter yield.

Table 4. Biological characteristics and resistance to *Cercospora beticola* Sacc.

Locality	Year	% of root in soil		Boltings (%)		*Cercospora beticola* (Score 1-9)	
		SR 072	Binal	SR 072	Binal	SR 072	Binal
Novi Sad	2008.	30	60	-	-	7.0	5.0
Kruševac	2007.	46.1	45.8	-		5.0	5.0
	2008.	49.9	58.2	-	-	5.0	5.0
	x	48	52.0	-	-	5.0	5.0
Sombor	2007.	90	90	-	-	0	0
	2008.	35	65	-	-	0	0
	x	62.5	77.5	-	-	0	0
Pancevo	2008.	70	60	-	-	7.0	7.0
X 2007-2008.		52.6	62.4	-	-	6.3	5.7

In terms of biological properties SR 072 hybrid showed the value (in %) of root in the soil characteristic of fodder beet plants (52.6 %), while the emergence of boltings was not found either in investigated hybrids or the standard one. The average tolerance grade to *Cercospora beticola* Sacc. (6.3) is at the level of standard and points out a relatively good tolerance to the economically significant genus Beta disease (Table 4).

Conclusion

On the basis of these results, we can conclude that these new fodder beet hybrids (by root yield) highly significantly surpass the current standard variety Binal. In terms of leaf yield, as well as dry matter yield the achieved results are at the level of standard. The investigated hybrids are in the process of registration by the Commission for the registration of newly created varieties and hybrids, so their registration and gradual introduction in the production of quality fodder can be expected. Further directions in fodder beet breeding are based on the increase of root yield with the desire to maintain a high level of dry matter content. That goal can be achieved by the introduction of male sterility in the plant breeding of fodder beet and creation of pure fodder beet hybrids.

Ispitivanje komponenti prinosa novostvorenih hibrida stočne repe

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Rezime

U radu su izneti rezultati ispitivanja dva novostvorena hibrida stočne repe (SR 071 i SR 072) i izvršeno upoređivanje sa standardnom sortom Binal. Određivani su prinos korena, prinos lista i prinos suve materije (kg ha^{-1}). Ispitivani hibridi su pokazali visoko značajno veći prinos korena u odnosu na standard, dok je prinos suve materije bio na nivou standarda. U pogledu prinosa lista hibrid SR 071 je ostvario visoko značajno manji prinos u odnosu na standard, dok je ostvareni prinos kod hibrida SR 072 bio na nivou standarda. U pogledu bioloških osobina i otpornosti na bolesti oba hibrida su pokazala svojstva karakteristična za stočnu repu.

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VARIABILITY OF FORAGE YIELD COMPONENTS OF MEADOW FESCUE (*Festuca pratensis* Huds.) POPULATIONS AND CULTIVARS

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Abstract: Dry matter yields of collection consisted of five breeding populations and six cultivars of meadow fescue (*Festuca pratensis* Huds.) were investigated. In two-year period, dry matter yield per plant in three cuts and annual dry matter yield per plant were analyzed. Statistically significant intra-population variability was determined for all yield components. By analysis of variance for each year of study, the significant differences between the studied populations and cultivars were found. Genetic relationship of studied meadow fescue genotypes was illustrated by cluster analysis. Average values of yield components obtained in this study suggest that studied genotypes are potentially valuable for further breeding.

Key words: meadow fescue, dry mater yield, populations, cultivars, variability

Introduction

Meadow fescue (*Festuca pratensis* Huds.) is grass species widespread on grasslands from lowland to upper areas in Serbia. It is a component of high quality and durable mixtures used for hay production, ensilaging and grazing, especially in the hilly area. It is characterized by a high resistance to cold climate, high tolerance to frost, so is ideal species for climatic conditions of Europe *(Kölliker, 1998)*. Also, it tolerates summer drought periods well, although prolonged droughts may reduce yield. It shows excellent response to irrigation. The meadow fescue is the dominant species in the natural grasslands on fertile and lime soils. It is common in the vicinity of forests and in thin woods.

It tolerates grazing very well and the pastures in which the meadow fescue is dominant are long-lasting. It can be grown as monoculture, but primarily it has been grown in mixtures with other grasses and legumes where it participates with 20-30%. By quality, meadow fescue is among the grasses of excellent quality, making it comparable to the species of the genus *Lolium (Kölliker, 1998)*. It is characterized by high crude protein content from 15 to 18 g kg^{-1} of dry matter, *(Niemeläinen et al., 2001)*.

The existence of genetic variability within the available breeding material (autochthonous populations, breeding populations and cultivars) influences the choice of breeding material and success of a plant breeding program *(Sokolović, 2001)*. Variability within populations and cultivars of meadow fescue were documented for important agronomical traits, forage yield and forage quality *(Kanapeckas et al., 2005)*. The primary aim in meadow fescue breeding is obtaining cultivars with a high yield and quality for livestock feed *(Casler and Santen, 2000)*. The aim of this paper was to examine the variability within populations and cultivars of meadow fescue in order to provide data which is important for breeding and for further investigations.

Materials and Methods

The studied collection consisted of five breeding populations and six cultivars of meadow fescue (*Festuca pratensis* Huds.). Breeding populations have passed 2 cycles of selection. Four originated from the local populations, collected mainly in Eastern Serbia (OP-1, OP-2 OP-3 and OP-4), and the population OP-5 originates from Eastern Europe (Czech Republic). Meadow fescue cultivars included in this study are: Kruševački 21 (K-21) (Serbia), Jabeljska (Slovenia), Premil, Pradel, Preval (Swicerland) and Rožnovska (Czech Republic).

The study was conducted on experimental field of the Institute for forage crops. The trial was set up as randomized block design in three replications with 30 plants per genotype. The sowing in containers was performed in early spring in the greenhouse and planting in the experimental field in April in plant nursery with plant to plant distance 60x60cm. Dry matter yield (DMY) per plant in three cuts and annual dry matter yield per plant were investigated over a two-year period. Mono-factorial analysis of variance was used for both years of research, due to large differences in agro-ecological conditions in studied years. Testing of differences between mean values of the studied traits was done using the least significant difference test (LSD test), with significance of 1 and 5% (α). Cluster analysis based on yield components was made by the Ward method, using the Euclid distances with software Statistica 7.0.

Results and Discussion

Dry matter yield per plant is one of the most important traits in the breeding programme. The main goal is to increase the DM yield or maintain the yield through improving of other traits. The yield is very variable trait, influenced by a large number of genes, and largely depends on the present agro-ecological conditions. It is indicated by the high variation coefficients and very large differences in average values of the realized yield per plant (both by cuts and by years of research). Noticeably lower average yield in 2007 can be explained by dry weather conditions.

In 2006, the highest dry matter yield in the first cut was determined in the cultivar Pradel, breeding population OP-2 and cultivar K-21 (Table 1). In 2007, dry matter yield in the first cut was much lower compared to 2006, which can be explained by the arid climatic conditions during the second year of research. The highest yield was obtained in breeding populations OP-2 (37.90 g per plant) and cultivar K-21 (36.54 g), while the lowest dry matter yield in the first cut was recorded in cultivar Jabeljska. The highest DMY in 2006 in the second cut was recorded in the Swiss cultivars Preval and Pradel, the breeding population OP-2 and cultivar K-21, which also had the highest yield in 2007 (Table 1). High variation coefficients indicate a high degree of variability within the genotypes for this trait.

Dry matter yield in the third cut was determined in 2006, since, due to dry weather conditions in 2007, plants did not have considerable re-growth after second cut (Table 1). The highest dry matter yield in the third cut was recorded in the cultivar K-21 and breeding population OP-2, while the lowest yield was in the cultivar Jabeljska.

Table 1. DMY per cut and the total annual yield of meadow fescue genotypes (g per plant)

Genotypes		I cut		II cut		IIII cut	Annual yield	
		2006.	2007.	2006.	2007.	2006.	2006.	2007.
OP-1		101.85	16.78	17.20	5.52	12.57	131.62	22.3
OP-2		133.61	37.90	24.44	18.58	15.50	173.55	56.48
OP-3		72.95	18.16	21.98	16.14	12.46	107.39	34.3
OP-4		77.15	29.46	14.53	13.34	7.43	99.11	42.8
OP-5		101.79	28.93	17.89	10.98	11.40	131.08	39.91
K-21		130.39	36.54	24.04	16.92	15.73	170.16	53.46
Jabeljska		87.34	12.13	13.85	10.60	7.37	108.56	22.73
Premil		109.24	15.62	21.48	10.46	11.21	141.93	26.08
Pradel		134.37	31.26	27.31	9.93	15.35	177.03	41.19
Preval		127.80	28.18	27.96	8.83	14.12	169.88	37.01
Rožnovska		127.79	32.81	22.41	8.18	14.21	164.41	40.99
Average		110.00	26.23	21.19	11.81	12.49	143.68	38.04
LSD	0,05	10.62	4.90	3.34	3.20	0.98	9.51	5.84
	0,01	14.49	6.68	4.56	4.37	1.34	12.97	7.97
CV (%)		46.44	53.76	53.75	60.51	53.24	42.24	52.24

The highest annual dry matter yield in 2006 was in the cultivar Pradel (179.39 g per plant) and the breeding population OP-2 (177.03 g) (Table 1). In

2007 the highest yield was determined in breeding population OP-2 (56.48 g) and cultivar K-21 (53.46 g). In paper of *Akgun et al. (2008),* the average dry matter yield of meadow fescue was 62.32 g per plant. Examining the wide collection of populations and cultivars of meadow fescue, *Casler and Santen (2000),* based on two-year study, determined that the average yield was 105g per plant. Considering the perennial grass yield, dry matter yield in the first cut participates with the highest percentage (about 70%) of the total annual DM yield *(Sokolović, 2001),* due to dry summer which reduces regeneration. In this study, the ratio of dry matter in the first cut in the total yield was 76.89% in 2006 and 68.95% in 2007, which is consistent with the results of *Sokolović (2001)* and *Stosić (1988)*.

Table 2. Ratio of DMY in the first, second and third cut in the annual DMY (%)

	2006.			2007.	
Genotypes	I cut	II cut	IIII cut	I cut	II cut
OP-1	78.47	13.25	8.27	76.25	24.75
OP-2	76.99	14.08	8.93	67.10	32.90
OP-3	67.92	20.47	11.61	52.94	47.06
OP-4	77.84	14.66	7.50	68.83	31.17
OP-5	77.65	13.65	8.70	72.49	27.51
K-21	76.63	14.13	9.24	68.35	31.65
Jabeljska	80.45	12.77	6.79	53.37	46.63
Premil	78.04	13.94	8.02	59.01	40.99
Pradel	76.13	15.17	8.70	75.89	24.11
Preval	76.28	15.72	8.00	76.61	23.39
Rožnovska	77.77	13.58	8.65	80.04	19.96
Average	76.89	14.55	8.57	68.95	31.05

In the study of *Leto et al. (2006)*, the share of the dry matter yield in the first cut of the total annual yield was about 55%. Also, *Kanapeckas et al. (2005)* and *Lemežiené et al. (2004)* state that the proportion of dry matter in the first cut of the total dry matter yield ranges in the interval of 52 to 55%. The ratio of the second cut in annual yield was approximately 15% in 2006 and in 2007 it was twice as high. It should be noted that in 2007 plants were not cut the third time, which certainly reflected on the yield share in the second cut in the annual dry matter yield. In the researches of *Lemežiené et al. (2004)*, ratio of dry matter yield from the second cut in the total annual dry matter yield was 25%.

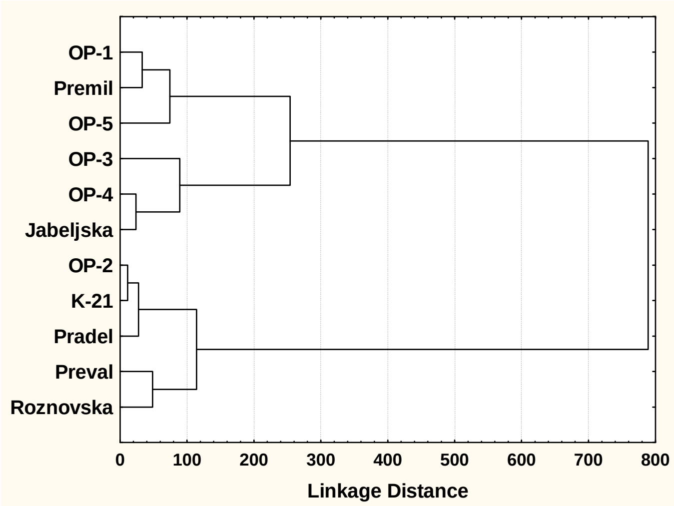

Figure 1. Cluster diagram of the meadow fescue genotypes based on biomass yield components

Using the hierarchical cluster analysis, grouping of meadow fescue was done and similar genotypes were classified in the same clusters. The results are shown as a cluster diagram (Figure 1). Genotypes with lower yield are grouped in the first cluster in two subgroups. The cultivar Premil and populations OP-1 and OP-5 are in the first subgroup. The second subgroup is consisted of the populations OP-3 and OP-4 and the cultivar Jabeljska. Populations OP-3 and OP-4 are characterized by the lowest annual dry matter yield. The second cluster is also composed of two subgroups, both characterized by a high annual dry matter yield. Breeding populations OP-2 and the cultivar K-21 were most similar with smaller distance. Their similarity can be explained by origin: OP-2 originated from Eastern Serbia and the cultivar K-21 was bred from populations originating from the same area.

Conclusion

This study demonstrates considerable differences in variability within meadow fescue populations and cultivars for forage yield traits. Coefficients of variation were 42.24% for annual DMY in 2006 and 52.24% in 2007. The highest annual yield was obtained for breeding population OP-2 and cultivars K-21 and Pradel. Swiss cultivar Pradel had the highest dry matter yield in favourable 2006, but in unfavourable 2007, it was ranked as the genotype with the medium yield. In the 2007, the highest dry matter yield was determined in the Serbian genotypes (population OP-2 and cultivar K-21), which indicates the dominance of domestic in relation to the introduced genotypes. Cluster analysis showed that the material included in this study had significant variability.

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Varijabilnost komponenti prinosa populacija i sorti livadskog vijuka (*Festuca pratensis* Huds.)

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Rezime

Istraživanja su obuhvatila kolekciju od pet oplemenjivačkih populacija i šest sorti livadskog vijuka (*Festuca pratensis* Huds.). U dvogodišnjem periodu proučavan je prinos suve suve materije po biljci po otkosima i ukupan prinos suve materije po biljci. Za sve komponente prinosa ustanovljena je statistički značajna unutar populacijska varijabilnost. Analizom varijanse za svaku godinu istraživanja utvrđene su značajne razlike između ispitivanih populacija i sorti. Velika varijabilnost proučavanih genotipova livadskog vijuka potvrđena je klaster analizom. Prosečne vrednosti komponenti prinosa dobijene u ovim istraživanjima ukazuju na perspektivnost proučavanih genotipova za dalji proces oplemenjivanja ove vrste.

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ACHIEVEMENTS IN THE CONSERVATION OF ANNUAL LEGUMES GENETIC RESOURCES IN SLOVAKIA AND SERBIA

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Communication

Abstract: During 2008 and 2009, a project *Conservation of the Genetic Resources of Annual Forage and Grain Legumes in Slovakia and Serbia* was carried out within the bilateral scientific cooperation between Slovakia and Serbia. Its participants were the Research Institute of Plant Production in Piešťany on behalf of Slovakia and Institute of Field and Vegetable Crops and Faculty of Agriculture in Novi Sad on behalf of Serbia. Annual legumes are important components of both wild and agricultural flora in both countries. Many of them, such as faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.) and grass pea (*Lathyrus sativus* L.) are becoming neglected and underutilised. There are also many wild species, such as narrow-leafed (*Vicia sativa* subsp. *nigra* (L.) Ehrh.), large-flowered (*Vicia grandiflora* Scop.), hairy (*Vicia villosa* Roth) and Hungarian (*Vicia pannonica* Crantz) vetches, as well as tall pea (*Pisum sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn. var. *elatius* (Steven ex M. Bieb.) Meikle), that may be important in breeding as a source of tolerance to various stresses or as having a great potential for forage production. The activities in both years involved *in situ* research, mainly expeditions, aimed at collecting herbarium seed samples~~,~~ and *ex situ* conservation, with emphasis on developing a passport data, characterization and evaluation. The collected accessions will be included in the participants' collections and evaluated for forage and seed yields and the tolerance to low temperatures, drought and other abiotic and biotic stress.

Key words: annual legumes, conservation, genetic resources, Serbia, Slovakia.

Annual legumes in Slovakia and Serbia

Several annual legumes such as chickpea (*Cicer arietunum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) are counted among the oldest crops and have begun to distribute across Europe ten millennia ago *(Ljuština & Mikić, 2008)*. Together with other vetches, vetchlings (*Lathyrus* spp.) and lupins (*Lupinus* spp.), these species originated in the Near Eastern and Mediterranean centres of diversity, while faba bean (*Vicia faba* L.) originated in the Central Asian centre *(Zeven & Zhukovsky, 1975)*.

Vetches and vetchlings are rather rich genera in both Slovakia and Serbia. For instance, in the flora of Serbia, there is about 30 species of vetchlings *(Kojić, 1972)* and about 30 vetch species. Also, Serbia could represent the northern border of the distribution of tall pea (*Pisum sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn. var. *elatius* (Steven ex M. Bieb.) Meikle) in the Balkan Peninsula *(Diklić, 1972)*.

The temperate annual legumes, such as those from Slovakia and Serbia, are both a valuable component of natural grassland systems and quality field crops that can be used as green forage, hay, forage meal, silage, haylage, immature grain, mature (dry) grain and straw, as well as green manure *(Mikić et al., 2006)*.

The Research Institute of Plant Production in Piešťany and Institute of Field and Vegetable Crops and Faculty of Agriculture in Novi Sad each carry out long-term programmes on annual legume genetic resources in Slovakia and Serbia. Their joint efforts have been articulated through a bilateral project *Conservation of the Genetic Resources of Annual Forage and Grain Legumes in Slovakia and Serbia*, with duration of 2008 and 2009 *(Hauptvogel et al., 2008)*.

Collections of annual legumes in Slovakia and Serbia

Regarding the fact that the project participants possess the largest number of accessions of diverse temperate annual legumes in both Slovakia and Serbia, the first milestone of the project was to maintain the present collections.

In early 2010, the Annual Forage Legumes Collection (AFLCNS) in Novi Sad, maintained in the Forage Crops Department of the Institute of Field and Vegetable Crops contained more than 2,200 accessions of about 20 genera and 70 species. The most numerous among its genera were vetches, with about 1,300 accessions (Table 1).

Table 1. The Vicia collection within the Annual Forage Legumes Collection in Novi Sad (Mikić et al., 2008a)

Species	No. of accessions
V. bithynica (L.) L.	2
V. cracca L.	1
V. disperma DC.	1
V. ervilia (L.) Willd.	4
V. faba L.	121
V. galilaea Plitm. & D. Zoh. in Plitm.	1
V. grandiflora Scop.	462
V. hirsuta (L.) Gray	46
V. hyaeniscyamus Mouterde	2
V. lathyroides L.	1
V. lutea L.	4
V. michauxii Spreng.	1
V. narbonensis L.	45
V. noeana Reut. ex Boiss.	11
V. pannonica Crantz	44
V. parviflora Cav.	1
V. pisiformis L.	3
V. sativa L.	496
V. sylvatica L.	1
V. tetrasperma (L.) Schreb.	1
V. vicioides (Desf.) Cout.	1
V. villosa Roth	27
Total	1,276

In search for annual legumes in Slovakia and Serbia

The next task of the project development was to enhance the current annual forage and grain legumes collections in Piešťany and Novi Sad by collecting both wild populations and local landraces of these species. The collecting had its peak during the duration of the project, that is, in spring and summer of both 2008 and 2009.

The most visited locations in Serbia with wild populations of mostly vetches and vetchlings, from 2002 onwards, were wider regions of Novi Sad, with emphasis upon the mountain of Fruška Gora, and Belgrade *(Ćupina et al., 2006)*. Species such as narrow-leafed vetch (*Vicia sativa* subsp. *nigra* (L.) Ehrh.), hairy vetch (*Vicia villosa* Roth) and large-flowered vetch (*Vicia grandiflora* Scop.) were often found growing together and in some locations joined by Hungarian vetch (*Vicia pannonica* Crantz), tiny vetch (*Vicia hirsuta* (L.) Gray) and yellow vetchling (*Lathyrus aphaca* L.). These vetch species were proven as rather winter hardy and the earliest of all annual legumes present in local wild floras *(Ćupina et al., 2007)*.

In Serbia, in the late spring and early summers of both 2008 and 2009, numerous wild populations of several vetch species in a wider region of Novi Sad and the mountains of Tara and Zlatibor in western Serbia were *ex situ* conserved in the form of collecting their seeds from mid- and late May in lowlands such as in Novi Sad until late June and early July in mountains such as Tara with an altitude of about 1,000 m. Apart from this, about 30 local landraces of faba bean, vegetable pea and lentil were purchased at local markets in towns and villages of the wide regions of Novi Sad and Kruševac, where these crops are still grown mostly to answer the needs of single households and having become officially forgotten. One accession of fenugreek (*Trigonella phoenum-graecum* L.), originally from mid-Banat and used as a medicine plant, was also collected.

In Slovakia, where the growing period of all collected annual legume species was late at least one month in comparison to their populations in Serbia, the expeditions were aimed at the meadow and pasture species, where the most significant were perennial vetches, such as bush vetch (*Vicia sepium* L.) and cow vetch (*Vicia cracca* L.). In August 2009, during the expedition in Tatra Mountains, samples of several species of lupins (*Lupinus* spp.), clovers (*Trifolium* spp.) and vetches *(Hauptvogel et al., 2009)* were collected.

Passport databases, characterisation and evaluation

One of the essential deliverables of the project was the building of a joint collection of wild populations and local landraces collected in Slovakia and Serbia before and during the duration of the project.

Each accession of the joint collection has its passport data, following the recommendations made within the Grain Legume Passport Descriptors (*IPGRI, 2000*), such as (1) institute code; (2) accession number; (3) collecting number; (4) genus; (5) species; (6) subtaxa; (7) accession name; (8) country of origin; (9) location of collecting site; (10) latitude of collecting site; (11) longitude of collecting site; (12) elevation of collecting site; (13) collecting date of original sample, (14) status of sample, (15) collecting source, (16) donor institute code, (17) donor number, (18) other number(s) associated with accession and (19) remarks *(Mihailović et al., 2006b)*.

Among the first tests of each accession is the characterisation of highly heritable traits that do not depend on environmental conditions, such as flower colour, seed shape, seed coat colour, and cotyledon colour *(Mihailović et al., 2007)*.

The fact that many wild annual legume species have a great potential for both forage or grain production *(Krstić et al., 2007)* makes the evaluation of diverse agronomic characteristics a strategic link between genetic resources and their sustainable utilisation in plant breeding.

A long-term evaluation of wild vetch species has proven their potential to be introduced as forage crops (Table 3).

Table 2. Selected results of the evaluation of forage yields in wild populations of four wild vetch species (Mihailović et al., 2008; Mihailović et al., 2009; Mikić et al., 2008b; Mikić et al., 2009a)

Species	Population	Green forage yield (t ha^{-1})	Forage dry matter yield (t ha^{-1})	Forage dry matter proportion
Narrow-leafed vetch	MM 02/02	33.4	7.3	0.22
	MM 02/03	13.7	4.3	0.32
	MM 02/04	14.5	4.4	0.30
	MM 03/08	17.4	5.1	0.29
	MM 03/09	15.8	5.1	0.32
Hairy vetch	MM 03/26	47.3	9.5	0.20
	MM 04/19	23.3	7.7	0.33
	MM 04/13	29.4	6.0	0.20
	MM 04/18	45.6	10.4	0.23
	MM 04/19	50.9	11.7	0.23
Hungarian vetch	MM 04/28	20.9	4.8	0.23
	MM 04/29	21.2	4.4	0.21
	MM 04/30	27.3	5.5	0.20
	MM 04/31	45.7	11.0	0.24
	MM 04/32	46.0	12.0	0.26
Large-flowered vetch	MM 03/19	25.0	5.5	0.22
	MM 03/25	38.6	9.7	0.25
	MM 04/08	39.8	11.6	0.29
	MM 05/07	40.3	13.8	0.34
	MM 06/24	29.8	9.9	0.33

The collected local landraces of both food and feed faba bean have a great potential for grain production and may be included as an excellent breeding material in the development of advanced cultivars (Table 3).

Table 3. Agronomic characteristics of five local landraces of faba bean from Serbia (Mihailović et al., 2006a)

Accession	Thousand grains mass (g)	Grain yield (kg ha^{-1})	Harvest index
PP 1	506	5247	0.45
PP 2	469	4607	0.43
PP 4	398	5190	0.47
PP 3	517	6150	0.46
PP 5	428	5727	0.48

Many accessions of the collected vetch and vetchling species proved to be tolerant to diverse stress. The most detailed evaluation that has been carried out was related to the tolerance to low temperatures, where species such as narrow-leafed, large-flowered and hairy vetches demonstrated an ability to survive rather harsh winter conditions, with an average absolute minimum temperature of nearly -20 °C in certain years *(Mikić et al., 2009b)*.

Future of the bilateral cooperation

The started cooperation will be continued through a new one, entitled *Preservation and sustainable utilization of wild and agricultural legume flora in Slovakia and Serbia* (LEGUMFLORA) and with duration of 2010 and 2011. This project will include both annual and perennial traditional legumes in both countries and will have several goals:

- improvement of the *ex situ* conservation and unification of the methodology of maintenance;
- further study on the seed and plant physiology of diverse legume species;
- comparative research on forage and grain/seed yields and quality parameters.

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Dostignuća u očuvanju genetičkih resursa jednogodišnjih mahunarki u Slovačkoj i Srbiji

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Rezime

Tokom 2008.i 2009. godine, u okviru bilateralne naučne saradnje između Slovačke i Srbije, sproveden je projekat *Očuvanje genetičkih resursa*

jednogodišnjih krmnih i zrnenih mahunarki u Slovačkoj i Srbiji. U njemu su učestvovali Naučni institut za biljnu proizvodnju u Pješćanima, u ime Slovačke, i Institut za ratarstvo i povrtarstvo i Poljoprivredni fakultet u Novom Sadu, u ime Srbije. Jednogodišnje mahunarke su važne komponente samonikle i poljoprivredne flore obe zemlje. Mnoge od njih, poput boba (*Vicia faba* L.), sočiva (*Lens culinaris* Medik.) ili sastrice (*Lathyrus sativus* L.), postaju zapostavljene i nedovoljno korišćene. Takođe, postoje i samonikle vrste, poput uskolisne (*Vicia sativa* subsp. *nigra* (L.) Ehrh.), krupnocvetne (*Vicia grandiflora* Scop.), maljave (*Vicia villosa* Roth) i panonske (*Vicia pannonica* Crantz) grahorice, kao i poput visokog graška (*Pisum sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn. var. *elatius* (Steven ex M. Bieb.) Meikle), koje mogu da budu od značaja za oplemenjivanje kao izvor tolerantnosti na različite vidove stresa ili usled potencijala za proizvodnju krme. Aktivnosti u obe godine projekta obuhvatile su istraživanja *in situ*, uglavnom ekspedicije, sa ciljem sakupljanja herbarskog materijala i uzoraka semena, kao i očuvanje *ex situ*, sa naglaskom na stvaranje baze pasoških podataka, karakterizaciju i evaluaciju. Sakupljene akcesije biće uključene u zbrike učesnika i evaluaciju prinosa krme i semena i tolerantnosti na niske temperature, sušu i ostale vidove abiotičkog i biotičkog stresa.

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GRAIN LEGUMES TECHNOLOGY TRANSFER PLATFORM (GL-TTP): A STEP TOWARDS THE INTEGRATION OF GRAIN, FORAGE AND OTHER LEGUME COMMUNITIES

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Communication

Abstract: Grain Legumes Technology Transfer Platform (GL-TTP) is an independent and non-profit international organisation with an aim facilitates the exploitation of the research results of the EU FP6 Grain Legumes Integrated Project (GLIP, www.eugrainlegumes.org). The GL-TTP members come from both public and private sector and constitute a network evaluating the needs and constraints of grain and other legume breeding programmes, assessing the relevance of new technologies and discoveries for the grain legume industry, collecting and disseminating appropriate information within and between research and industry, setting up public and private and research and industry partnerships, organising training workshops and proposing technology transfer projects. GL-TTP focuses on genomic technologies to characterise the genetic diversity, identifies new genes of a potential agronomic interest, validates the function of candidate genes using high-throughput functional screens of mutagenic populations and generates molecular markers for breeders. GL-TTP is open for an active interchange of the results with the forage and other legume communities to the mutual benefit.

Key words: forage legumes, grain legumes, technology transfer platform.

Introduction

Grain legumes: so good, yet in decline. Grain legume crops are widely recognised as an essential component of sustainable agricultural systems and as a healthy alternative source of protein for animal feed and human consumption. However, these crops are underused and in decrease in Europe, while in non-European countries such as Canada they represent an efficient amalgam of research and production, resulting in an increase of both harvested area and yield *(Warkentin et al., 2008)*. Grain legume crops suffer from inconsistent yields, mostly due to poor resistance to diseases in the field, and lack of tolerance to abiotic stress.

From models to crops. The top priority of GL-TTP is to facilitate and expedite grain legume breeding programmes through the use of molecular technologies. Due to complex, diverse and large genomes, molecular biology and genetics studies have been slow in cultivated legumes. In contrast, molecular research is expanding fast in the model legumes *Medicago truncatula* and *Lotus japonicus*. Several studies of comparative genomics have highlighted significant syntenic relationships between legume species, which will allow the transfer of genomics data from model to crops.

Need to broaden genetic base of modern varieties. It is recognised that the genetic base of cultivated plants has narrowed down through the past thousands of years of domestication, migration bottlenecks and breeding activities. As a consequence, current varieties have less potential to respond to changing conditions, such as climate change, land degradation, water scarcity or pathogen evolution. One of the goals of GL-TTP is to facilitate the characterisation and exploitation of genetic resources for their use in international breeding programmes.

An initiative of GLIP. GL-TTP was launched by the Grain Legumes Integrated Project (GLIP, www.eugrainlegumes.org) primarily to work at the integration, assessment and commercial exploitation of the results of GLIP research. GLIP was a €24-M, 4-years (2004-2008) European Project that involved 18 countries, 54 partners, in an effort to boost the European production of grain legumes for animal feed and human consumption (*Ellis et al., 2005*). The research programme of GLIP encompassed economic, environmental, agricultural and nutritional issues of crop legumes, as well as the production of genetic resources and genomic tools in model legumes. GL-TTP was set up to work in close collaboration with the European Association for Grain Legume Research (AEP, www.grainlegumes.com).

Objectives and Mission

Major focus. GL-TTP has been created to facilitate collaborative efforts for carrying out scientific survey and making value of technology watch for the purposes of applied legume crop breeding. It aims to facilitate and expedite the genetic improvement of grain legume varieties to suit the needs by the grain legume producers and end-users *(Golstein et al., 2006)*.

GL-TTP mission. The mission of GL-TTP is to provide enabling tools in order to: 1) increase grain legume production through the use of more robust varieties and improved crop management; 2) improve and diversify grain legume products for the feed and food industry through better grain quality and new processing techniques.

The way to go. To reach these objectives, GL-TTP commits, as written in its statutes: 1) to form a durable partnership between research and industry in the international grain legume community; 2) to provide its members with all information and expertise necessary for their exploitation of the results of GLIP and of any other research programmes relevant to grain legumes; 3) to propose and to commission research and development activities for its members; 4) to contribute to the design of tools, methodology and know-how for commercial application of the results of grain legume research *(Smýkal et al., 2008)*. Combining efforts of the GL-TTP members reduce the costs of joint activities and strengthen the attempts for raising complementary funds for joint projects *(Schneider, 2007)*.

Structure

Membership. is open to research institutes and grain legume industries that want to contribute to, and benefit from, the international network of GL-TTP. The termination of GLIP in early 2008 has its impact to the composition of GL-TTP members. Today, GL-TTP gathers together 27 organisations from 18 countries (Table 1).

Table 1. GL-TTP members in 2009

Organisation full name	Country
Agricultural Institute of Slovenia	Slovenia
Agritec Plant Research Ltd.	Czech Republic
All-Russia Research Institute for Agricultural Microbiology	Russia
BASF Plant Science	Germany
Centro per le Produzioni Foraggere e Lattiero-Casearie	Italy
Crop Development Centre	Canada
Faculty of Agriculture in Novi Sad	Serbia
Faculty of Agriculture in Osijek	Croatia
Feed Research Institute	Ukraine
GenXPro	Germany
Green Lane Agricultural Assistance NGO	Armenia
Institute for Forage Crops	Serbia
Institute for Lowland Forestry and Environment	Serbia
Institute of Field and Vegetable Crops	Serbia
Institute of Plant Genetic Resources	Bulgaria
Institute of Soil Science	Serbia
John Innes Centre	UK
Lentil Research Association	India
Limagrain Verneuil Holding	France
Maize Research Institute	Serbia
Nepal Agricultural Research Council	Nepal
Norddeutsche Pflanzenzucht Hans-Georg Lembke KG	Germany
North Dakota State University	USA
Research Institute for Fodder Crops	Czech Republic
Saskatchewan Pulse Growers	Canada
University of Helsinki	Finland
University of Ibadan	Nigeria

Entity. GL-TTP is a not-for-profit association under the French law, so-called “Association loi 1901”. GL-TTP has been created by eleven foundation members. Following the decisions of the Fourth General Assembly in Novi Sad, Serbia, in November 2008, GL-TTP has been decided to undergo statute changes and thus transform into a more efficient entity with an improved ability to answer current challenges to the benefit of its members.

Management. GL-TTP is managed by the Executive Committee on behalf of the Council. The first Executive Committee was chaired by Noel Ellis (John Innes Centre, UK). The members of the current Executive Committee are Petr Smýkal (Agritec Plant Research Ltd., Czech Republic) as President, Noel Ellis as Past President, Anne-Marie Bochard (Limagrain Verneuil Holding, France) as Treasurer, Tom Warkentin (Crop Development Centre, Canada) and Aleksandar Mikić (Institute of Field and Vegetable Crops, Serbia) as Vice-Presidents and Anne Schneider (GL-TTP foundation member, France) as Executive Secretary.

Workshops

So far, there have been two GL-TTP Workshops. The first one, under the title *Targeting Science to Real Needs*, was held in Paris, France, 23-25 April 2007, while the second one, entitled *Integrating Legume Science and Crop Breeding*, was held in Novi Sad, Serbia, 27 and 28 November 2008.

Integrating grain and forage legume research

The facts that most of grain legume species may be used for forage production *(Mikić et al., 2006)*, as well as that the results of the model legume research are equally applicable in breeding all cultivated legumes, naturally led to an increased interest in GL-TTP activities of the institutions that deal with breeding forage, tree legume species and soybean *(Smýkal et al., 2009)*. One of the goals GL-TTP has is to develop joint strategies in legume research with the organisations such as the European Grassland Federation (EGF), especially with its members involved in forage and grassland legume breeding. Such activities might result in the transformation of GL-TTP into broader-based organisation tentatively called *International Legumes Society*.

Platforma za prenos tehnologije zrnenih mahunarki (GL-TTP): korak ka integraciji zajednica zrnenih, krmnih i ostalih mahunarki

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Rezime

Platforma za prenos tehnologije zrnenih mahunarki (Grain Legumes Technology Transfer Platform, GL-TTP) jeste nezavisna i neprofitabilna međunarodna organizacija sa ciljem da olakša korišćenje istraživačkih rezultata EU FP6 Integrisanog projekta zrnenih mahunarki (Grain Legumes Integrated Project, GLIP, www.eugrainlegumes.org). Članovi GL-TTP dolaze iz javnog i privatnog sektora i sačinjavaju mrežu koja procenjuje potrebe i poteškoće oplemenjivačkih programa zrnenih i drugih mahunarki, ispituje važnost novih tehnologija i otkrića za industriju zrnenih mahunarki, sakuplja i cirkuliše odgovarajuće informacije između istraživača i proizvodnje, uspostavlja partnerstvo između javnog i privatnog i između istraživača i proizvodnje, organizuje radionice za obuku i predlaže projekte prenosa tehnologije. GL-TTP se usredsređuje na genomičke tehnologije u cilju karakterizacije genetičke raznolikosti, identifikuje nove gene od moguće agronomske važnosti, ocenjuje funkciju gena kandidata kroz visokoprotočne funkcionalne skrinove mutagenih populacija i stvara molekularne markere za oplemenjivače. GL-TTP je otvorena za aktivnu razmenu rezultata sa zajednicama krmnih i ostalih mahunarki na obostranu korist.

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RESULTS IN ALFALFA BREEDING AT NARDI FUNDULEA

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Abstract: This paper presents the results which were obtained in alfalfa breeding programme in Romania, at NARDI Fundulea during last 10 years. Alfalfa is the most important forage crop in Romania. The main objective in alfalfa breeding is to improve dry matter yield, as well as biotic and abiotic stress tolerance. Among 2000-2009, in Romania 10 new cultivars were developed. They represent a progress of the breeding research and they are result of selection for high fodder and seed yield, quality and adaptability to different environmental conditions. These cultivars are characterized by a rapid growth in the spring, a faster re-growth after cutting, a good resistance to more common diseases spread in Romania and a very good level of winter hardiness. They are synthetic cultivars obtained by recombination of foreign and Romanian germplasm. The objective of this work was the evaluation of forage yield and quality of new synthetic cultivars selected at NARDI Fundulea.

Key words: alfalfa, synthetic cultivars, forage, seed, yield, fodder quality.

Introduction

Introduction and spread of suitable alfalfa cultivars are very important, because alfalfa is a perennial plant, and has been known that, it live for 5 years or more. However, most important traits of alfalfa cultivars is high productivity, quality and resistance to biotic and abiotic stresses, especially winter hardiness for Romanian weather conditions. Alfalfa breeders have also been aware of the importance of forage quality on improving animal performance. The term forage quality is a board term referring to a number of factors that affect nutritive value of the forage. Among these factors, dry matter digestibility is considered to be the most important one *(Posselt, 1994).*

The improvement of the nutritive value of forage in the last period became the main objective in alfalfa breeding programs in Romania *(Varga et al., 1998; Schitea et al., 2006).* Forage quality can be improved by increasing the leaf/stem ratio, which could be achieved by selecting genotypes with short internodes. New cultivars should also combine high fodder yield and quality with good seed yields *(Bolanos-Aguilar et al., 2002; Julier et al., 2000).*

Alfalfa is the most important forage crop in Romania. During last two years, from a total of 1.2 millions ha cultivated with forage crops, the area covered by alfalfa was about 450.000 ha, which is 4.8% of arable land of Romania. The main objective in forage crops breeding is to improve dry matter yield, quality as well as biotic and abiotic stress tolerance *(Varga et al., 1998; Rotili, 2002; Schitea, 2002; Schitea, 2008).* The objective of this work was the evaluation of forage yield and quality of new synthetic cultivars selected at NARDI Fundulea.

Materials and Methods

The new Romanian alfalfa cultivars are synthetic ones obtained by poly-cross method and derived from hybrids between Romanian germplasm and different foreign sources (Table 1). They are constituted from different number of progenies, from 5 components (Daniela, Aurora cultivars) to 22-23 components (Dorinela and Madalina cultivars). One of the most important aims of Romanian breeding programme in alfalfa was to utilized different sources of germoplasm (Table 1) in order to avoid genetic vulnerability, but in the same time, that new cultivars have to characterize with a good phenotypic uniformity in concordance with UPOV requirements. The trials were carried out during 1999–2009, in 3-6 locations of NARDI Fundulea network (NARDI Fundulea, ARDS Simnic and ARDS Caracal under both irrigated conditions and dry land) and ARDS Teleorman, ARDS Lovrin and ARDS Podu Iloaiei under dry land conditions.

Table 1. The origin of germplasm sources used in alfalfa breeding program, during 2000-2009

No.	Cultivar	Genealogy no.	No. components	Year of registration	Origin of germplasm sources (%)
1	Magnat	F 8003-88	6	1996	Romania (50%), USA (50%)
2	Daniela	Syn-85	5	2000	China (100%)
3	Alina	F 65-94	5	2001	Romania (100%)
4	Dorinela	F522-93	22	2002	Romania (70%), USA (30%)
5	Madalina	F 523-93	23	2002	Romania (100%)
6	Sandra	F 660-94	10	2003	Romania (50%), France (50%)
7	Cosmina	F 815-96	15	2004	USA (100%)
8	Adin	F 912-97	12	2006	Romania (58%), Germany
9	Catinca	F 1008-98	8	2006	Romania (50%), France (20%),
10	Mihaela	F 1111-99	11	2009	Romania (100%)
11	Roxana	F1320T-01	10	2009	Romania (100%), USA (40%),

The experimental design was a randomized complete blocks with 4 replications, with 10 m^2 per plots. The evaluated traits were: dry matter yield (DMY, t ha^{-1}) per year, regrowth at 14^{th} day (RGW, cm), plant height at cut (PLH, cm), leaf:stem ratio (LSR %), vigor (cm at 2 weeks after equinox) and winter hardiness (notes: 1- very resistant, 9-very sensitive). The cultivars were harvested at the early flowering stage.

Chemical analysis was performed only on the first cut, every year. A random sample of forage for quality analysis was taken from each plot, dried in a forced air oven at 60^0C for 48 h and then grounded in a Cyclotec mill to pass a 1-mm screen. Crude protein (CP %) was obtained multiplying the N present obtained by micro-Kjeldahl analysis by 6.25, Neutral detergent fiber (NDF, %) acid detergent fiber (ADF, %) were determined by methods of *Goring and Van Soest (1970),* digestible organic matter (DOM, g/kg) and net energy (NE, kcal.) were determined by methods of *Terry and Tilley (1964)*, meat fodder units (MFU) were determined by the methods of *Georgescu (1995).* Resistance to fusarium wilt has been appreciated under natural conditions in monoculture of alfalfa after six years cultivation.

Results and Discussion

In Official Romanian Catalogue of cultivars, during last ten years, ten new alfalfa cultivars are registered, excepting Magnat, which was registered in 1996 (Table 1). Despite of this, Magnat cultivar is still “on market” due to its very good behaviour, being during last years, the most spread alfalfa cultivars in Romania.

The genetic progress achieved in the alfalfa breeding programme of NARDI Fundulea is indicated by a gain of 5.2% of the dry matter yield (average for three years) of the new cultivars compared with Magnat (control). The dry matter yield ranged for the first vegetation year from 13.7 t ha^{-1} (cultivar Magnat) to 15.5 t ha^{-1} DMY for cultivars Madalina followed by Dorinela (15.1 t ha^{-1}). Cultivar Madalina maintained the first place in the second year of vegetation with 14.7 t ha^{-1} DMY (a gain 12.5% above control) (Table 2). The favourable precipitation from June (103 mm) and July (119.5 mm) 2009 contributed to an increased dry matter yield for all cultivars. In the third year, the highest dry matter yield obtained by Madalina, Cosmina and Roxana (20.9-21 t ha^{-1}).

For the all three years of vegetation, Madalina was the best cultivar with 17.1 t ha^{-1} (11% above control). Good results were given by Adin, Catinca and Roxana with 16.3-16.4 t ha^{-1} (6% above control) followed Dorinela, Sandra and Cosmina with 16.2 t ha^{-1} (5% above control).

Table 2. Fodder yield of the Romanian alfalfa cultivars, average of 3 years during 2007-2009, NARDI Fundulea

No	CULTIVAR	2007		2008		2009		Average	
		Dry matter		Dry matter		Dry matter		Dry matter	
		t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
1	Madalina	15.5	113.0	14.7	112.5	21.0	108.2	17.1	111.0
2	Adin	14.8	107.8	13.8	105.6	20.6	106.2	16.4	106.5
3	Catinca	14.7	107.1	14.1	1079	20.1	103.6	16.3	105.8
4	Roxana	14.3	103.9	13.7	105.0	20.9	107.7	16.3	105.8
5	Dorinela	15.1	110.1	13.9	106.4	19.7	101.5	16.2	105.2
6	Sandra	14.5	105.6	13.9	106.4	20.2	104.1	16.2	105.2
7	Cosmina	13.8	100.5	13.6	103.7	21.0	108.2	16.1	104.5
8	Alina	14.2	103.1	13.1	100.5	206	106.2	16.0	103.9
9	Magnat	13.7	100.0	13.1	100.0	19.4	100.0	15.4	100.0
10	Daniela	13.9	101.4	12.7	96.9	19.2	99.0	15.3	99.4
11	Selena (check 1)	13.5	98.5	12.7	96.9	18.9	97.4	15.0	97.4
	Average	14.4	105.2	13.7	104.5	20.3	104.5	16.1	104.5
	LDS 5%	0.6	4.4	0.5	3.8	1.0	5.2	0.7	4.5

The cultivar Cosmina outyielded the Selena with 7.3% at NARDI Fundulea and 7.1% under all ecological network conditions (Tables 3-4). The cultivars Adin and Catinca registered in 2006, were superior to Selena as well as to cultivars Sandra and Cosmina. Under NARDI Fundulea conditions, as well as across six locations, they outyielded the control of 10.3% (Table 4).

Table 3 Fodder yield of the Romanian alfalfa cultivars, average of 3 years (2007-2009) at NARDI Fundulea

No.	CULTIVAR	Dry matter			Meat Fodder Units			
		(t ha^{-1})	%	%	MFU	MFU$^{*/ha}$	%	%
1	MADALINA	17.1	114.0	111.0	0.93	15903	117.8	114.7
2	ROXANA	16.3	108.7	1058	0.95	15485	114.7	111.7
3	COSMINA	16.1	107.3	104.5	0.96	15456	114.5	111.5
4	ADIN	16.4	109.3	106.5	0.94	15416	114.2	111.2
5	CATINCA	16.3	108.7	105.8	0.94	15322	113.5	110.5
6	DORINELA	16.2	108.0	105.2	0.94	15228	112.8	109.9
7	ALINA	16.0	106.7	103.9	0.94	15040	111.4	108.5
8	SANDRA	16.2	108.0	105.2	0.92	14904	110.4	107.5
9	DANIELA	15.3	102.0	99.4	0.92	14076	104.3	101.6
10	MAGNAT (Check 2)	15.4	102.7	100.0	0.90	13860	102.7	100.0
11	SELENA (Check 1)	15.0	100.0	97.4	0.90	13500	100.0	97.4
	AVERAGE	16.1	107.3	10.5	0.93	15069	111.6	108.7
	LDS 5%	0.7	4.7	4.5		942	7.0	6.8

The new alfalfa cultivars have a very good quality, the digestibility coefficient, on three years average, was 73% for Mihaela and Cosmina, 72% for Madalina, Roxana, Catinca, Adin, Alina, Dorinela, Sandra, 71% for cultivars Daniela and Selena and 70% for Magnat, respectively, with an increasing of 1-3%. Superior values registered the new cultivars Adin, Carina and Sandra for other quality indicators, such as net energy value of 1424-1495 kcal, vs. 1406 at cultivar Magnat and 1401 at cultivar Selena. Regarding the meat fodder units, it ranged between 0.96 at Cosmina and Mihaela cultivars and 0.95 at Roxana, as compared to the control, 0.90 (Table 5). The differences registered in NDF, ADF and CP between the new cultivars and the check contributed to the fodder value of them.

Table 4. Fodder yield of the Romanian alfalfa cultivars, average of 3 years, during 1999-2009, average of 3-6 experimental stations

No	Cultivar	Dry matter				Meat Fodder Units*		
		t ha^{-1}	%	%	MFU	MFU per	%	%
1	Adin, 2003-2005	15,6	110.3	106.1	0.94	14619	115.2	110.6
2	Catinca, 2003-2005	15,6	110.3	106.1	0.94	14619	115.2	110.6
3	Cosmina, 2002-2004	15,1	107.1	102.9	0.96	14497	114.2	109.6
4	Mihaela(2004-2006)	15,3	108.8	104.6	0.94	14420	113.6	109.1
5	Roxana (2007-2009)	15,0	106.3	102.1	0.95	14239	112.2	107.7
6	Madalina (2007-2009)	15,3	108.3	104.1	0.93	14201	111.9	107.4
7	Sandra, 2007-2009)	15,2	107,5	103.3	0.92	13945	109.9	105.5
8	Dorinela(1999-2001)	14,8	105.3	101,1	0.94	13956	110.0	1055
9	Alina (2006-2008)	14,7	104.2	100.0	0.94	13811	108.8	104.4
10	Daniela (2004-2007)	14,7	104.4	100.2	0.92	13543	106.7	102.4
11	Magnat (check 2)	14.7	104.2	100.0	0.90	13223	104.2	100.0
12	Selena (check 1)	14.1	100.0	96.0	0.90	12690	100.0	96.0
	AVERAGE	15.0			0.93	13980	110.1	105.7
	LSD 5%		6.3	6.0		797	6.3	6.0

Concerning of this, the data presented in the table 5 colligated with the data presented in tables 3-4, all cultivars, except Daniela, show average 10% (7.5-17.8%) progress realized over the control by the new cultivars as regards the quantity of meat fodder units ha^{-1}, at both Fundulea and experimentation network. On the average of three years and six locations, the cultivar Sandra yielded 13956 MFU ha^{-1}, (gain of 8.3 % vs. check), Cosmina 14495 MFU ha^{-1} and Adin and Catinca 14619 MFU/ha. Adin and Catinca are followed in the top by Cosmina, Mihaela and Roxana with 14239-14497 MFU, 12.2-14.2 gain vs. Selena and 7.7-9.6 gain vs. Magnat.

As regards as the recent testing into network (2007-2009, Table 6), in the South of Romania (Fundulea, Simnic, Caracal), presents the yields achieved by cultivars introduced already into production (Magnat and Sandra) or during introduction (Madalina). The yields ranged between 11.4 t ha^{-1}, in the first year of vegetation (ARDS Caracal) and 23.0 t ha^{-1}, in the second year of vegetation (ARDS Simnic), Sandra cultivar. On three years and three stations average, the cultivars Madalina and Sandra achieved 17.2 – 17.3 t ha^{-1} DM, vs. 16.6 t ha^{-1} Magnat one, and 15824-16089 MFU.

It is known that, between fodder yield and quality and seed yield there is a negative correlation. The development of cultivars with good yield, good quality and good seed yielding depending of the ability of the breeders to select genotypes where this correlation is not too strong. Concerning this, the data presented in the figure 1 show a good seed yielding for the Romanian alfalfa cultivars, especially Mihaela, and Roxana. These cultivars outyielded the check Selena with 9-10% and the check Magnat with 6-7%. The seed yielding ability of the new cultivars cumulate with intensive technology to grow will permit introduction and extension of them in the farms *(Moga and Schitea, 2005)*.

Table 5. Feed quality of new alfalfa cultivar as compared to the official check, average 3 years (2007-2009)

No.	Cultivar	DOM (%)	NE (Kcal)	FU (oat)	MFU	NDF (%)	ADF (%)	CP (%)
1	MIHAELA	73	1484	1.05	0.96	-	-	20.90
2	COSMINA	73	1495	1.06	0.96	34.04	27.26	19.84
3	MADALINA	72	1453	1.02	0.93	31.12	23.68	20.58
4	ROXANA	72	1468	1.04	0.95	35.06	27.82	19.68
5	CATINCA	72	1462	1.03	0.94	32.07	24.98	19.69
6	ADIN	72	1457	1.03	0.94	34.57	26.21	19.45
7	ALINA	72	1457	1.03	0.94	27.00	22.25	19.26
8	DORINELA	72	1457	1.03	0.94	31.36	2309	19.40
9	SANDRA	72	1444	1.01	0.93	31.55	23.61	19.75
10	DANIELA	71	1424	1.01	0.92	22.79	22.91	19.50
11	MAGNAT	70	1406	0.99	0.90	35.48	26.25	18.74
12	SELENA	71	1401	0.99	0.90	32.45	28.75	19.36
	Average	72	1451	1.02	0.93	31.59	25.16	19.68

Table 6. Fodder yield (t ha^{-1}) of the cultivars Madalina, Sandra and Magnat (control)

CULTIVAR	ARDS CARACAL			ARDS SIMNIC			NARDI FUNDULEA			*Average*		Meat Fodder Units	
	2007	2008	2009	2007	2008	2009	2007	2008	2009	t ha^{-}	%	no.ha^{-1}	%
MÃDÃLINA	11,7	19,4	14,7	12,8	22,7	19,6	15,5	18,5	20,8	*17,3*	*104,1*	16089	107,7
SANDRA	11,4	18,9	15,1	12,9	23,0	19,2	15,5	18,5	19,9	*17,2*	*103,3*	15824	105,9
MAGNAT	11,5	18,9	14,4	12,8	22,2	18,6	13,7	17,7	19,7	*16,6*	*100*	14940	100,0
LSD 5%	0.6	0.9	0.8	0.5	0.9	0.9	0.6	0.5	1.0	*0.7*	*4.2*	117	7.2

In alfalfa, forage quality has two main components, digestibility and protein content. Both of them are positively correlated to the proportion of leaves in the forage.

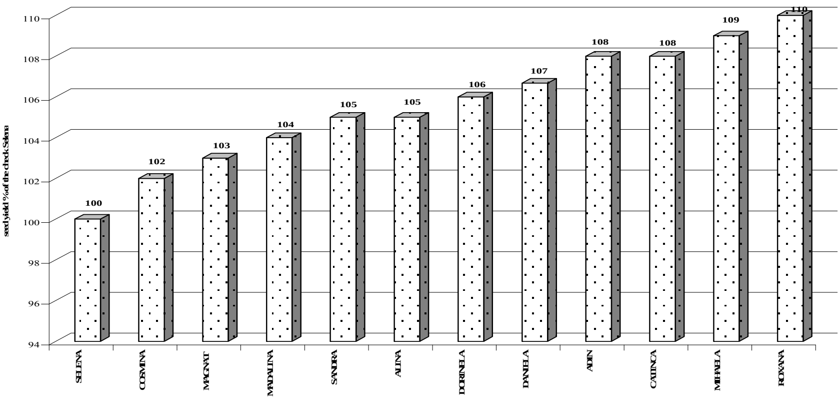

Figure 1. Seed yield of the alfalfa cultivars, average of 3 years, during 1999-2009, Average of 3-6 experimental stations

Morphological traits, such as leaf:stem ratio and number of internodes are related to fodder quality (Table 7). Leaf:stem ratio was 36% for Cosmina, 38% for Sandra and Carina and was highest for Adin (39%), with 1-4% more then the standard Selena. Similar differences were noticed regarding the number of internodes, but not for plant height. The new cultivars proved to be superior to the check Selena also for vigor, regrowth after cutting, and disease resistance, especially to *Fusarium* (table 7).

Table 7. **Some characteristics of Romanian alfalfa cultivars**

Cultivar	Leaf:stem ratio (%)	Number of intern.	Plant height (cm)	Vigor	Regrow after cutting	Winter hardiness	Fall dormancy	Resistance to Fusarium oxysporum
Mihaela	37	10.3	65.0	2.1	1.8	2.0	4	2.0
Cosmina	39	10.0	63.0	2.5	1.9	2.0	4	2.0
Madalina	38	11.1	64.0	2.0	1.7	2.0	4	2.0
Roxana	38	11.0	64.0	2.2	1.8	2.0	4	2.0
Catinca	38	10.3	60.0	2.2	1.7	1.8	4	2.4
Adin	39	10.5	62.3	2.3	1.8	1.9	4	2.2
Alina	36	10.2	60.0	2.2	2.5	2.0	4	3.0
Dorinela	36	10.5	62.0	2.4	2.2	2.5	4.5	2.2
Sandra	38	11.0	64.0	2.2	2.0	2.0	4	2.5
Daniela	36	10.5	63.0	2.0	2,8	2.5	5	3.0
Magnat	36	10.0	62.0	2.5	2.2	2.0	4	3.0
Selena	36	10.1	61.8	2.9	3.0	2.0	4	3.0
Average	37	10.5	62.6	2.3	2.1	2.2	4.1	2.4

For breeding of alfalfa it is important to obtain cultivars with a good distribution of yield between cuttings. Higher yield values for the first and last cut are expected for cultivars with early spring growth and late growth in autumn (long period of vegetation), cultivars with high level of fall dormancy, than for cultivars with a shorter vegetation period. But, the cultivars with long vegetation period are most susceptible to winter damages and this makes them less suitable for Romanian conditions. However, Daniela and Sandra cultivars achieved 37.1-38% of the annual yield from the first cut (Figure 2).

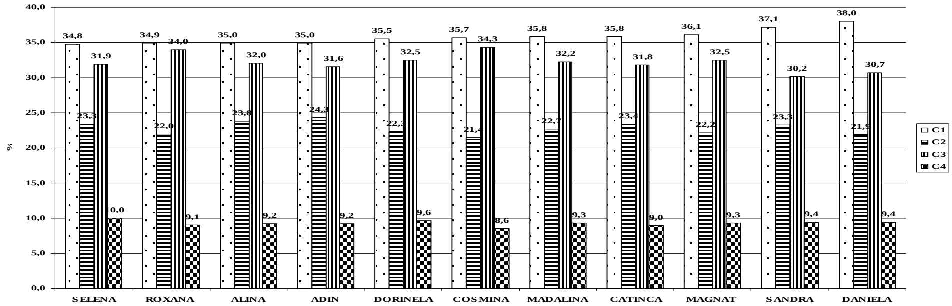

Figure 2. Distribution of yield/cuttings, cultivars tested at NARDI Fundulea

It is obviously the decrease of dry matter yield at the second cut. The reason of this decrease was the water stress registered during the second yield formation. Resistance to drought means the ability of the cultivars to pass easier under the water stress and the good capacity for re-growth after stress, (*Schitea, 2002; Petcu et al. 2009),* better in the case of the cultivars Cosmina and Roxana, at which the third cutting represented 34.0 - 34.3% of the total yield, vs. Daniela and Sandra, 30,2-30,7%, (Figure 2).

Conclusion

The new Romanian alfalfa cultivars represent a genetic progress achieved in the alfalfa breeding program of NARDI Fundulea.The cultivars Madalina, Cosmina, Adin, Roxana, Madalina and Catinca achieved good fodder yield and nutritive value compared with the checks Selena and Magnat. The cultivars Mihaela and Roxana gave high seed yields. All new cultivars have demonstrated a good stability of yield due by the high level of resistance to diseases and winter hardiness.

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Rezultati oplemenjivanja lucerke u NARDI Fundulea

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Rezime

Rad predstavlja rezultate dobijene u programu oplemenjivanja lucerke u NARDI Fundulea u Rumuniji. Glavni cilj oplemenjivanja lucerke u Rumuniji je poboljšanje prinosa biomase, kao i otpornost na biotičke i abiotičke stresne uslove u toku vegetacije. U periodu od 2000-2009. u Institutuje selekcionisano 10 novih sorti. Sorte su rezultat selekcije na visok prinos krme i semena, kvalitet i adaptabilnost na različite uslove sredine i predstavljaju značajan napredak u oblasti oplemenjivanja lucerke u Rumuniji. Sorte se karakterišu brzim rastom u proleće, odličnom regeneracijim posle otkosa i dobrom otpornošću na najraširenije bolesti lucerke u Rumuniji. To su sintetičke sorte dobijene rekombinacijom strane i Rumunske germplazme. U radu su prikazane važnije osobine, kao i rezultati za prinos krme i semena, i kvaliteta novih sintetičkih sorti NARDI instituta u Rumuniji.

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GENETIC ANALYSIS OF TILLERING IN A TWO-ROW X MULTIPLE-ROW CROSS OF BARLEY

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Original scientific paper

Abstract: A study was conducted on a diallel cross involving five divergent genotypes of multiple- and two-row winter barley to estimate the mode of inheritance, gene action and general and specific combining abilities of parents for productive tillering in the F1 and F2 generations. The modes of inheritance of tillering in the F1 generation were partial dominance, dominance and over dominance, depending on the cross combination, whereas partial dominance was observed in most combinations in the F2 generation. An analysis of the combining abilities showed that productive tillering was induced by additive and non-additive gene action. The GCA/SCA ratio in both F1 and F2 indicated that the inheritance of this trait was predominantly governed by the additive gene action. The cultivar Jagodinac, had the highest mean for productive tillering and was identified as the best general combiner, which suggests that it can be used as a parent in multiple-row barley breeding programmes.

Key words: tillering, barley, inheritance, combining ability

Introduction

Tillering as a process of underground stem branching and plant shoot production is a major grain yield component. Shoot number is formed at early stages of ontogenesis and it has a large effect on crop uniformity and density, and lodging resistance *(Borojević, 1972; Kraljević-Balalić and Petrović, 1991)*. The trait is substantially affected not only by genetic but also by environmental factors and mineral nutrition, particularly nitrogen supply *(Pavlović, 1997; Paunović, 2002; Paunović et al,. 2006).* Apart from mineral nutrition, tillering rate is also largely related to the amount of growing space (crop density). Hence, at equal nitrogen supply, low-density crops will show a higher rate of tiller emergence. An increase in spike number or productive tillers above a certain level induces a decrease in grain number per spike and vice versa, suggesting that the highest grain yields can be obtained at high values of a single yield component or at balanced values of all components *(Madić et al. 2005)*. Increased tillering has a favourable effect on yield up to a certain point (530 spikes per m^2), beyond which it has adverse or no effect *(Đokić, 1988)*. The number of productive tillers is also dependent upon crop water supply. Therefore, tiller dynamics under water stress can be used as a selection criterion for breeding for drought tolerance *(Mosaad et al. 1995)*.

The objective of this study was to use a diallel analysis to estimate the mode of inheritance, gene action and combining abilities of parents selected for productive tillering. This would help bring greater certainty to breeding for tillering in generations following hybridization.

Materials and Methods

The multiple-row barley genotypes KG-9/4 and Partisan and two-row genotypes NS-293, Jagodinac and Sladoran were studied for mode of inheritance, gene action and combining abilities for productive tillering.

Diallel crosses, without reciprocals, were conducted in 2001/2002 and a set of 6 hybrid combinations of the F1 and F2 generations were produced. The hybrid material and the parents were planted in the same year in a randomized block design with three replications at the experimental field of the Small Grains Research Centre, Kragujevac. The planting was done in 1-m-long rows at an inter-row and intra-row spacing of 20 cm and 10 cm, respectively (sparse planting density), providing identical growing conditions for all generation plants. Two random samples of 30 plants (3x10) for parents and F1, and 150 (3x50) plants for F2 were taken at full maturity for crop analysis.

Mode of inheritance was determined by a significance test of generation means relative to the parental average *(Kraljević-Balalić et al. 1991)*. The analysis of variance for combining abilities was performed using *Griffing's (1956)* method for incomplete diallel crosses.

Results and Discussion

Mean values and inheritance of productive tillering – The parental cultivars used in the crossing programme showed significant differences in productive tillering. Tiller number was lowest in cv. Sladoran (7.3) and highest in cv. Jagodinac (11.1), as given in Table 1.

The number of tillers in the F1 generation ranged from 7.5 in the KG-1/9 x Sladoran combination to 11.3 in the Partisan x Jagodinac combination. The mean values for tiller number in the F2 generation were somewhat lower in most combinations than those in the F1 generation and varied from 6.8 to 9.6 in the Partisan x Sladoran and Partisan x Jagodinac combination, respectively.

Different modes of inheritance of productive tillering in the F1 generation were observed: intermediate inheritance was found in the NS-293 x Sladoran combination, and over dominance in the Partisan x NS-293 combination, whereas the predominant mode of inheritance in the other combinations was partial dominance or dominance (Table 1).

Table 1. Mean values of parents and F1 and F2 generations for productive tillering

Parents and their hybrids	F_1	F_2
KG-9/4	8.5	
KG-9/4x PARTIZAN	8.7	8.8
KG-9/4x JAGODINAC	9.9 ***pd***	6.9 ***-d***
KG-9/4x NS-293	8.3	9.5 ***pd***
KG-9/4x SLADORAN	7.5 ***d***	8.4 ***d***
PARTIZAN	9.1	
PARTIZAN x JAGODINAC	11.3 ***d***	9. ***pd***
PARTIZAN x NS-293	11.1 ***od***	8.8 ***-d***
PARTIZAN x SLADORAN	8.9 ***d***	6.8 ***-d***
JAGODINAC	11.1	
JAGODINAC x NS-293	10.2 ***pd***	9.1 ***pd***
JAGODINAC x SLADORAN	8.3 ***pd***	7.5 ***d***
NS-293	9.6	
NS-293 x SLADORAN	8.4 ***i***	8.1 ***i***
SLADORAN	7.3	
LSD 0.05	1.36	1.02
0.01	1.84	1.38

Pd – partial dominance, ***d*** - dominance, ***i*** – intermediate inheritance and ***od*** – over dominance

The mode of inheritance of productive tillering in the F2 generation was partial dominance or dominance of parents with lower tiller number in most combinations. Partial dominance was also exhibited by a cross combination where the parents showed substantial differences in the trait concerned (Jagodinac x Sladoran). *Pržulj et al. (1999)* also reported the overdominance, dominance or intermediate inheritance, using the additive-dominant model when analyzing productive tillering in the F1 and F2 generations.

Analysis of variance for combining abilities for productive tillering – An analysis of variance for combining abilities in the F1 generation revealed significant differences for general (GCA) and specific combining abilities (SCA), with the GCA variance, however, being considerably higher. This suggests that productive tillering in F1 generation is mostly governed by additive gene action and to a much lesser extent by non-additive gene action (dominance and epistasis).

Table 2. ANOVA for combining abilities for productive tillering in barley

Source of variation	Generation	DF	MS	F_e	F_t	
					0.01	0.05
GCA	F_1	4	4.56	20.64**	4.08	2.72
SCA		10	0.86	3.88**	3.04	2.19
E		28	0.22			
GCA/SCA				5.31		
GCA	F_2	4	2.88	23.23**		
SCA		10	0.20	1.62		
E		28	0.12			
GCA/SCA				14.33		

Significant differences in the F2 generation were observed only for GCA, which indicates that productive tillering was induced by additive gene action. The GCA/SCA value was very high, confirming the additive gene action (Table 2).

The value of GCA in the F1 and F2 generations was much higher in cv. Jagodinac than in the other genotypes studied. Hence, the cultivar is a good combiner for productive tillering. Low values of GCA were recorded for cv. Sladoran and KG9/4 line (Table 3).

A significantly high value of SCA in the F1 generation was found in the Partisan x Jagodinac cross as compared to the other combinations, whereas the KG-9/4 x NS-293 cross had a significant negative value. A positive but non-significant value for SCA in the F2 generation was observed in the crosses between a good general combiner and a bad general combiner (Table 4).

Table 3. GCA values for productive tillering

Parents	F1	Rank	SE	F2	Rank	SE
KG-9/4	-0.600	4		-0.360	4	
PARTIZAN	0.451	2		-0.005	2	
JAGODINAC	1.027**	1	0.159	1.093**	1	0.243
NS-293	0.111	3		-0.195	3	
SLADORAN	-0.988**	5		-0.533**	5	

F1	LSD	0.05	0.386	F2	LSD	0.05	0.862
		0.01	0.520			0.01	1.163

Highly significant GCA and SCA variances were reported by *Kraljević-Balalić and Petrović (1991), Madić and Đurović (1996)* and *Madić et al. (2006)*, who obtained higher GCA values, which suggested a higher contribution of additive gene action in the inheritance of productive tillering. Conversely, in their study on combining abilities for productive tillering, *Zečević et al. (1995)* underlined the higher contribution of non-additive gene action, with the GCA/SCA ratio being lower than unity.

Table 4. SCA values for productive tillering

Cross combination	F1	SE	F2	SE
KG-9/4x PARTIZAN	-0.980		0.003	
KG-9/4x JAGODINAC	0.183		-0.906	
KG-9/4x NS-293	-1.576**		-0.378	
KG-9/4x SLADORAN	0.539		0.098	
PARTIZAN x JAGODINAC	1.342*	0.342	-0.361	0.243
PARTIZAN x NS-293	0.783		-0.216	
PARTIZAN x SLADORAN	0.222		0.210	
JAGODINAC x NS-293	-0.187		0.241	
JAGODINAC x SLADORAN	0.112		0.113	
NS-293 x SLADORAN	-0.862		-0.099	

F1	LSD	0.05	1.151	F2	LSD	0.05	0.862
		0.01	1.552			0.01	1.163

Conclusion

The mode of inheritance of productive tillering of barley in the F1 generation was partial dominance, dominance or over dominance, depending on the cross combination, whereas partial dominance was observed in most combinations in the F2 generation. An analysis of variance for combining abilities in the F1 generation revealed significant differences among the parents for both general and specific combining abilities. Productive tillering in the F1 generation is mostly induced by additive gene action and to a much lesser extent by non-additive gene action (dominance and epistasis).

Substantial differences in the F2 generation were observed only for general combining abilities, indicating that productive tillering is induced by additive gene action.

The GCA/SCA value (14.33) was very high, which confirmed the dditive gene action. The Jagodinac cultivar was the best general combiner. The Partisan x Jagodinac and Jagodinac x Sladoran combinations are recommended to be used in further breeding activities.

Genetička analiza bokorenja u ukrštanju dvoredog i višerednog ječma

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Rezime

Na osnovu rezultata dialelnog ukrštanja pet divergentnih genotipova ozimog višerednog i dvoredog ječma ocenjen je način nasleđivanja, efekat gena i opšte i posebne kombinacione sposobnosti roditelja za produktivno bokorenje u F1 i F2 generaciji. Način nasleđivanja bokorenja u F1 generaciji je parcijalna dominacija, dominacija i superdominacija zavisno od kombinacije ukrštanja, dok je u F2 generaciji u većini kombinacija ispoljena parcijalna dominacija. Analiza kombinacionih sposobnosti je pokazala da je produktivno bokorenje uslovljeno genima sa aditivnim i neaditivnim delovanjem. Odnos OKS/PKS u F1 i F2 generaciji ukazuje da u nasleđivanju ove osobine preovladavaju geni sa aditivnim efektom. Sorta Jagodinac je imala najveću srednju vrednost za produktivno bokorenje i pokazala se kao najbolji opšti kombinator, tako da se može koristiti kao roditelj u programima oplemenjivanja višeredog ječma.

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EVALUATION OF LOCAL AND INTRODUCED PEA CULTIVARS

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Abstract: A Study of 19 pea cultivars according to the following morphological characters was carried out: plant height (cm), first pod height (cm), internode length (cm), unproductive nodes number, total node number per plant, pod number per plant, length and width of pod (cm), weight of grain green from plant (g), plump grains per plant (%) and average grain number in pod. The study was performed during the period 2003-2005 on the experimental field at the Maritsa Vegetable Crops research Institute, Plovdiv. Pea cultivars included in this test are suitable for mechanical harvesting with plant height from 50.2 cm to 86.0 cm, first pod setting above 27.7 cm and 14.9 pods per plant. The average pod length and width is 7.9 cm and 1.3 cm, respectively. The cultivar Senator possess the best biological potential, expressed by high values of the main yield components: dimension of green pod (11.3/1.6 cm), green grain weight per plant (47.4 g) and average grain number in pod (8).

Key words: *P. sativum* L., morphological traits, yield, crude protein

Introduction

A great diversity of plant material from pea is introduced and kept in the national collection *(Angelova and Guteva, 2001; Koeva et al., 1994)*. The permanent extension of the existing collection as well as accumulation, systematization and supplementing of the obtained information establishes the conditions for increasing the efficiency of plant breeding and other improvement activities *(Kalapchieva, 2005; Angelova and Guteva, 1995)*.

The source of genetic material is enriched annually with local and foreign pea accessions with diverse origin and different levels of selection, which allows researchers to create high quality and productive cultivars adapted to environmental conditions *(Angelova et al., 2001; Kalapchieva, 2005; Coyne et al., 2005)*.

The purpose of this study was to investigate the morphological and economic characteristics of local and foreign accessions for their successful including in breeding programs for new genetic pea plasma for food and agriculture.

Materials and Methods

During the period 2003-2005 in the “Maritsa” Vegetable Crops Research Institute (MVCRI), Plovdiv, Bulgaria 19 pea cultivars were tested: Alex, Baccara, Ballet, Casino, Countess and Ramir, from working collection of Institute of Plant Genetic Reassures (IRGP), Sadovo, Bulgaria; Kosmos, Atlas, Trikala and Sima from Germany; Senator, Piccolo Provenzale and Male Provenzalle from Italy; Onward and Merveille de Kelvedon from France and the local pea cultivars Vyara, Reyna, Vechernitsa and № G/03. The experiment was conducted according to the technology adopted for growing peas. At the technological maturity 10 plants of each cultivar were analyzed.

The cultivars of IPGR, Sadovo were with smooth seed test and afila leaf, type. They were chosen from the collection by a complex of valuable characters - productivity, earliness and resistance to lodging. The rest of the accessions had wrinkled seeds and with a normal leaf type.

The main morphological characteristics, such as plant height (cm), first pod height (cm), internodes length (cm), unproductive nodes number, total node number per plant, pod number per plant, length and width of pod (cm), weight of grain green from plant (g), plump grains per plant (%) and average grain number in pod, were investigated. The yield of dry grain per hectare was measured and the percentage of crude protein in the grains for six afila pea cultivars was determined. The obtained results are processed by the Duncan’s test and the two-factor analysis of variance (ANOVA).

Results and Discussion

The dispersion analysis showed significant differences between investigated pea cultivars ($P \leq 0{,}001$) for all studied morphological characteristics (Table 1). Differences between years were not statistically significant except for first pod height. The interaction cultivar x year demonstrated differences for all characteristics except average grain number in pod. Such influence of these factors was reported in other studies *(Angelova and Guteva, 1995; Jovicevic et al., 2002; Kalapchieva, 2005)*.

Table 1. Two-way analysis of variance on morphological characteristics of pea cultivars

Sources for variation	Degree of freedom	Plant height, cm	First pod height, cm	Internodes length, cm	Unproductive nodes number	Total nodes number per plant	Pods number per plant	Length of pod, cm	Width of pod, cm	Weight of green grain per plant, g	Plump grains per plant,%	Average grain number in pod
Cultivar	18	1030.5***	1327.7***	9.0***	79.7***	76.0***	593.6***	14.3***	0.64***	945.7***	474,2***	11.8***
Year	2	683.7***	10.1$^{n.s.}$	39.5***	6.3*	28.0***	1347.1***	2.4**	0.05***	8703.9***	1731,1***	119.8***
Cultivar x Year	36	158.2***	75.4***	4.0***	5.8***	9.5***	216.1***	0.9***	0.04***	755.5***	308,1***	4.9***.
Residual	171	26.3	28.9	0.6	1.7	2.7	21.1	0.4	0.003	73.2	112,1	0.9

The percentage of cultivar influence in the general variation was higher than 50% for plant height, first pod height, unproductive nodes number and total number of nodes per plant, length and width of pod (Table 2).

Table2. Influence of factor of variation on morphological characteristics of pea cultivars, (%)

Sources for variation	Plant height, cm	First pod height, cm	Internodes length, cm	Unproductive nodes number	Total nodes number per plant	Pods number per plant	Length of pod, cm	Width of pod, cm	Weight of green grain per plant, g	Plump grains per plant,%	Average grain number in pod
Cultivar	61.6	75.7	33.0	73.4	61.4	43.1	72.0	86.1	23.0	20.2	16.6
Year	4.5	0.1	16.1	0.7	2.5	10.8	1.3	0.7	23.5	8.2	3.0
Cultivar x Year	18.9	8.6	29.6	10.6	15.3	31.4	9.4	9.9	36.7	26.3	12.0

These morphological characteristics were probably strongly genetically predetermined and their phenotypic choice would give good results. The influence of genotype was lowest for average grain number in pod (16.6%).

Year conditions had slight influence on the variability of the characteristics - from 0.1% to 23.5% and this is important for breeding since these conditions are unpredictable. This influence on green grain weight per plant (23.5%) and internodes length (16.1%) demonstrated the greatest effect.

The interaction cultivar x year was the second factor influencing the variation of the studied characteristics. It exceeded the influence of cultivar and year in weight of green grain from plant (36.7%) and the percentage of plump grains per plant (26.3%) while in pod number per plant (31.4%) and internodes length (29.6%) it exceeded only the influence of years. The phenotype choice would be less successful for the breeding work on these characteristics.

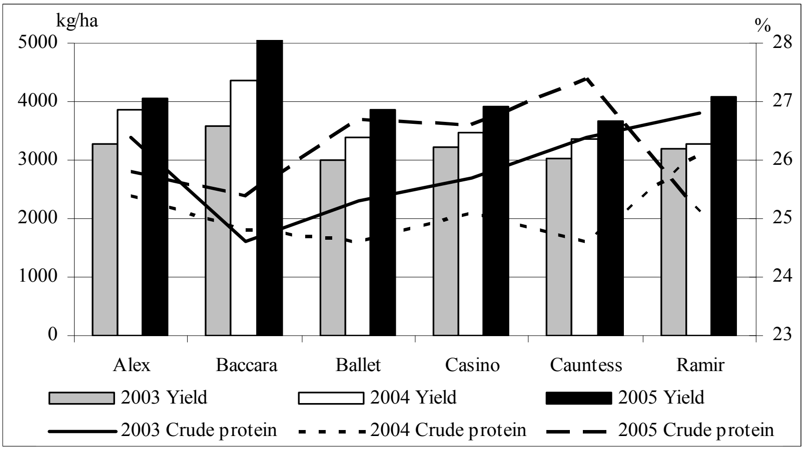

Figure 1. Average yield and crude protein in pea seeds kg ha^{-1}, 2003-2005.

Investigated pea cultivars have a mid-high, simple type stems reaching a height of 86 cm (№ G/03). The cultivars with smaller height are included in one group: Reyna, Baccara, Merveille de Kelvedon and P. provenzale (Table 3). The ranking by the character – height of first pod was the same. Reyna and P. provenzale set up the first pod at the lowest height, followed by Alex, Baccara and Trikala, divided into separate groups. The highest values of this characteristic were for the accession № G/03 and the cultivar Atlas. Height of the fertile part of plant was suitable for mechanized harvesting - over 25 cm for all accessions.

Average internode length was 4.9 cm. The cultivar Atlas is characterized by numerous and short internodes. Differences in this characteristic have not been established for the remaining studied accessions.

The number of unproductive nodes is comparatively stable characteristic and an indirect indicator for the determination of the vegetation period duration. In our investigation Reyna and P. provenzale were with small unproductive nodes number (respectively the earliest for ripening) while with the greatest number were Atlas and Sima. In the same sequence these cultivars formed the lowest and the highest total nodes number per plant.

According to Duncan's Multiple Range test, the cultivars were divided into three groups by pod number per plant: first group included the variety with the most number of pods - Trikala, the second - Merveille de Kelvedon and Vechernitsa and the third all remaining cultivars.

Table 3. Morphological characteristics of pea cultivars

Cultivars	Plant height, cm	First pod height, cm	Internodes length, cm	Unproductive nodes number	Total nodes number per plant	Pods number per plant	Length of pod, cm	Width of pod, cm	Weight of green grain per plant, g	Plump grains per plant,%	Aver. grain number in pod
Alex	58.8 gh	34.3 i	5.1 bcde	14.0 gh	22.3 fgh	15.2 def	6.8 ij	1.2 ef	25.9 cde	79.6 def	4.9 hi
Baccara	54.1 i	35.3 i	5.4 abcde	14.3 gh	21.6 gh	18.8 cd	6.8 i	1.2 ef	34.6 b	83.4 abcdef	5.6 efgh
Ballet	61.2 fg	42.3 efg	4.6 efg	16.1 def	23.0 efg	11.3 fghi	7.1 hij	1.2 ef	16.9 fgh	80.3 def	5.4 efgh
Casino	65.3 def	43.4 efg	5.5 abcd	16.3 def	23.6 def	12.5 efghi	7.2 ghij	1.2 ef	22.1 cdefg	84.0 abcdef	5.2 ghi
Cauntess	70.2 bcd	49.5 cd	5.0 cde	16.8 cde	24.8 bcd	12.1 efghi	7.3 ghij	1.3 c	20.0 efgh	66.8 g	4.5 i
Ramir	65.4 def	46.8 de	5.0 cde	15.5 efg	22.8 efgh	17.3 cde	7.1 hij	1.2 ef	29.6 bc	92.0 ab	6.0 cdef
Kosmos	72.5 b	46.6 def	4.2 fgh	17.9 c	25.5 abc	15.0 def	7.5 fghij	1.2 ef	24.6 cdef	90.8 abc	6.4 cde
Atlas	75.0 b	62.5 b	2.8 i	21.1 a	26.3 ab	7.4 i	7.4 efg	1.2 de	114.7 gh	92.1 a	6.1 cdef
Trikala	74.1 b	37.3 i	4.8 def	15.8 ef	26.4 ab	37.9 a	7.5 fgh	1.1 g	29.3 bcd	84.8 abcde	6.3 cde
Senator	74.8 b	53.0 c	5.9 ab	15.0 fgh	21.8 gh	12.5 efghi	11.3 a	1.6 b	47.4 a	88.8 abcd	8.0 a
P. provenzale	50.2 i	28.0 j	5.5 abcd	11.0 i	18.4 k	8.3 hi	8.9 c	1.3 c	29.0 bcd	82.4 abcdef	7.2 abc
M. provenzale	67.3 cde	52.2 c	4.8 def	15.3 fgh	23.1 efg	10.7 fghi	8.3 cde	1.2 de	17.2 fgh	81.8 cdef	7.8 a
Onward	62.8 efg	41.8 efg	6.1 a	14.3 gh	21.1 hi	10.5 fghi	9.6 b	1.6 b	34.1 b	83.3 abcdef	7.1 abc
Sima	71.5 bc	53.1 c	3.8 h	19.3 b	27.0 a	13.8 defg	7.6 fgh	1.1 g	13.7 h	85.3 abcde	6.1 defg
M. Kelvedon	54.9 i	40.6 gh	4.1 fgh	13.8 h	19.8 j	21.2 b	8.0 def	1.3 d	36.2 b	88.3 abcd	6.6 bcd
Vyara	71.8 bc	44.6 efh	6.0 a	14.9 fgh	24.2 cd	11.7 fghi	8.3 cde	1.2 ef	17.1 rgh	90.6 abc	6.7 bcd
Reyna	52.1 i	27.7 j	5.0 cde	9.8 i	18.1 k	8.8 ghi	7.5 fgh	1.3 c	34.2 b	75.2 f	7.5 ab
Vechernitsa	66.9 cde	50.8 cd	4.0 gh	16.8 cde	23.7 def	23.8 b	7.6 fgh	1.1 g	20.8 efgh	78.3 ef	5.9 cdef
№ G/03	86.0 a	68.3 a	5.8 abc	17.3 cd	22.1 fgh	13.3 efgh	8.5 cd	2.1 a	21.5 defgh	82.2 bcdef	5.3 fghi
Average	66.0	45.2	4.9	15.6	22.9	14.9	7.9	1.3	25.7	83.7	6.2

a,b... Duncan's Multiple Range test ($P \leq 0.05$)

The investigated pea accessions differed in morphological characteristic established pod size. The length varied from 6.8 (Baccara) to 11.3 cm (Senator), the width - from 1.1 cm (Trikala) to 2.1 cm (G/03).

The remaining three characteristics had no statistically significant differences, with the exception of Senator, which had the highest weight of green grain per plant, and Countess, with the lowest percentage of plump grains per plant.

All cultivars had white flowers. The study group had normal leaf type and stipules of medium size. The varieties Alex, Baccara, Ballet, Casino, Countess and Ramir had afila leaf type and were resistant to lodging.

The cultivar Baccara demonstrated the highest dry grain yield, from 3596 to 5061 kg ha^{-1} in the three years of study. The highest percentage of crude protein was in cultivar Ramir, in the first two years, and in Countess, in the third year.

Conclusion

The investigated accessions are of interest to breeding programs and agriculture.

Cultivar Senator is distinguished with the best biological potential, demonstrated with high values of the main yield components, making it suitable for the production of green grain.

Baccara is a variety with smooth seeds and high yields of dry grain, suitable for grain production. Breeding activities to enhance their technological qualities will contribute to its use in different directions.

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Evaluacija lokalnih i introdukovanih sorti graška

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Rezime

Proučavano je 19 sorti graška. Posmatrane karakteristike su bile: visina biljke (cm), visina do prve mahune (cm), dužina internodija (cm), broj

neproduktivnih nodusa, ukupan broj nodusa po biljci, broj mahuna po biljci, dužina i širina mahune (cm), masa zelenog semena (g), krupna zrna po biljci (%) i prosečni broj semena po mahuni. Istraživanje je vršeno u periodu 2003.-2005. godine na oglednom dobru Maritsa, Instituta za povrće, Plovdiv. Sorte graška, uključene u ovo testiranje, su pogodne za mehanizovanu žetvu. Visine biljaka su bile 50.2cm do 86.0cm, prvi plod je iznad 27,7 cm, a broj plodova po biljci je bio 14,9. Prosečna dužina mahune je 7,9 cm, a širina 1,3 cm. Sorta Senator je imala najveći biološki potencijal, izražen kroz visoke vrednosti glavnih komponenti prinosa: veličine zelene mahune (11,3/1,6 cm), mase zelenog semena po biljci (47,4 g) i prosečnog broja semena po mahuni (8).

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YIELD AND QUALITY OF ALFALFA SYNTHETIC POPULATIONS AND THEIR COMPONENTS

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Original scientific paper

Abstract: Dry matter yield and quality components have been studied in 12 experimental alfalfa populations and 2 commercial cultivars (Banat VS and NS Mediana ZMS V). Yield was determined over a three-year cultivation period (2006-2008), while quality components were determined in all five cuts of the third year (2008). The aim of this study was to determine the yield and quality of three synthetic alfalfa populations and compare them with the yield and quality of their components. Yield and quality of the synthetic alfalfa cultivars were at the average level of their components. Components should have manifest desirable traits, but they should still be genetically divergent. Synthetic should comprise a small number of components (but not less than four) characterized by high yield and quality.

Key words: quality, alfalfa, population, yield, synthetic

Introduction

Different methods of breeding (hybridization, backcrossing, selfing, mass selection, recurrent phenotypic selection and clonal selection, poly-cross, progeny test) have contributed to the development and maintenance of alfalfa varieties *(Woodfield and Brummer 2001)*. The need for cultivars resistant to different environmental conditions, diseases and pests, has led to the idea of developing synthetic cultivars.

Hayes and Garber (1919) were first to use this term for synthetic corn developed by crossing several inbred lines and selecting them in the F_1 and subsequent generations. Today, the term 'synthetic' is used for cultivars developed and multiplied by random crossing under conditions of open (random) pollination. Synthetic cultivars partially exploit the effect of heterosis, less than it is the case with hybrids, but provide a practical method for seed multiplication *(Rumbaugh et al., 1988)*. Parents of synthetic alfalfa cultivars are developed by different breeding methods, which are applied in order to fix genes for desirable traits.

Production of synthetic cultivars and their multiplication are relatively simple, but the selection of parents for the syn 0 generation is crucial, because selection of these parents determines the performance of their synthetics *(Rumbaugh et al., 1988; Stjepanović 1998)*. Best parents for synthetic cultivars are those that produce highly productive progenies when selfed and crossed, thanks to the additive and non-additive gene actions of their genes *(Busbice et al., 1974).*

Yield of dry matter is the most important target in alfalfa breeding. Different methods are applied in breeding for yield *per se*, but the results are modest, which is explained by a complex genetic structure and other biological traits of the species *(Bingham et al., 1994; Rotili et al., 1999; Guines et al., 2002).*

Dry matter quality is evaluated on the basis of digestibility, crude protein content and the content of structural carbohydrates. Little progress has been made in breeding for quality improvement *(Veronesi et al., 2006)*, because this too is a complex trait that is negatively correlated with dry matter yield *(Katić et al., 2005)*.

The objective of this study was to determine the yield and quality of three synthetic alfalfa populations and compare their yield and quality with those of their parent components.

Materials and Methods

The experiment included 12 experimental populations made in a single cycle of mass selection (Niva 122 04, 124 Zuzana 04) and after one generation of selfing (Concorde 128 04, Warotte 141 04 Europe II 130 and 04). These experimental populations were used to make a 5 syn 1 population (SINES). By crossing inbred lines derived from American varieties Vernal, Saranac and Iroqoui, we obtained experimental populations 12 x 18 90 04; 12 x 9 94 04; 10 x 10 x 4 108 04; 19 x 12 96 04 which were used to make a 4 syn 1 population (SINUSA). By combining all these components we made a 9 syn 1 population (SINEA). Two commercial alfalfa varieties developed at Institute of Field and Vegetable Crops, NS Mediana ZMS V and Banat VS, were used as controls.

A field experiment was established in the spring of 2006, on slightly calcareous soil, at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops. The experiment was set up in a randomized block design in five replications. The experimental unit size was 5 m^2, the units spaced 40 cm apart and the blocks spaced 100 cm apart.

Three cuts were made in 2006, five in 2007 and 2008. The yield of green forage was determined by on-site measuring the weight of harvested aboveground plant parts and converting the obtained values into t ha^{-1}. After that, 0.5-kg samples were taken for determination of dry matter content which served for calculation of dry matter yield (t ha^{-1}).

In 2008, samples were taken from all five cuts for *in vitro* analysis of digestibility of dry matter and cell wall content (NDF, ADF and lignin). Plants were cut at the height of 5 cm and the sampled area was around 0.02 m^2, so that the samples amounted to about 500 g of green forage. Plant material was dried at 60˚C for 48 hours. Digestibility of organic matter (OM) was calculated by the formula: OM = OM – non-digestible OM/OM x 100

Digestibility analysis was performed by the method of *Hvelplund et al. (1999)* in a laboratory of Institute for Food Technology in Novi Sad. Chemical analyses were carried out using standard methods.

The results were processed by the analysis of variance for two-factorial experiment where the experimental populations and cultivars were fixed effects and year was the random effect. Differences in yield, quality and digestibility were evaluated by the LSD test and variations presented as variation coefficients.

Results and Discussion

The analysis of the yields of green forage and hay produced by the synthetics and their components in the period 2006-2008 showed that the synthetic populations were close to average values of these parameters produced by their components (Table 1).

Table 1. Average green forage and dry matter yields (t ha^{-1}) of alfalfa genotypes in the period 2006-2008

Population	Green forage	Hay
12 x 18 90 04	92.0*	19.4
12 x 9 94 04	85.2	18.1
19 x 12 96 04	86.4	19.3
10 x 10 x 4 108 04	85.2	17.8
Average of USA components	87.2	18.7
SINUSA	90.0	19.9*
Niva 122 04	84.5	18.0
Zuzana 124 04	85.3	18.7
Concorde 128 04	83.5	18.3
Evropa II 130 04	83.8	19.1
Warotte 141 04	83.1	19.3
Average of EURO components	84.0	18.7
SINES	81.4	18.5
SINEA	83.2	18.6
All components average	85.4	18.7
Banat VS	77.9	17.6
NS Mediana ZMS V	83.0	18.3
Average of the controls	80.5	17.9
Average	84.6	18.6
CV%	5.4	5.3
LSD 0.05	5.8	1.3
LSD 0.01	7.7	1.7

SINUSA, composed of four components, was a better yielder than the other synthetic cultivars. However, this synthetic was not superior in yield performance in relation to the best components (12 h 18 90 04). SINES, composed of six components, was also similar in yield performance to its components and their averages. SINEA, composed of all nine components, had a similar yield to the average of these components, but the synthetic is significantly lower yielding than the two best components. In relation to the average of the standard cultivars (Banat VS and NS Mediana ZMS V) SINUSA was significantly better in both, the yields of green forage or hay. In 2008, in addition to yield of dry matter, we analyzed also the yield of digestible dry matter (Table 2).

Table 2. Yields (t ha^{-1}) of green forage, dry matter and digestible dry matter yield of alfalfa genotypes in 2008

Population	Green forage	Hay	*In vitro* digestible dry matter yield
12 x 18 90 04	112.1	19.9	13.7
12 x 9 94 04	106.5	20.2	14.1
19 x 12 96 04	109.4	22.4	15.2
10 x 10 x 4 108 04	105.9	20.7	14.7
Average of USA components	108.5	20.8	14.4
SINUSA	111.3	22.6	15.7
Niva 122 04	103.8	18.9	13.1
Zuzana 124 04	109.0	20.5	14.3
Concorde 128 04	106.4	21.8	14.8
Evropa II 130 04	109.3	22.3	14.9
Warotte 141 04	104.1	21.8	15.2
Average of EURO components	107.3	21.3	14.5
SINES	102.7	21.4	15.0
SINEA	104.2	21.8	14.9
All components average	107.8	21.1	14.4
Banat VS	99.7	20.5	14.2
NS Mediana ZMS V	110.0	21.9	15.3
Average of the controls	104.9	21.2	14.8
Average	106.7	21.2	14.7
CV%	10.9	10.9	10.9
LSD 0.05	6.5	1.3	0.9
LSD 0.01	8.6	1.7	1.2

The obtained results indicated that the yield of digestible dry matter of SIN USA was significantly higher than the average of the standard cultivars as well as the averages of its components and the other synthetics. In the case of SIN USA, the increase in yield did not reduce the quality, so that the yield of digestible dry matter increased cumulatively. SIN ES and SIN EA had increased yields of *in vitro* digestible dry matter, but they were not significantly higher than the averages of their components and the control cultivars.

NDF and ADF contents in the synthetic population SINUSA were close to the average of its components, but the lignin content was significantly higher compared with those of the components. However, *in vitro* digestible dry matter content was close to the average of the components (Table 3). The synthetic population SINES had lower NDF and lignin contents than its components, while ADF content and digestibility were higher but not statistically significant.

The synthetic population SINEA had a similar NDF content, significantly higher ADF and lignin contents and significantly lower *in vitro* digestible dry matter content than the averages of these parameters in their components (Table 3).

Our results confirm the earlier view that the inheritance of yield components and quality is controlled by additive genes *(Bingham et al., 1994; Rotili et al., 1999)*. However, the increase in digestibility contributed to a significant yield increase of in vitro digestible dry matter. Our results indicate that it is possible to increase alfalfa yield (*per se*) while maintaining alfalfa quality. Our results also confirm that a crucial point for the development of synthetics is the choice of parents for the Syn 0 generation.

Table 3. Content of structural carbohydrates and *in vitro* digestibility (%) in alfalfa genotypes in 2008

Population	NDF %	ADF %	Lignin %	*In vitro* digestibility
12 x 18 90 04	42.81	35.38	8.85	69.17
12 x 9 94 04	41.01	34.88	7.57	69.91
19 x 12 96 04	42.08	36.17	8.44	67.98
10 x 10 x 4 108 04	40.92	34.70	8.81	71.19
Average of USA components	41.71	35.28	8.42	69.56
SINUSA	41.70	35.39	8.83	70.33
Niva 122 04	42.87	36.18	8.92	69.14
Zuzana 124 04	41.00	35.78	8.69	70.08
Concorde 128 04	41.18	35.06	8.74	67.72
Evropa II 130 04	43.20	35.91	9.19	67.82
Warotte 141 04	40.59	34.05	8.41	69.67
Average of EURO components	41.77	35.40	8.79	68.89
SINES	41.03	35.62	8.37	69.42
SINEA	41.78	35.98	9.16	68.01
All components average	41.74	35.35	8.62	69.19
Banat VS	42.36	35.3	8.32	69.17
NS Mediana ZMS V	42.31	35.25	8.91	69.06
Average	42.34	35.28	8.62	69.12
Average	41.78	35.4	8.66	69.19
CV %	1.6	1.7	3.6	1.7
LSD 0.05	0.6	0.5	0.3	1.0
LSD 0.01	0.8	0.7	0.4	1.4

Synthetic alfalfa cultivars are developed in order to increase yield stability and cultivar adaptability, as well as to avoid inbreeding depression in the generations of maintenance *(Holland and Bingham 1994)*. Therefore, it is suggested for the development of synthetic cultivars to use more than 4 and up to 100 parents (components) *(Rumbaugh et al., 1988)*. Our results confirm the results of the previous research but they give priority to synthetics with fewer components. Parents to be used in synthetics should be genetically divergent, but too large a diversity can lead to outbreeding depression caused by crossing genetically distant parents *(Li and Brummer 2009)*.

It is therefore necessary to know as many characteristics of synthetics' parental components as possible, it is desirable to check their combining ability or to use progeny tests in order to select most desirable parents. Correct choice allows us to maintain yield level over generations of crossing (without occurrence of inbreeding depression), and to achieve higher yield and quality of alfalfa dry matter.

Conclusion

Regarding yield and quality, the synthetic alfalfa cultivars were at the level of the average of their components.

It is essential to know well the components intended for syn 0, because the performance of synthetics depends on the selected components. The synthetic populations composed of four components (SINUSA), had a yield higher than the average, and its quality was at the level of the average of its components. Components should have clearly superior in desirable yield and quality traits, and they should be genetically divergent.

Synthetic should be composed of not less than four components, which in their turn should have superior yield and quality.

Prinos i kvalitet sintetičkih populacija lucerke i njihovih komponenti

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Rezime

Proučavan je prinos i komponente kvaliteta suve materije lucerke kod 12 eksperimentalnih populacija i 2 komercijalne sorte (Banat VS i NS Mediana ZMS V). Prinos je određen kroz tri godine života biljaka lucerke (2006-2008), dok su

komponente kvaliteta određene u svih pet otkosa treće godine (2008). Cilj rada je bio da se odredi prinos i kvalitet tri sintetičke populacije lucerke i uporede sa prinosom i kvalitetom njihovih komponenti. Sintetičke sorte lucerke su u prinosu i kvalitetu na nivou proseka njihovih komponenti. Komponente treba da imaju ispoljene poželjne osobine, ali i da su genetički divergentne. Za sintetičke sorte treba koristiti manji broj (ne manje od četiri) komponente koje će se odlikovati prinosom i kvalitetom.

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ISOZYME POLYMORPHISM IN PROSPEROUS GENOTYPES OF PERENNIAL RYEGRASS (*Lolium perenne* L.)

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Original scientific paper

Abstract: Previously selected upon the analysis of the most important agronomic and morphological traits, the promising perennial ryegrass genotypes were analyzed by isozymes in order to determinate their genetic relationship. Analyzed enzyme systems were: phosphoglucose isomerase (PGI) and acid phosphatase (ACP) since, according to literature data, their loci are highly polymorphic in the *Lolium* genus. For PGI the presence of 5 alleles was established. The average frequency of occurrence of allele b was the highest (0.58), followed by frequencies of the allele a (0.35), d (0.03), c (0.024) and the lowest is e allele (0.019). Frequency varied considerably depending on the populations. For ACP 3 alleles were observed. All alleles were present in all 10 genotypes with frequencies of 0.16 (b), 0.29 (a) and 0.55 (c). The enzyme PGI in all of the studied genotypes occurred in most phenotypes (average 5.4) with average heterozygosity of 59.8%. ACP enzyme showed average heterozygosity of 42.7% with phenotypes occurence in average of 4.6. Based on the frequency of alleles of the studied enzyme systems, the cluster diagram was formed in order to evaluate genotypes ability to give superior hybrid progeny.

Keywords: perennial ryegrass, isozymes, polymorphism, genetic distance

Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the agronomically most important forage grasses. During the breeding of this grass species in Serbia, a special attention is given to the selection of genotypes with improved tolerance to drought and high temperatures and also with sufficient dry matter productivity and quality in those conditions *(Sokolović et al., 2007a; Tomić and Sokolović, 2007)*.

Source of promising genotypes, that can form new varieties adapted to agro-ecological conditions in Serbia, is broad germplasm formed of different local *(Sokolović et al., 2007b; 2009a)* and introduced populations *(Sokolović et al.,* *2009b)*. The possibility that genetically distant genotypes of perennial ryegrass can give heterotic offspring brings the studies of genotype genetic relationship in first plan.

Genetic distance can be determined by biochemical markers, such as isozyme protein markers. Numerous studies using electrophoresis have revealed the occurrence of genetically controlled enzyme variants in many plant species *(Zlokolica and Taški, 2005)*. For the *Lolium* genus a number of different isozymes loci and alleles have been identified and used in the collection characterization and for allied breeding purpose *(Hayward at al., 1995)*.

The objective of this study was to determine the genetic distance of 10 selected genotypes of Perennial ryegrass, originating from different populations, by analyzing high polymorphic enzyme systems: phosphoglucose isomerase (PGI) and acid phosphatase (ACP).

Materials and Methods

Based on the analyses of the most important agronomic and morphological traits of improved perennial ryegrass population, 10 genotypes were selected for isozymes analyses. Dry matter yield per plant, number of tillers per plant and parameters of leaf size were crucial traits for genotype selection. Their origins are populations from surroundings of Krusevac (localities Jastrebac, Lomnica, Bagdala) and Valjevo (locality Divci).

Analysis of isozymes. On average 40 plants were studied for each genotype. Stem tissues of 7 day old seedlings were homogenized in 50 mM Tris-HCl buffer pH 6.8 with 1% β-mercaptoethanol. Paper wicks (2x11; Whatman N°3) were immersed in the homogenized tissues and after absorption of crude extract set at 12% starch gel. Electrophoresis was done in 0.065M L-histidine-citric buffer pH 6.5 over 3.5 hours at constant power of 16W at 4 °C. After electrophoresis ran, the gel was sliced horizontally into slabs which were separately incubated in specific staining solutions for visualization of phosphoglucose isomerase (PGI; E.C. 5.3.1.9.) and acid phosphatase (ACP; E.C. 3.1.3.2.) according to *Stuber et al. (1988)*.

Data analysis. Allelic frequencies were determined by direct allele counting. A cluster analysis was processed based on isozyme allele frequency data using the unweighted pair group average method (UPGMA).

Results and Discussion

Since the PGI and ACP are the most polymorfic *(Charmet and Balfourer, 1994; Balfourer et al., 1998)* they were chosen for this study. In analyses of the obtained zymograms, 9 bands were detected for isozyme PGI and 6 for isozyme ACP (Figure 1). Zymograms showed a single band and three banded pattern, which what is the expected pattern in the diploid perennial ryegrass *(Hayward and McAdam, 1977)* for the analyzed isozymes.

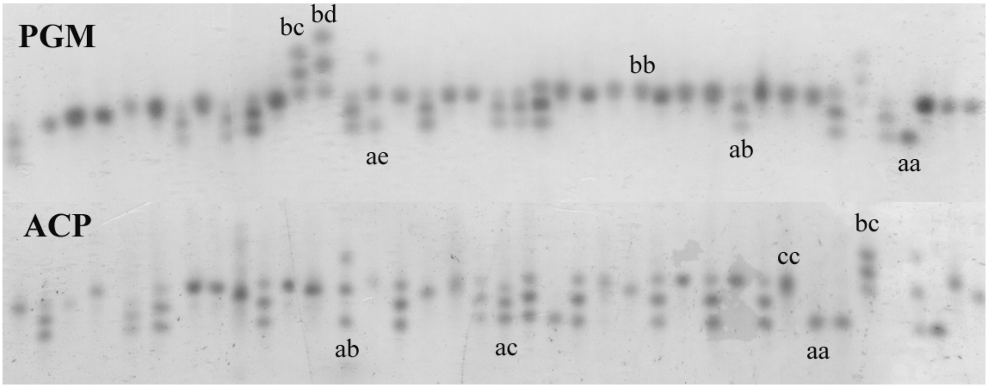

Figure 1. The PGM and ACP phenotypes in genotype number 10

Number of the various phenotypes for each enzyme system is presented in the Table 1.

Table 1. Numbers of individuals in the phenotypic classes of PGI and ACP enzymes

Isozymes	Genotype	Phenotypic classes											
		aa	bb	cc	ab	bc	bd	be	ac	ad	ae	Number of phenotypes	Percent of heterozygosity
PGI	1	2	11		11	1	2	6	2	1	2	9	65.8
	2	-	24		12	1	-	3	-	-	-	4	40
	3	1	18		19	-	-	-	1	1	-	5	52.5
	4	7	12		17	2	-	-	1	-	-	5	51.3
	5	-	12		28	-	-	-	-	-	-	2	70
	6	7	1		31	-	-	-	2	1	-	5	80.9
	7	1	11		21	1	1	2	-	2	1	8	70
	8	3	9		21	1	1	-	4	-	-	6	69.2
	9	17	-		15	1	8	-	-	-	-	4	58.5
	10	1	23		12	2	1	-	-	-	1	6	40
												5.4	59.8
ACP	1	4	3	13	5	6			7			6	47.3
	2	-	-	27	-	7			6			3	32.5
	3	-	-	18	6	1			5			5	35
	4	8	-	14	2	-			16			4	45
	5	13	-	3	1	-			23			4	60
	6	10	2	8	3	-			18			5	51.2
	7	4	3	13	2	4			9			6	42
	8	1	-	24	1	4			8			5	34.2
	9	-	-	23	-	6			11			3	42.5
	10	5	-	20	2	1			12			5	37.5
												4.6	42.7

Ten genotypes used in this study consisted of 9 phenotypes for isozyme PGI (mean 5.4). For isozyme ACP six phenotypes were confirmed (mean 4.6). Among confirmed phenotypes, 7 types for PGI and three for ACP are heterozygote. The observed heterozygosity for PGI varied from 40% in genotypes 10 and 2 to 80.9 in genotype 5, on average 59.8. For ACP heterozygosity varied from 32.5% in genotype 2 to 60 % in genotype 5, on average 42.7. The high percentage of heterozygosity is in opposition to the results of *Charmet et al.*, *(1993)* who found a significant deficit of heterozygotes for ACP in 38.4% of the studied populations.

The frequencies of the alleles were calculated based on the number of individuals in the phenotypic classes and presented in the Table 2. For PGI five alleles were recorded and denoted **a-e**. The average frequency of occurrence for allele **b** was the highest (0.58), followed by frequencies of the allele **a** (0.35), **d** (0.03), **c** (0.024) and the lowest was allele **e** (0.019). The frequencies of allele **a** ranged from 0.57 in the genotype 6, where it was the most common allele, to 0.15 in genotype 2. The second allele **b** was most frequent in genotype 2 and lowest in genotype 9. The allele **c** and **d** occurred infrequently except in genotypes 8 and 9, but even here their frequency was up to 0.1. The allele **e** was very rare, except in genotype 1 and it was also found in genotypes 2, 7 and 10.

Table 2. Allelic frequencies observed among perennial ryegrasses genotypes

	Genotype	Alleles						Genotype	Alleles		
		a	b	c	d	e			a	b	c
PGI	1	0.26	0.56	0.04	0.04	0.1	ACP	1	0.26	0.23	0.51
	2	0.15	0.8	0.01	-	0.04		2	0.07	0.09	0.84
	3	0.29	0.69	0.01	0.01	-		3	0.14	0.29	0.57
	4	0.41	0.55	0.04	-	-		4	0.42	0.03	0.55
	5	0.35	0.65	-	-	-		5	0.63	0.01	0.36
	6	0.57	0.39	0.03	0.01	-		6	0.5	0.09	0.41
	7	0.33	0.58	0.01	0.04	0.04		7	0.27	0.17	0.56
	8	0.4	0.52	0.07	0.01	-		8	0.14	0.07	0.79
	9	0.6	0.29	0.01	0.1	-		9	0.14	0.07	0.79
	10	0.19	0.76	0.03	0.01	0.01		10	0.3	0.54	0.16
	average	0.35	0.58	0.024	0.03	0.02		average	0.29	0.16	0.55

Three alleles were found for ACP. All three alleles were recorded in each of the tested genotypes. The most frequent occurrence of the fast allele **a** was in the genotype 5, while it was rarest in genotype 2. Allele **b** ranged from 0.54 in genotype 10, while it ranged to 0.01 in genotype 5. The third allele **c** revealed a heterogeneous distribution within this set of genotypes, ranging from highest 0.84 in genotype 2 to lowest 0.16 in genotype 10. According to *Hayward et al. (1995)*, the number of alleles determined by starch gel electrophoresis ranges from 2 to 7 for PGI and from 2 to 5 for ACP, depending on which system is used for the analysis.

Similar results for PGI and ACP enzymes were supplied by several authors. *Charmet et al. (1993)* present in their work that the PGI and the ACP had 6 alleles with the highest average frequency for allele **b** (0.516) of PGI, and 0.460 for allele **a** of ACP. *Charmet and Balfourier (1994)* found the presence of 7 alleles for PGI (**a** and **b** have significant frequency of occurrence) and 4 alleles for ACP with significant frequencies for all alleles. *Loos (1994)* stated similar results with 7 PGI alleles (two minor) and 4 alleles of ACP.

Based on allelic frequencies observed among perennial ryegrasses genotypes dendrogram of their genetic relationship was formed (Figure 2). It is noticeable that genotypes are grouped in two clusters. Therefore, genotypes for further crossing will be selected from those different clusters, accordingly they are most genetically divergent.

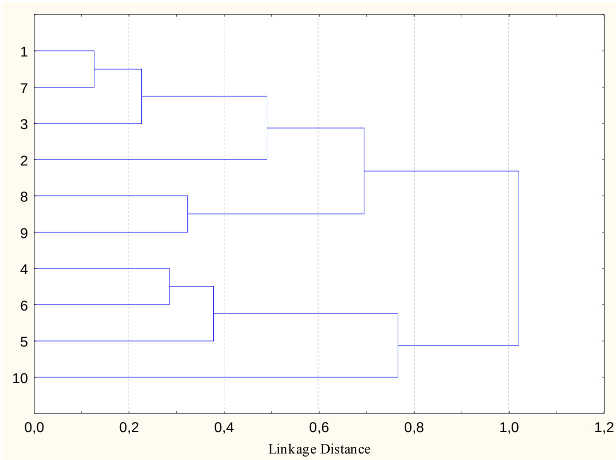

Figure 2. Dendrogram of UPGMA cluster analisis based on isozyme allele frequency data

Conclusion

Presented results show that there is an important variation at the loci of the analyzed enzyme systems. Therefore, genotypes, which are pre-selected for high yield and finest morphological traits, have significant genetic variability. This provides them high breeding value for further inter-crosses and achieving heterosis.

Pairs of genotypes for hybridisation will be chosen from different clusters and hybrids will be analised for heterotic effect.

Izoenzimski polimorfizam perspektivnih genotipova engleskog ljulja

K. Taški-Ajduković, D. Sokolović, M., Vujaković, S. Babić, J. Radović, A. Mikić, B. Živković

Rezime

Deset perspektivnih genotipova engleskog ljulja, odabranih na osnovu analiza najbitnijih agronomskih i morfoloških osobina, analizirano je pomoću izoenzima da bi se odredila njihova genetička srodnost u cilju procene sposobnosti da daju superiorna hibridna potomstva. Analizirani su enzimski sistemi fosfo-gluko izomeraza (PGI) i kisela fosfataza (ACP) budući da su prema literarnim podacima u rodu *Lolium* njihovi lokusi visokopolimorfni. Za PGI je utvrđeno prisustvo pet alela. Prosečna frekvencija pojavljivanja najveća je kod alela **b** (0,58), sledi frekvencija alela **a** (0,35), **d** (0.03) i **c** (0.024), dok je najmanja utvrđena frekvencija kod alela **e** (0,019). Frekvencije znatno variraju u zavisnosti od genotipa. Enzimski sistem ACP u ispitivanom materijalu karakteriše tri alela. Sva tri alela su prisutna u svih 10 genotipova sa frekvencijama od 0,16 (**b**), 0.29 (**a**) do 0,55 (**c**). Enzim PGI se u svim proučavanim genotipovima javlja u najviše alelnih kombinacija (u proseku 5,4) sa prosečnom alelnom heterozigotnosti od 59,8%. Enzim ACP je pokazao prosečnu heterozigotnost od 42,7% sa prosečno 4,6 alelnih kombinacija. Na osnovu frekvencija alela proučavanih enzimskih sistema formiran je klaster dijagram na osnovu koga će se odabrati genetički udaljeni genotipovi za dalju hibridizaciju.

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EFFECT OF SOWING METHOD AND SEEDING RATE ON YIELD COMPONENTS AND SEED YIELDS IN RED CLOVER (*Trifolium pretense* L.)

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Original scientific paper

Abstract: A two-year (2008-2009) two-factor experiment with the red clover cultivar Una was conducted at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad. The objective of the study was to determine the effects of planting method and seeding rate on seed yields and some seed yield components (number of inflorescences per stem, flower number per inflorescence, seed number per inflorescence) under the growing conditions of the South Bačka region. The method of planting, i.e. row-to-row spacing (20, 40 and 60 cm) was Factor A. Seeding rate per experimental unit row (0.7 and 1.4 g/row) was Factor B. The highest seed yields (686.2 kg ha^{-1} and 680.7 kg ha^{-1}, respectively) were obtained in 2008 with a spacing of 60 cm between rows and a seeding rate of 4.2 kg ha^{-1} and a row-to-row spacing of 40 cm and a seeding rate of 3.5 kg ha^{-1}.

Key words: red clover, seed yield, row-to-row spacing, seeding rate.

Introduction

Temperate climates provide the best conditions for growing red clover for both forage and seed. Areas in Serbia in which red clover is grown for seed almost completely overlap with those where the crop is grown for feed production purposes and those where alfalfa is grown. *Lugić et al. (1996)* studied the effects of the method of sowing of red clover intended for combined use (hay + seed) on a parapodzol soil in Kruševac, and their recommendation was to use a seeding rate of 14 kg ha^{-1} and a row-to-row spacing of 12.5 to 25 cm.

In contrast to this, a large group of Russian researchers *(Артеменко* and *Кулька,1988; Кишенков* and *Путников, 1983; Переправо* and *Акатышев, 1989)* argue in favor of a specialized approach when growing red clover for seed that involves widely spaced rows and low seeding rates. This, according to them, produces an optimum plant density by preventing the stand from becoming overcrowded and thus makes it possible to obtain high seed yields. According to *Taylor and Quesenberry (1996)*, growers in France also have a special approach to red clover seed production.

Due to the great importance an optimum plant population has for obtaining high seed yields in red clover, the objective of our study was to investigate how the method of planting and seeding rate affected seed yields and certain seed yield components of the crop under the growing conditions of the South Bačka region in the Serbian province of Vojvodina.

Materials and Methods

A two-factor experiment with the red clover cultivar Una was set up in 2008 at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad. A randomized block design with three replications was used. The experimental unit was a plot 5 m long by 4 m wide (20 m^2). Sowing was done with a precision hand seeder on April 1, 2008.

The first factor (A) was the method of planting, i.e. the inter-row spacing. There were three treatments:

A1-20 cm between the rows (20 rows per plot):
A2-40 cm between the rows (10 rows per plot)
A3-60 cm between the rows (6 rows per plot)

The second factor (B) was the seeding rate per experimental plot row (5 m):

B1-0.7 g per experimental plot row
B2-1.4 g per experimental plot row

Thus, the seeding rates in the different treatments were as follows:

A1B1 = 20 rows x 0.7 g/per row = 14 g/20 m^2 = **7** kg ha^{-1}
A1B2 = 20 rows x 1.4 g/per row = 28 g/20 m^2 = 14 kg ha^{-1}
A2B1 = 10 rows x 0.7 g/ per row = 7 g/20 m^2 = 3.5 kg ha^{-1}
A2B2 = 10 rows x 1.4 g/per row = 14 g/20 m^2 = 7 kg ha^{-1}
A3B1 = 6 rows x 0.7 g/per row = 4.2 g/20 m^2 = 2.1 kg ha^{-1}
A3B2 = 6 rows x 1.4 g/per row = 8.4 g/20 m^2 = 4.2 kg ha^{-1}

In the seeding year, analysis of seed yield components was performed in the first cutting (at full bloom – 10/07/2008 and at full pod maturity – 08/08/2008), whereas in the second year the analysis was carried out in the second cut (at full bloom -16/07/2009 and at full pod maturity – 19/08/2009). At flowering, the following components of seed yield were analyzed on a 1 m^2 sample: number of inflorescences per stem, flower number per inflorescence. At full pod maturity, seed number per inflorescence and total seed yield per hectare were determined. The data was statistically analyzed by two-way ANOVA and the LSD procedure.

Weather conditions

Novi Sad and the surrounding area have a temperate continental climate. During the growing season, the long-term average rainfall (1964-2008) is 367 mm and the mean temperature 17.9 ^{o}C. Table 1 shows the two main meteorological parameters (rainfall and temperature) recorded during the 2008-2009 growing seasons as compared to the long-term average (1964-2008).

Table 1. Rainfall and mean monthly temperatures during the growing season (2008-2009) as compared to the long-term average (1964-2008)

Month	Rainfall (mm)			Temperature ^{o}C		
	2008	2009	1964-2008	2008	2009	1964-2008
IV	21.9	3.6	47	13.0	15.0	11.5
V	46.2	50.4	59	18.3	18.1	16.9
VI	115.9	127.2	87	21.8	20.3	19.9
VII	41.6	58.1	67	21.7	23.0	21.5
VIII	14.0	19.1	59	22.3	23.2	21.1
IX	93.6	13.1	48	16.0	20.1	16.8
Total	333.2	271.5	367	/	/	/
Mean	/	/	/	18.9	19.9	17.9

In the two years of the study, the mean monthly air temperature during the growing season was 1°C (2008) and 2°C (2009) higher than the long-term average (Table 1). In both years, but especially in 2009, there was less rainfall in growing season than the long-term average. Looking at the summer months in particular, in both years the most rainfall was recorded in June and the least in August, so the distribution of rainfall can be said to have been very uneven. Extremely high rainfall deficits were observed in April and September in 2009 (Table 1).

Soil conditions

Chemical analyses of the soil from our study showed that $CaCO_3$ had leached from the humus-accumulating layer and that the soil was thus a slightly alkaline non-calcareous chernozem (Table 2). Chemical analysis also showed that the soil was well-supplied with phosphorus and potassium, which is of particular importance for the production of red clover seeds.

Table 2. Chemical properties of the soil (Source: Institute of Field and Vegetable Crops, Novi Sad, Laboratory for Agro ecology, 2008)

Soil depth (cm)	$CaCO_3$ (%)	pH		Humus (%)	N (%)	mg/100 g soil	
		in H_2O	in KCL			P_2O_5	K_2O
30	2.52	8.25	7.28	2.58	0.19	32.4	30.6

Results and Discussion

The tables below show the results of our study on the effects of seeding rate and planting method on red clover seed yields and yield components.

Table 3. Effects of planting method and seeding rate per experimental unit row on the number of inflorescences per stem at full bloom (2008-2009)

	2008			2009		
Inter-row spacing (A)	Seeding rate per row (B)					
	0.7 g (B1)	1.4 g (B2)	Mean (A)	0.7 g (B1)	1.4 g (B2)	Mean (A)
20 cm (A1)	4.3	3.0	3.6	8.3	5.3	6.8
40 cm (A2)	5.3	5.3	5.3	7.0	6.7	6.8
60 cm (A3)	6.7	5.0	5.8	11.3	8.3	9.8
Mean (B)	5.4	4.4		8.9	6.8	

	A**	B**	A**	B**
LSD 0.05	0.8		1.5	
0.01	1.3		2.2	

Over the two-year period (2008-2009), both of the factors studied produced highly significant differences among the treatments in the number of inflorescences per stem at full flowering (Table 3)

Flower number per inflorescence at full bloom did not vary significantly from one treatment to another (Table 4). The number of flowers (Table 4) and seeds (Table 5) per inflorescence was higher in 2008 due to the fact that growing conditions during seed yield formation were more favorable that year (there was more rainfall). It is interesting to note that significant differences in seed number per flower cluster were observed among the treatments only in the seeding year (Table 5).

Table 4. Effects of planting method and seeding rate per experimental unit row on flower number per inflorescence at full bloom (2008-2009)

	2008			2009		
Inter-row spacing (A)	Seeding rate per row (B)					
	0.7 g (B1)	1.4 g (B2)	Mean (A)	0.7 g (B1)	1.4 g (B2)	Mean (A)
20 cm (A1)	120.7	123.0	121.8	108.7	117.3	113.0
40 cm (A2)	115.7	118.0	116.8	109.7	99.3	104.5
60 cm (A3)	122.3	129.7	126.0	99.0	106.0	102.5
Mean (B)	119.5	123.5		105.8	107.5	

	A	B	A	B
LSD 0.05	15.5		24.7	
0.01	22.1		35.1	

Table 5. Effects of planting method and seeding rate per experimental unit row on seed number per inflorescence at full pod maturity (2008-2009)

	2008			2009		
Inter-row spacing (A)	Seeding rate per row (B)					
	0.7 g (B1)	1.4 g (B2)	Mean (A)	0.7 g (B1)	1,4 g (B2)	Mean (A)
20 cm (A1)	104.7	73.7	89.2	59.7	70.7	65.2
40 cm (A2)	96.0	77.7	86.8	57.0	66.7	61.8
60 cm (A3)	106.7	108.0	107.3	58.7	52.0	55.3
Mean (B)	102.4	86.4		58.4	63.1	

	A**	B**	A	B
LSD 0.05	14.5		19.5	
0.01	20.5		27.7	

The highest seed yields (686.2 kg ha^{-1} and 680.7 kg ha^{-1}, respectively (Table 6) were obtained in 2008 with a spacing of 60 cm between rows and a seeding rate of 4.2 kg ha^{-1} and a row-to-row spacing of 40 cm and a seeding rate of 3.5 kg ha^{-1}.

Table 6. Effects of planting method and seeding rate per experimental unit row on seed yield (kg ha^{1}) at full pod maturity (2008-2009)

	2008			2009		
Inter-row spacing (A)	Seeding rate per row (B)					
	0.7 g (B1)	1.4 g (B2)	Mean (A)	0.7 g (B1)	1,4 g (B2)	Mean (A)
20 cm (A1)	611.3	311.0	461.2	248.0	263.0	255.5
40 cm (A2)	680.7	378.7	529.7	274.0	193.0	233.5
60 cm (A3)	582.3	686.0	634.2	370.3	344.7	357.5
Mean (B)	624.8	458.6		297.4	266.9	

	A*	B**	A*	B
LSD 0.05	165.4		112.4	
0.01	235.2		159.8	

Vučković et al. (1997) studied the influence of planting method and seeding rate on red clover seed yields in the growing conditions of the western Srem region and obtained the highest yields (554.8 kg ha^{-1}) with a row-to-row spacing of 20 cm and a seeding rate of 10 kg ha^{-1}.

In arid climates, according to *Новоселова, (1986),* all categories of red clover seeds are by and large produced using wide row spacing.

Conclusion

In both years of the experiment, statistically significant differences were observed among the treatments with respect to the number of inflorescences per stem. A significantly higher seed number per inflorescence and total seed yield were obtained with wide row spacing (40-60 cm) and lower seeding rates (3.5-4.2 kg ha^{-1}) in the seeding year. In 2009, unfavorable growing conditions accompanied by a severe drought during early seed formation and at the seed filling stage brought about significant seed yield losses in all the treatments, although the one with wide row spacing (60 cm) did produce significantly higher seed yields than the rest.

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Rezime

U dvogodišnjim istraživanjima (2008-2009) na oglednom polju Instituta za ratarstvo i povrtarstvo, na Rimskim Šančevima postavljen je dvofaktorijalni ogled sa sortom crvene deteline Una. Cilj ogleda je bio da se u agroekološkim uslovima Južnobačkog regiona ispita uticaj načina setve i setvene norme, na neke komponente prinosa (broj cvasti po stabljici, broj cvetova po cvasti, broj semena po cvasti) i prinos semena crvene deteline. Način setve, odnosno međuredno rastojanje (20, 40 i 60 cm) je faktor A, a drugi faktor (B) je količina semena po redu

elementarne parcele (0.7 i 1.4 g po redu). Najveći prinosi semena crvene deteline (686.2 kg ha^{-1}, 680.7 kg ha^{-1}) su ostvareni u 2008. godini, setvom na međuredno rastojanje od 60 cm, sa setvenom normom od 4.2 kg ha^{-1}, kao i setvom 3.5 kg ha^{-1} na rastojanju od 40 cm.

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THE INFLUENCE OF THE HIVE DISTANCE AND THE USE OF CORN SYRUP ON POLLINATOR VISITS AND RED CLOVER SEED YIELD

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Original scientific paper

Abstract: During two-year study on the red clover, dependence of the honey bee distribution on seed crops on the hive distance from crops was researched. In addition, the presence and prevalence of other pollinators of red clover was recorded. Also, the influence of the sugar syrup application to the attraction of honey bees on seed crops was researched. It was determined that the honey bee is prevalent in relation to the other pollinators of red clover. The largest number of honey bees was counted in the immediate vicinity of the hives in the first year and 100 m from the hive in the second year. Seed yield and yield components were the highest at 100 m from the hive. The lots treated with sugar syrup gave almost 20% more seeds. Second year of observation was much more favourable for seed production and the achieved yield was for 30% higher.

Key words: red clover, honey bee, seed yield, sugar syrup

Introduction

Red clover is species that is almost entirely reproduced by cross pollination (97-98%). The auto-sterility is conditioned by the incompatibility of gametophytes. The morphological structure of red clover flower allows the entomophily by honey bee (*Apis mellifera* L.) and bumblebee (*Bombus spp.*), *(Taylor and Smith, 1979)*. There is still a divided opinion on which of the pollinators is primary for this forage crop. *Palmer-Jones (1967)* gives a primary role to the honey bee. In the study done by *Jevtić et al. (2006)* in 2003, during the ten day period a large number of honey bees were noted, although the colonies were not purposefully brought for pollination. Also, a significant number of other pollinators (solitary bees) were noted, but bumblebees were not found.

The amount of nectar in the flower tube has the main influence on the honey bee visits to the red clover. *Miladinović (1968)* found that in the second cut 71% of flower tubes had more than 2 mm of nectar, and the 29% had nectar level less than 2 mm. Also, the diploid cultivars of red clover (2n=14 chromosomes) are easier to pollinate than tetraploid cultivars (4n=28), since these have a deeper floral tubes, and so the seed production is considerably lower. Due to this, the increased interest in the honey bee for the pollination of red clover is quite justified.

Materials and Methods

The research was conducted in 2005 and 2006. In the first year, the research was performed in the Dobričevo near Ćuprija, and in the second in Kruševac. Red clover cultivar K-39 was used for research in both years. Crop was established with 16 kg ha^{-1}. Tests were performed in the second year of crop utilization, in the second cut. In the first year, the lot size was 16 ha, and in the second, it was 1 ha. The number of 22 honey bee colonies was included in the pollination. Honey bee colonies were distributed on the edges of the lots in the beginning of the flowering period. Five plants were selected for the analysis of yield components and seed yield at the specific distance from the hives (50 m, 100 m and 200 m). Also, at these distances, red clover plants were sprayed with sugar syrup in order to increase the attractiveness to the honey bees and improve their activity. At the same time, five plants from sugar syrup-treated areas were selected in order to determine yield components and seed yield. The counting of pollinators was done 10 times during the experiment, AM (09:00-11:30) and PM (14:30-16:30). After the seed ripening, seed yield components (number of fertile inflorescences, number of pods per inflorescence, number of seeds per inflorescence) were studied. Also, at three distances from the bee hives, seed yield per area unit is determined.

Results and Discussion

For an average time of 30 minutes, the 54.01 pollinators were counted (Table 1), of which 45.13 were honey bees and 8.88 were other pollinators (ratio of 5:1 in favour of honey bees). *Foster and Hadfield (1958)* found that honey bee makes the 77% to 89% or red clover pollinators in New Zealand. *Jevtić et al. (2002)* determined that of average 76.2 pollinators, 57.9 are honey bees (75.98%) and 18.3 are the other pollinators (24.02%). In the research of *Jevtić et al. (2006)* on the red clover, the average number of 25.4 pollinators was found, of which 21.3 were bees (83.86%) and 4.1 were the other pollinators (16.14%). In the second year, there were 32.7% more pollinators than in the first year. In the both years, more honey bees (4.6 and 4.4) were recorded in AM period. The time of day did not significantly affect the distribution of the other pollinators of seed crops. The distance of the hives from seed crops affected the number of honey bees. In the first year, the largest number of honey bees was at 50 m, and in the second at 100 meters, and the lowest number of honey bees was recorded at the distance of 200 m from the hive. The difference in the number of bees was especially high in the second year at the 100 and 200 m from the hive (10.35 honey bees). The distance of the honey bee hives did not affect distribution of the other pollinators. Slightly more pollinators were counted on the edges of the lot (50 and 200m), and slightly less in the middle (100 m). The larger number of the other pollinators at the edge of the lot is related to their natural habitats that are in shrublands, uncultivated fields and grasslands that were located around the lots with seed crops. The only exception was in the second year in the counting in the AM period of day when the largest number of other pollinators was counted in the middle of the lot.

Table 1. Number of honey bees and other red clover pollinators on m^2 for 30 minutes in 2005., 2006.

Distance	Observing time					
	Before noon		After noon		Average	
	Honey bee	Other poll.	Honey bee	Other poll.	Honey bee	Other poll.
	2005 (A)					
50m	43.4	9.3	37.5	9.1	40.45	9.20
100m	41.7	7.4	34.6	7.3	38.15	7.35
200m	36.1	8.8	35.8	7.4	35.95	8.20
$\overline{X}$	40.40	8.50	35.97	7.93	38.18	8.22
Total	48.90		43.90		46.40	
	2006 (B)					
50m	56.8	9.4	45.6	10.6	51.20	10.00
100m	59.8	10.1	55.6	8.2	57.70	9.15
200m	46.3	9.8	48.4	9.1	47.35	9.45
$\overline{X}$	54.30	9.77	49.87	9.30	52.08	9.53
Total	64.07		59.17		61.61	
Average (A+B)	47.35	9.14	42.92	8.62	45.13	8.88
	56.49		51.54		54.01	

Higher seed yield, the larger number of inflorescences per plant, seeds per inflorescence and better seed development were achieved in the second year of observation (Tables 2 and 3). In the first year, the treatment of red clover crops with sugar syrup had the significant influence on the seed yield and yield components, and the higher yield was achieved (18%). Additionally, the treated areas that were visited by pollinators especially honey bees, achieved significantly greater seed production compared to the untreated surface. The yield and yield components were also influenced by distance from the bee hives. In not treated lots, the highest yield was achieved in the immediate vicinity of the hive (50 m) due to the large number of young bees, which fly near the hive in the first few days, and later start to move away from the hive. In lots that were treated with sugar syrup, the highest yield was achieved at a distance of 100 m. It is obvious that, at this distance, after exiting the bee hive, honey bees first find nectar flora and stay the longest on it. There are both young and older forager bees at this distance.

Table 2. The yield components and seed yield of red clover at the various distances from the bee hives in 2005

Distance / Traits	Without sugar syrup				With sugar syrup			
	50m	100m	200m	$\overline{X}$	50m	100m	200m	$\overline{X}$
	2005							
N. inf. p. plant	4.0	4.2	3.8	4.00	5.0	5.4	5.0	5.13
N. pods per inf.	36.6	38.0	36.2	36.93	40.0	42.8	36.6	39.80
N. seed. per inf.	26.4	31.4	24.4	27.40	35.6	37.8	31.4	34.93
% of prod. seed	72.13	82.63	67.40	74.05	89.00	88.32	83.07	86.80
S. yield (kg ha^{-1})	584.1	524.3	458.8	522.57	646.9	661.2	544.3	617.47

Results obtained in the first year were only confirmed in the second year of study (Table 3). The difference is that in the second year was much more favourable for seed production. On the lots not treated with sugar syrup 156.23 kg ha^{-1} (29.8%) was gained, and on the treated lots was gained 175.39 kg ha^{-1} (28.4%) more seed than in the first year. In the second year, higher seed yield was achieved in lots that were treated with sugar syrup for 114,26 kg ha^{-1} (16.8%) compared to untreated lots. The seed production was better in the treated lots, as well as a number of inflorescences per plant and seeds per inflorescence.

Table 3. The yield components and seed yield of red clover at the various distances from the bee hives in 2006

Distance / Traits	Without sugar syrup				With sugar syrup			
	50m	100m	200m	$\overline{X}$	50m	100m	200m	$\overline{X}$
	2006							
N. inf. p. plant	5.4	5.5	4.8	5.23	5.8	6.1	5.3	5.73
N. pods per inf.	40.4	42.2	42.9	41.83	43.5	45.4	46.5	45.13
N. seed. per inf.	35.5	38.8	34.8	36.37	38.9	42.5	40.6	40.67
% of prod. seed	87.87	91.94	81.12	86.98	89.42	93.50	87.31	90.07
S. yield (kg ha^{-1})	685.2	712.5	608.1	678.60	785.4	824.8	768.4	792.86

Seeds yield of red clover can vary much and depends on the exploitation year and can reach a maximum of 700-1000 kg ha^{-1} *(Miladinović, 2001)*. Seed yield in research of *Lugićet al. (1996)* depended on the seed norms and interrow distance. For the combined utilization (hay-seed), it is recommended to sow 14 kg ha^{-1} with inter-row distance of 12.5 and 25 cm. In the research of *Jevtić et al. (2006)*, the best yield was achieved in the treatment with sugar syrup; a slightly smaller yield was achieved in the control (treatment without the syrup) and in the isolation with bees, and the lowest yield was achieved in isolation without the bees. Yield components and yield were not highest in the nearest vicinity of hives, but have gradually increased, and the best were at the distance of 100 m from the hives, and furthermore decreases. The obtained results can be interpreted by the activity of honey bees. It was determined that in honey bee pollination, especially the crops that are not attractive enough for it, honey bee is the most active on the distance of 100m (*Jevtić et al., 2004*). With further distance from the hive all the parameters of seed yield are decreased.

Conclusion

Based on the monitoring of the impact of the bee hive distance from seed crops, and using sugar syrup to attract honey bees to the red clover seed crop, following conclusions can be made:

Among all observed pollinators, honey bee had the dominant role (ratio 5:1) compared to the other pollinators.

The distance of hives from the seed crops also had influence on seed yield and yield components. The highest yield was achieved at 100m from the bee hives.

The treatment of the red clover crops with sugar syrup yielded positive results because in the first (18%) and in the second year (16.8%). More seed was harvested from lots treated with sugar syrup.

Also, the agro-meteorological conditions are very significant. The second was more favourable, and nearly 30% more seed was gained in this year.

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Uticaj rastojanja košnica i upotrebe šećernog sirupa na posetu oprašivača, oprašivanje i prinos semena crvene deteline

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Rezime

Tokom dvogodišnjeg istraživanja na crvenoj detelini praćena je distribucija medonosne pčele na semenskom usevu u zavisnosti od udaljenosti košnica od useva. Pored toga evidentirano je prisustvo i rasprostranjenost ostalih oprašivača

crvene deteline. Osim navedenog, utvrđivano je i kako utiče primena šećernog sirupa na privlačenje medonosne pčele na semenski usev. Utvrđeno je da je medonosna pčela znatno brojnija u odnosu na ostale oprašivače crvene deteline. Najviše pčela izbrojano je u neposrednoj blizini košnica u prvoj i na 100 m od košnica u drugoj posmatranoj godini. Prinos i komponente prinosa semena bili su najveći na 100 m od košnica. Parcele koje su tretirane šećernim sirupom dale su skoro 20% više semena. Druga godina posmatranja bila je znatno povoljnija za proizvodnju semena jer je ostvareni prinos bio za 30% veći.

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THE INFLUENCE OF ALFALFA FLOWER COLOURATION AND THE PERIOD OF THE DAY ON THE POLLINATOR VISITS

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Abstract: For the successful production of alfalfa seed it is necessary to provide satisfactory pollination, which is one of most important factors in the alfalfa seed production, and thus the research of pollinator behaviour is of key significance for further alfalfa selection. There are many factors that have impact on pollination activities and behaviour of insects. The aim of this study was to determine the visitation of pollinators in relation to the colouration of alfalfa flower (dark purple and light purple) as well as the period of the day. Four most important genera of alfalfa pollinators (*Apis*, *Osmia, Megachile,* and *Bombus*) were selected for this study. The flower colouration was significant factor only for *Apis*, and was not significant for all other studied pollinators. It was determined that the period of day was the most significant of studied factors, as the pollinators were most active in midday. On the contrary, the least activity was determined in morning period.

Key words: *Apis, Osmia*, *Megachile*, *Bombus*, alfalfa, pollination

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in Serbia. *Pedersen et al. (1956)*, have presented that 46.7% of flowers can grow (germinate) pods, therefore it is possible to realize up to 2000 kg ha^{-1} of seed yield. Such high yield of seed is rarely achieved not only in experimental conditions, but also in production primarily due to poor fertilization of flowers and insufficient presence of adequate pollinators. Yield of alfalfa seed is often more influenced by agro-meteorological factors and production technology, than by genetic potential (*Erić, 1995, Mihajlović et al. 1999, Bapka and Ćupina 1999*).

Alfalfa is largely self-fertile, but for mechanical reasons, flowers require insect visitations for pollination (reviewed in *Bohart 1957, Richards, 1996*). Alfalfa flowers resemble small pea flowers, with a sexual column of fused stamens and pistil held under tension within the keel. When a visiting bee inadvertently "trips" the flower, the sexual column snaps upward, sometimes striking the bee. The sexual column is inevitably driven against the banner petal. The flower can no longer be pollinated if once tripped *(Cane, 2002)*.

Commercial pollination of alfalfa is distinctive for an additional reason: both in Europe and the United States, most of the seed is produced using bees other than the honey bee, *Apis mellifera* L. Elsewhere, the honey bee has been shown to rarely trip the alfalfa flowers that it visits *(Tysdal 1940; Bohart 1957; Dylewska et al. 1970)*.

A large number of authors have proved that honey bee is not as effective in pollination of alfalfa, primarily due to the anatomy of the alfalfa flower. In addition, it was found that many other pollinator species are significantly more effective in pollination of this important forage crop *(Stanisavljević et al., 2003)*. However, these bees have their shortcomings because they are very dependent on meteorological conditions and anthropogenic factors. In addition, alfalfa is grown on large areas where there is no natural habitat for spontaneous pollinators. The pollination of alfalfa can not be left only to spontaneous pollinators because their number is insufficient, and very dependent on various factors.

The results of numerous scientific researches points that the certain pollinators tend to pollinate specific plants. For example, the insects of super family *Apoidea*, due to their long tongue, have the affinity towards the red clover rather than alfalfa. The studies of season dynamic of genera *Eucera*, *Andrena* and *Melitta* have shown that the some species of genus *Andrena* have the longest period of activity (mid April to September), while some species of genus *Melitta* have the shortest (July to October).

The analysis of the efficiency of alfalfa pollinators have shown that the most effective are *Melitta leporine* Pz., *Megachille rotundata* P. and *Andrena* sp.. To improve the alfalfa seed production it is needed to complement the period of alfalfa flowering with the period of increased pollinator activity *(Milojević, 1992)*.

Honey bee (*Apis mellifera* L.) is one of the most important pollinators. This is species of social insects, known for foraging both pollen and nectar in large quantities.

The alfalfa leafcutter bees of genus *Megachile* (most common is *Megachile rotundata* P.), are solitary bees that do not build colonies or store honey, but are very efficient pollinator of alfalfa, carrots and some other vegetables. While these bees do not store honey, females do collect pollen which they store in the cells of their nests.

The pollinators of genus *Osmia* (*Osmia cornuta* Latreille) is the most common), are a solitary, spring-flying mason bees. They forage for pollen and nectar for their brood.

Insects of genus *Bombus* are known to be good pollinators due to their long proboscis. They pollinate by "buzzing", meaning that they disperse pollen by vibrating their wings as they fly *(Stanisavljević et al., 2003)*.

The aim of this study was to determine the number of different pollinators on the alfalfa crops, in relation to flower colour and the period of day.

Materials and Methods

The experiment was conducted in experimental field of the Institute for forage crops, Krusevac, during 2008 and 2009. Total of four different genera of pollinators were studied (*Apis*, *Osmia, Megachile,* and *Bombus*). The experiment lasted for 7 days in each year of study. The alfalfa plants were sampled based on the colour of their flowers in two groups (light purple flowers and dark purple flowers). The counting of the pollinators was done in three periods of a day (08:00-09:00am, 11:00-12:00am and 14:00-15:00pm). The average temperatures for those periods were 16.2°C, 22.4°C and 27.6°C, respectively, in the first year, and 15.3°C, 24.2°C and 28.1°C in the second year.

The experiment was set as completely randomized plan. Statistical data analysis was done using the computer program Costat.

Results and Discussion

In the first year of research, it has been determined that the plants with lighter coloured flowers had slightly more visits (average 0.97 insect per plant) than ones with darker flowers (average 0.81 insect per plant). Similar results were determined in the second year. Again, the pollinators preferred lighter flowers (average 1.04 insect per plant) in contrast to darker flowers (average 0.99 insect per plant).

Table 1. The number of pollinators in relation to flower colour and time of the day in 2008

Pollinator	Light flower colour				Dark flower colour			
	08:00am	11:00am	14:00pm	Average	08:00am	11:00am	14:00pm	Average
Apis	0.00	1.00	1.03	0.68	0.00	0.76	0.81	0.52
Megachile	0.05	1.52	1.76	1.11	0.05	2.14	1.38	1.19
Osmia	0.00	2.24	2.57	1.60	0.14	1.52	1.14	0.94
Bombus	0.00	0.62	0.43	0.35	0.10	0.57	0.48	0.38
Average	0.02	1.42	1.47	0.97	0.07	1.29	1.06	0.81

Table 2. The number of pollinators in relation to flower colour and time of the day in 2009

Pollinator	Light flower colour				Dark flower colour			
	08:00am	11:00am	14:00pm	Average	08:00am	11:00am	14:00pm	Average
Apis	0.07	1.20	0.93	0.73	0.00	0.92	1.02	0.65
Megachile	0.10	1.86	2.10	1.35	0.19	2.29	1.90	1.46
Osmia	0.00	2.14	2.52	1.56	0.19	1.57	1.90	1.22
Bombus	0.05	0.52	0.62	0.40	0,10	0.62	0.43	0.38
Average	0.09	1.49	1.54	1.04	0.14	1.43	1.39	0.99

The insects of genus *Osmia* were the most frequent visitors to the light purple flowers in both years (average 1.60 and 1.56), while insects of genus *Megachile* were the most frequent in the dark purple flowers (average 1.19 and 1.46). On the contrary, the lowest number of visitors to both colour variants in both years was determined for the insects of genus *Bombus* (average 0.35 and 0.38 in the first year and 0.40 and 0.38 in the second year) (Tables 1 and 2).

In relation to the time of day, most of the studied pollinators showed greater activity in the afternoon counting (14:00-15:00 pm), when temperatures were the highest. This is due to the physiological adaptations of insects, as they show greater activity as the temperature increases, so these results were expected. In the study by *Jevtić et al. (2005)*, it is shown that the temperature is not the only factor that may influence the pollination.

The colouration of flowers was statistically significant only for *Apis* in both years, while it was not significant for all other pollinators (Tables 3 and 4).

The time of the day was statistically very significant in both years for all pollinators except for *Bombus* in 2008, where the time of day did not show significance at all. The lowest activity for all pollinators was determined in the morning counting (08:00 am) (Tables 3. and 4.).

Pollinators of genus *Apis* were the most active in the third period on the light coloured flowers in 2008, and in the second period, also on light coloured flowers, in 2009. On the contrary, *Megachile* had the highest activity on dark flowers, though their activity was highest in the second period (11:00-12:00am) in both years.

Osmia showed increased activity in the second period in 2008, and in the third period in 2009. In both years, *Osmia* mostly visited light coloured flowers.

Bombus was the most active in the second period of day in the both years. Considering flower colouration, *Bombus* had greater affinity toward darker flowers in 2008 and toward lighter in 2009.

Table 3. The significance of flower colour and time of the day regarding pollinators in 2008

Apis						*Megachille*					
	F	Time		Colour			F	Time		Colour	
Time***	258.805	Lsd$_{0.05}$=0.099 Lsd$_{0.01}$=0.139		Lsd$_{0,05}$=0.081 Lsd$_{0,01}$=0.114		Time***	57.201	Lsd$_{0.05}$=0.393 Lsd$_{0.01}$=0.551		Lsd$_{0,05}$=0.321 Lsd$_{0,01}$=0.449	
Colour**	16.728	3	0.917^{a}	2	0.674^{a}	Colour^{ns}	0.287	2	1.833^{a}	2	1.19^{a}
Interact.*	4.199	2	0.878^{a}	1	0.522^{b}	Interact.*	3.937	3	1.572^{a}	1	1.111^{a}
		1	0^{b}					1	0.047^{b}		
Osmia						*Bombus*					
	F	Time		Colour			F	Time		Colour	
Time***	13.172	Lsd$_{0.05}$=0.882 Lsd$_{0.01}$=1.236		Lsd$_{0,05}$=0.720 Lsd$_{0,01}$=1.009		Time^{ns}	3.442	Lsd$_{0.05}$=0.471 Lsd$_{0.01}$=0.661		Lsd$_{0,05}$=0.385 Lsd$_{0,01}$=0.539	
Colour^{ns}	4.076	2	1.88^{a}	1	1.603^{a}	Colour^{ns}	0.031	2	0.595^{a}	2	0.381^{a}
Interact.^{ns}	1.897	3	1.858^{a}	2	0.937^{a}	Interact.^{ns}	0.059	3	0.453^{a}	1	0.35^{a}
		1	0.072^{b}					1	0.048^{a}		

Table 4. The significance of flower colour and time of the day regarding pollinators in 2009

Apis						*Megachille*					
	F	Time		Colour			F	Time		Colour	
Time***	295.835	Lsd$_{0.05}$=0.102 Lsd$_{0.01}$=0.143		Lsd$_{0,05}$=0.083 Lsd$_{0,01}$=0.117		Time***	94.406	Lsd$_{0.05}$=0.347 Lsd$_{0.01}$=0.486		Lsd$_{0,05}$=0.283 Lsd$_{0,01}$=0.397	
Colour**	5.125	2	1,06^{a}	1	0,733^{a}	Colour^{ns}	0.745	2	2.072^{a}	2	1.460^{a}
Interact.*	7.714	3	0,977^{a}	2	0,647^{b}	Interact.^{ns}	1.899	3	1.998^{a}	1	1.348^{a}
		1	0,033^{b}					1	0.142^{b}		
Osmia						*Bombus*					
	F	Time		Colour			F	Time		Colour	
Time***	71.419	Lsd$_{0.05}$=0.413 Lsd$_{0.01}$=0.579		Lsd$_{0,05}$=0.338 Lsd$_{0,01}$=0.473		Time**	11.671	Lsd$_{0.05}$=0.249 Lsd$_{0.01}$=0.349		Lsd$_{0,05}$=0.203 Lsd$_{0,01}$=0.285	
Colour^{ns}	4.658	3	2.213^{a}	1	1.555^{a}	Colour^{ns}	0.024	2	0.572^{a}	2	0.397^{a}
Interact.^{ns}	2.871	2	1.857^{a}	2	1.221^{a}	Interact.^{ns}	0.905	3	0.525^{a}	1	0.382^{a}
		1	0.059^{b}					1	0.072^{b}		

Conclusion

The pollinator behaviour is highly influenced by the period of day, which was statistically significant in all cases except for *Bombus* in 2008. The highest activity of pollinators was determined in midday in both years, while the pollinators were least active in the morning.

The colouration of flowers was only statistically significant for *Apis mellifera*, but had no significance to the other pollinators.

Megachile and *Osmia* were the most frequent pollinators in all variants, while *Bombus* species were the rarest visitors, both regarding both flower colour and period of the day.

Uticaj boje cveta lucerke i doba dana na posetu oprašivača

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Rezime

Za uspešnu proizvodnju semena lucerke, neophodno je obezbediti zadovoljavajuće oprašivanje, što predstavlja jedan od najbitnijih faktora u produkciji semena lucerke. Samim tim, proučavanje ponašanja polinatora je od ključnog značaja za proizvodnju semena lucerke. Postoji više faktora koji utiču na oprašivačku aktivnost i ponašanje insekata. Cilj ovog rada je da se utvrdi poseta oprašivača u odnosu na boju cveta lucerke (tamno ljubičasta i svetlo ljubičasta) i na doba dana. U ovom istraživanju korišćena su četiri najznačajnija roda oprašivača lucerke (*Apis*, *Osmia, Megachile,* and *Bombus*). Boja cveta je značajno uticala samo na rod *Apis*, a za ostale proučavane polinatore nije bila značajna. Utvrđeno je da je doba dana bilo najznačajniji faktor, jer su oprašivači bili najaktivniji oko podneva. Nasuprot, najmanja aktivnost je utvrđena u jutarnjem periodu.

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HARVEST INDEX OF ITALIAN RYEGRASS FOR SEED IN THE FIRST HARVEST YEAR

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Original scientific paper

Abstract: Italian ryegrass (*Lolium multiflorum* Lam.) cv. Tetraflorum sown at different inter-row spacings (20, 40 and 60 cm), seeding rates (5, 10, 15 and 20 kg ha^{-1}) and spring nitrogen (N) fertilization (0, 50, 100 and 150 kg ha^{-1}), was tested under the agro ecological conditions of Western Serbia. The field experiments were carried out from 2002 to 2006. Seed and straw yield were measured and harvest index was calculated during the first production year. The highest harvest index varied among treatments depending on seasonal conditions. Harvest index was impacted by inter-row spacing, marked, however, by an opposite impact in arid and humid weather conditions. The inter-row spacing of 40 cm was found to be the least risky for seed production. The increase in seed rates in stand establishment provided higher harvest index in years with unfavourable weather conditions. However, the seed rate had either no impact on harvest index, or decreased the index as a result of ryegrass lodging following seed shedding in the years with favourable weather conditions. Spring nitrogen fertilization decreased harvest index by increasing development of vegetative biomass, as well as, ryegrass lodging following seed shedding. There was positive correlation between harvest index and seed yield during experimental years.

Key words: Italian ryegrass, seed yield, straw yield, harvest index

Introduction

Italian ryegrass is important forage grass in Serbia, producing high-quality forage from early spring to late summer *(Tomić et al., 2007; Simić et al., 2009c)*. According to *Simić et al. (2009a)* excellent ryegrass seed yield achieved in Serbia in the first production year is partially the result of high rate of seedling growth, the highest among cultivated cool season grasses. The harvest index is an indicator of the grass seed production efficiency. Harvest index reflects the partitioning of photosynthate between the grain and the vegetative plant and improvements in harvest index emphasize the importance of carbon allocation in grain production *(Sinclair, 1998)*. Ryegrass harvest index generally followed similar trends to those seen in seed yield *(Mueller-Warrant and Caprice Rosato, 2002)*. The range between the best and the worst treatments tend to be smaller for harvest index than for seed yield. A narrower range in harvest index than in seed yield could be explained by the fact that treatments that reduced seed yield also tend to reduce total aboveground biomass. An alternate formulation of this concept would be that whatever damage was done to ryegrass plants by the treatments, this damage had more effect on vegetative growth than on reproductive allocation. Low ryegrass harvest index could be attributed to cultivation of non-recommended cultivars, late sowing, imperfect sowing methods, low plant population, poor plant protection, and proliferation of weeds, imbalanced use of fertilizer and non-availability of water for irrigation at critical crop growth stages. Balanced use of inputs like seed, fertilizers and moisture is essential for improving harvest index of grass seed crops. This field study was conducted to determine the harvest index of Italian ryegrass seed crop in the first production year using different sowing rates, row spacing and spring nitrogen application.

Materials and Methods

The study was maintained for 4 consecutive years, between 2002 and 2006, near Šabac, Serbia (44°47’ N, 19°35’ E, 80 m asl.) which is located in a semi-humid region (with very variable years). Seed from the primary growth of tetraploid Italian ryegrass cv. Tetraflorum was harvested in the first production year after the establishment. Italian ryegrass was planted (20, 40 and 60 cm row spaces) each autumn (first to third decade of October) prior to the preceding summer seed harvest. Seeding rate was equivalent to 5, 10, 15 and 20 kg ha^{-1}, providing 12 plant spatial treatments in combination with inter-row spacing. The plot for harvest was 10 m^2, and replicated four times in a randomized complete block design. Phosphorus, potassium and a portion of nitrogen were applied in the fall before each seed production year (250 kg ha^{-1} of 8-16-24 fertilizer NPK), with additional application of N (0, 50, 100 and 150 kg ha^{-1}) in the spring. After seed threshing, the straw was collected and weighed as dry shoot mass. This measurement was needed for calculating the harvest index, by using the formula HI =[SY/(SY+SDM)]*100, where HI is harvest index (%), SY – seed yield (kg ha^{-1}) and SDM – shoot dry mass (straw yield) (kg ha^{-1}). Soil in the experimental area was humofluvisol (2.54% humus), with rinsed limestone. The main characteristics of the soil (depth: 0-30 cm) were the following: soil texture: clay; $CaCO_3$: 0.36%; pH in KCl: 5.25; K_2O: 15 mg kg^{-1} and P_2O_5: 3 mg kg^{-1}. Monthly precipitations during the 4 years of the experiment were very contrasting. Accumulated precipitation during the vegetation period was compared and presented (Figure 1).

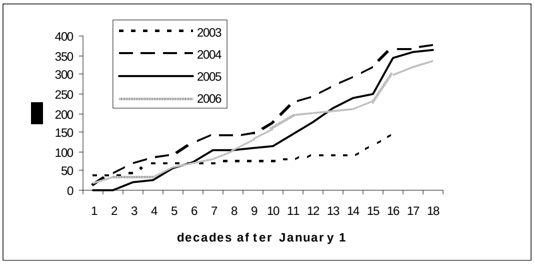

Figure 1. Accumulated precipitation during four consecutive years in Italian ryegrass grown for seed

Results and Discussion

The harvest index (HI), as an indicator of the seed production efficiency, was impacted significantly by applied treatments. The highest HI was reached in 2003 (29.56±0.24%), which was unexpected and the least in 2004 (12.62±0.23%) (Table 1). Regarding the low seed yield and reduced dry mass production, the harvest index could not be considered as an objective indicator in 2003 year, especially taking into account that positive correlation coefficient between straw yield and harvest index was only in that year (Table 1).

Table 1. Harvest index during experimental years – average index, variation by years (%) and correlation coefficients between seed and straw yield

Years	$\bar{x}$	X_{min}	X_{max}	Sd	$S\bar{x}$	Cv (%)	$r_{seed\ yield}$	$r_{straw\ yield}$
2003	29.56	20.8	35.7	3.38	0.24	11.4	0.58	0.36
2004	12.62	6.1	25.1	3.20	0.23	25.4	0.72	-0.58
2005	21.13	11.0	30.5	4.08	0.29	19.3	0.49	-0.61
2006	20.82	11.0	32.0	5.28	0.44	25.4	0.31	-0.88

Also, in 2003 the least harvest index variation was obtained (11.4%), indicating dominant influence of environmental conditions, particularly rainfall shortage. Index was more influenced by treatments in 2004 and 2006, with variation coefficient 25.4%. Correlation, which was expected, between HI and seed yield was positive, but negative for straw yield, except in 2003.

The results indicated that all applied factors influenced harvest index during experimental years (Table 2), with significant effect of the factors interaction in 2005 and 2006. Applied factors influenced HI during the years in different ways, depending on environmental conditions, mostly on rainfall. The least HI was in 2003 at 60 cm row spacing (Figure 2). While in 2004 and 2005 the highest HI was at 60 cm, in 2006 maximum was at medium inter row spacing. In contrast with the previous years, where higher tiller density had a deleterious effect on seed yield, environmental conditions (flooding) during 2006 forced stands with greatly varied tiller populations to reach a similar stand density and productivity.

Table 2. Statistical summary of harvest index responses to stand density and varied spring applied nitrogen (LSD test) during the period of 2003-2006

Factors and interaction	2003	2004	2005	2006
Inter row spacing A	**	**	**	**
Seeding rate B	**	**	**	**
Spring N rates C	NS	**	**	**
A X B	NS	NS	NS	NS
A X C	NS	NS	NS	NS
B X C	NS	NS	*	**
A X B X C	NS	NS	NS	*

[1]*NS* = not significant *P* value 0.05, * = *P* value < *0.05* ** = *P* value <*0.01*

The optimum density appears to be within a seeding rate range of 15 to 20 kg ha^{-1}. This is within the normal seeding rate for Italian ryegrass although under-sowing after harvest can exceed this optimum by 10 times or more. Even when unburned fields are ploughed, all of the shattered seed from the previous crop is mixed into the soil during seed bed preparation, thus, many commercial Italian ryegrass stands are too dense to achieve maximum seed yield *(Young, 1996)*. There was more expressed moisture loss at wider row spacing in 2003, plants accelerated maturing and that decreased HI in those spacings. Lodging decreased at the highest row spacing in humid years, which resulted in higher HI. Sparse canopy at medium row spacing due to high spring moisture resulting in decreased development of vegetative biomass, and HI increased despite the least achieved seed yield.

In 2003 the higher amount of sown seed, the higher seed yield was achieved, resulting in harvest index increase (Figure 2). Also, in the same year straw yield increased, raising seeding rates, but seed yield increase was higher than straw yield increase, which resulted in harvest index increase. According to *Mueller-Warrant and Caprice Rosato (2002)*, drought damage had more effect on vegetative growth than on reproductive allocation. During the following years harvest index decreased while seeding rates increased. In 2004 index increased prior to seeding rate 10 kg ha^{-1}, while in 2005 and 2006 prior to 15 kg ha^{-1}. High harvest index in 2004 and 2005 was primarily achieved due to the least developed biomass and the least crop lodging, because seed yield was not significantly different at seeding rates of 5 kg ha^{-1} from other treatments. Larger amounts of sowing seed increased biomass and lodging, which with uniform seed yield led to harvest index decrease.

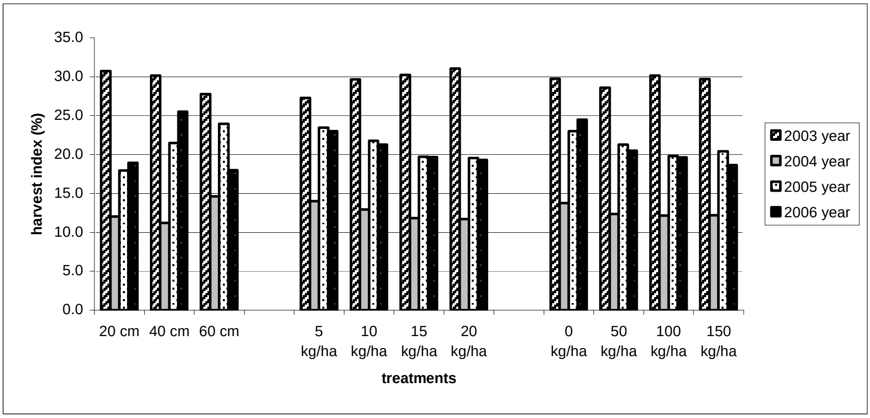

Figure 2. Impact of applied treatments on Italian ryegrass harvest index during the period of 2003-2006

The highest harvest index was reached at treatments without fertilizing, except in 2003, when nitrogen application had no influence on harvest index. Total biomass substantially increased up to the highest N rates. This increase in biomass did not result in higher seed yield and caused a decrease in harvest index. The cause of decreased yield from higher N rates may be from excessive plant growth resulting in early lodging and shading of the crop thereby diminishing the realized yield potential *(Young, 2001).* Negative effects of increasing spring N caused a decrease in harvest index and an increase in straw yield.

These results are in accordance with the results of seed production, where N application caused lodging and consequently negative influence on seed yield, achieving the highest yields during the three years in variants without N application. The highest harvest index was recorded in control fertilizer treatment except for the year of 2003, when N application had no influence on harvest index. Similarly, *Young et al. (2001)* while investigating seed production of Italian ryegrass on two locations at different nitrogen levels had harvest index without statistically significant differences during treatments. *Silberstein et al. (2002)* while applying nitrogenous fertilizers did not have considerable differences in harvest index of ryegrass and it ranged from 14 to 15,5%. The same authors recommend application of plant growth regulators (PGR), which decreases lengthening (thereby biomass), but increases stem hardiness raising resistance to lodging. Harvest index increased with PGR applications *(Silberstein et al., 2002*), which was confirmed by recent trials in Serbian conditions *(Simić et al., 2009b).*

Conclusion

The results of the present study indicate that in order to maximize seed productivity in the first production year harvest index must be taken into account. Harvest index varied during experimental years due to environmental conditions, but it can be influnced by growing mode as well. Similarly to the measures for achieving maximum seed yield, for good HI a high seeding rate is preferable (15-20 kg ha^{-1}), and medium row spacing (40 cm) is the least risky. The results of the present study support the use of a relatively low nitrogen rates and seeding rate for Italian ryegrass seed production in the first harvest year. The harvest index of Italian ryegrass as an indicator of successful seed production cannot be observed separately from other harvest indicators (seed and straw yield), for achieved low and high values must be monitored/observed in the context of weather conditions in the given year. Future studies should focus on including additional sites with different soil drainage classes in contrasting climatic years.

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Žetveni indeks semena italijanskog ljulja u prvoj godini proizvodnje

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Rezime

Italijanski ljulj, sorta Tetraflorum, je ispitivan u agroekološkim uslovima zapadne Srbije pri setvi na različitom međurednom rastojanju (20, 40 i 60 cm), različitom normom semena (5, 10, 15 i 20 kg ha^{-1}) i prolećnom prihranom (0, 50, 100 i 150 kg ha^{-1}) u periodu 2002-2006. Mereni su prinos semena i slame, a žetveni indeks je izračunat za prvu proizvodnu godinu. Najveći žetveni indeks je varirao između primenjenih tretmana zavisno od vremenskih uslova godine. Žetveni indeks je bio pod značajnim uticajem međurednog rastojanja, mada sa suprotnim efektom u sušnim i vlažnim vremenskim uslovima. Međuredno rastojanje od 40 cm se može uzeti kao najmanje rizično za semensku proizvodnju ljulja. Povećanje količine semena pri zasnivanju je dalo veći žetveni indeks u godinama sa nepovoljnim

vremenskim uslovima. Međutim, setvena norma nije imala uticaja na žetveni indeks ili ga je smanjivala usled poleganja ljulja na koji se nadovezivalo osipanje semena u godinama sa povoljnim vremenskim uslovima. Prolećna prihrana azotom je smanjivala žetveni indeks sa povećanjem razvoja vegetativne biomase, kao i usled poleganja ljulja uz osipanje semena. Postojala je pozitivna korelacija između žetvenog indeksa i prinosa semena tokom eksperimentalnih godina.

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THE INFLUENCE OF ALFALFA SEED PURITY ON THE ELECTRIC POWER CONSUMPTION DURING PROCESSING

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Original scientific paper

Abstract: The paper shows the results of the research obtained through the processing of alfalfa seed in the processing centre of the Institute for Forage Crops in Kruševac. From three lots of naturalized seed of different purity, processed by the same equipment, in three repetitions, the following relevant parameters have been determined: the processing time, electric power consumption and the quantity of the processed seed. The processing I lot seed was of 59.0% average grain purity with 39.3% of inert matter and 1.7% of weeds. The average II lot seed purity was 78.0% with 17.9% of inert substance and 4.1% of weeds and a greater amount of quarantine weeds with 35 grains of dodder (*Cuscuta spp.*) and 4 grains of curly docks (*Rumex spp.*) in the sample of 5 g, foxtail millet (*Setaria spp.*) and Johnson grass (*Sorghum halapense*). The III lot seed had 85.4% of pure grain, 13.4% of inert matter and 1.2% of weeds. Analyzing the results after processing each lot of seeds of different purity by the same equipment, all relevant parameters were different and depended on the initial seed purity as well as on the quantity and types of existing weeds in natural seed. During the processing, the main problem was the presence of quarantine weeds of dodders and docks as well as foxtail millet in the seed of alfalfa.

Key words: alfalfa, purity, seed processing, equipment, weeds

Introduction

Alfalfa (*Medicago sativa* L.) is one of the oldest and most important perennial forage legume due to its high forage quality and area in which it is grown. It is also significant for seed production due to its high price on both domestic and foreign market *(Mišković, 1986)*.

In combine harvesting of the alfalfa seeds, the obtained material is a mixture of the species of this seed, the seeds of other plants and weeds, as well as various waste of organic and inorganic origin. The weeds in alfalfa seed crops make the harvesting harder, contaminate the seed and make the processing more difficult *(Erić et al.,1993)*. The purpose of processing is to set aside all the grains of alien origin and various waste and separate the pure grain of primary plant so as to adequately prepare the seed for sowing machine and good seeding, sprouting and growing, storing and preserving until the time of seeding. The processing of alfalfa seed is done by several machines and devices using different technological methods depending on natural seed purity. An inadequate equipment and technology usage may result in an increase in processing time and power consumption and lead to higher percentage of seed losses.

The processing is based on the physical features of the seed. The processors have to be careful in analyzing each seed lot that comes from field and decide upon which equipment to use in order to achieve the best results in seed purifying *(Smith 1988; Copeland and McDonald, 2004*; *Black et al., 2006)*.

The Law on seeds and planting material *(Official Journal of RS, 2005)*, which is in accordance with The Constitution of International Seed Testing Association *(ISTA, 1999)*, defines the conditions and ways of producing, processing and distribution of seed. The consumption of electric power per production unit is one of the basic measures for the quality of a production process and an index which highly influences the products competitiveness on the market *(Oros et al., 2008)*. During the alfalfa seed processing, the difference between the amount of pure seed, estimated in a laboratory, and the amount got after processing, should be as low as possible *(Djokić et al., 2008)*.

The objective of this paper is to determine the amount of power consumption, processing time and the quantity of seed left after processing three lots of seed of different purity, processed by the same equipment.

Materials and Methods

The research was carried out in the processing centre of the Institute for Forage Crops in Kruševac. In three repetitions, three lots of seeds with purity values of: 59.0% - I, 78.0% - II, and 85.4% - III, were being processed (Table 1). The quantity of seed in each pass was 300 kg, i.e. 900 kg for each purity value (2700 kg in total).

The analysis of the contents of the additions in the seed was performed in a laboratory, assessing seed masses of 5 g and 50 g on an electronic scale. For each repetition and each seed purity, the following parameters were measured: clean seed (%), seed of other plants (%), inert matter (%), weed seed (%), the amount of the processed seed (kg), seed processing time (h), active power consumption (kWh) and reactive power consumption (kVArh), output of processing and seed losses on the processing equipment (%).

Table 1. An average purity of alfalfa natural seed in I, II and III lot

Natural alfalfa seed						
Lot	I		II		III	
Content	%	Weed species	%	Weed species	%	Weed species
Pure seed	59.0		78.0		85.4	
Weed	1.7	*Convolvulus arvensis*, *Amaranthus retroflexus* L., *Daucus carota* L., 4 *Cuscuta spp.* in 5 g	4.1	*Amaranthus retroflexus* L., *Setaria spp.*, *Sorghum spp.*, 4 *Rumex spp.* in 5 g , 35 *Cuscuta spp.* in 5 g	1.2	*Amaranthus retroflexus* L., *Daucus carota* L., 3 *Cuscuta spp.* in 5 g, 6 *Rumex spp.* in 5 g, *Lolium perenne* L.
Other species	0	red clover in traces	0		0	
Inert matter	39.3	pods, sickly grains, harvest residues	17.9	pods, sickly grains harvest residues, soil	13.4	sickly grains, harvest residues

The readings of power consumption were done by multifunctional digital three - phase power meter DMG2. The time was measured by a stopwatch. The equipment used in a processing centre of the Institute for Forage Crops in Krusevac, comprised machines and devices made by Danish producers "Damas", "Kongskilde" and by a German producer "Emceka Gompper".

Results and Discussion

An average number of passes through the processing equipment, the processing time and the power consumption for I lot seed with a purity value of 59.0%, are all shown in a Table 2.

Table 2. The power consumption and the time of the processing of I lot seed

Average	Number of passes	The time of the processing (min)	Power consumption	
			Active (kWh)	Reactive (kVArh)
$\overline{X}$	2	137.7	53.2	73.13
$\overline{X}^*$	1	51.33	7.6	16.8
$\sum$	3	189.0	60.8	89.93

$\overline{X}^*$ waste processing

This seed underwent processing three times. The total time was 189.0 min. The active power consumption was 60.8 kWh and reactive 89.93 kVArh. In Table 3. average values for the number of passes, processing time and the power consumption for the seed with purity value of 78.0% (II lot) with a large amount of weed are presented.

Table 3. The power consumption and the time of the processing of II lot seed

Average	Number of passes	The time of the processing (min)	Power consumption Active (kWh)	Reactive (kVArh)
$\overline{X}$	3	192.0	77.93	111.57
$\overline{X}^{*}$	1	74.4	37.53	46.53
$\overline{X}^{**}$	1	47.0	17.17	21.17
$\sum$	5	313.7	132.6	179.27

$\overline{X}^{*}$the seed processing after demagnetizing, $\overline{X}^{**}$the waste processing after demagnetizing

The II lot seed before demagnetizing underwent processing three times. Since it had contained a considerable quantity of quarantine weeds it couldn't be processed even after two passes through magnetic machine. Therefore it was left to demagnetize for three months in order to be processed again. This seed passed the processing five times with an average processing time of 313.7 min. The active power consumption was 132.6 kWh and for reactive it was 179.27 kVArh.

The seed from III lot with the highest content of pure seed (85.4%) and the lowest content of weeds and inert matter, was underwent processing two times, during that process average time of processing was 166.3 min. The active power consumption was 59.87 kWh and for reactive it was 85.27 kVArh (Table 4).

Table 4. The power consumption and the time of the processing of III lot seed

Average	Number of passes	The time of the processing (min)	Power consumption Active (kWh)	Reactive (kVArh)
$\overline{X}$	1	111.3	40.27	61.6
$\overline{X}^{*}$	1	55.0	19.6	23.67
$\sum$	2	166.3	59.87	85.27

$\overline{X}^{*}$ waste processing

Table 5 shows that out of 300 kg of naturalized I lot seed with purity value of 59.0%, after processing, an average of 142.67 kg of processed seed was obtained. By processing waste seed the additional 22.67 kg clean seed are attained. At the end of processing an average attained amount reached 165.3 kg. The processed amount of seed compared to initial amount shows the output of processing of 55.1%, with a clean grain loss of 6.61% (Table 5).

By processing 300 kg of naturalized II lot seed with purity value of 78.0% and large amount of dodder (35 grains in a sample of 5 g), an average of 176.0 kg was obtained. The analysis of a 50 g sample on a small magnetic machine showed the presence of dodder in spite of a high purity value of 98.1%. This seed was left to demagnetize for three months.

Table 5. The time of the processing, power consumption, average quantity of processed seed, output of processing and seed loss for I, II and III lot during the alfalfa seed processing

Lot	Processing time (min)	Power consumption		Seed quantity (kg)			Processing output (%)	Grain loss (%)
		Active (kWh)	Reactive (kVArh)	From processing	From waste	∑		
I	189.0	60.8	89.97	142.67	22.67	165.3	55.1	6.61
II	313.7	132.6	179.27	142.7	20.33	163,0	54.33	30,34
III	166.3	59.87	85.27	221.7	13.17	234.8	78.27	8.39
CV(%)	35.6	49.4	44.8	27.0	26.4	21.7	21.7	87.5

After processing the waste seed from II lot, which contained a considerable amount of quarantine weeds, an adequate quality seed couldn't be obtained and would have made any further processing the waste of time and energy. The seed that had been left to demagnetize was being processed again and an average of 142.7 kg of a quality seed was obtained and
20.33 kg out of waste. At the end of processing, an average quantity of processed seed was 163.0 kg. Compared to initial quantity of 300 kg of 78.0% purity value, output of processing was 54.33% with a clean grain loss of 30.34%.

By processing III lot seed of 85.4% purity value, an average of 221.7 kg of a quality seed was obtained and additional 13.17 kg out of waste, i.e. 234.8 kg in total. Output of processing was 78.27%, and overall losses reached 8.39%. The seed purity had the most significant influence on the seed losses (CV=87.5%). Less significant was the influence on active electric power (CV=49.4%), reactive (CV=44.8%) and on the processing time (CV=35.6%). The least significant influence, the seed purity had on the overall quantity of obtained seed (CV=21.7%).

Conclusion

The longest processing time (313.7 min) was in II lot seed with a large amount of dodder (35 grains in 5 g) and the shortest (166.3 min) was in III lot seed of high purity value and the smallest amount of weeds and inert matter.

Due to demagnetizing II lot seed, electric power consumption was the highest, with 132 kWh of active and 179.27 kVArh of reactive electric power. III lot seed with the highest purity value of 85.4% had the lowest power consumption with 58.87 kWh of active and 85.27 kVArh of reactive electric power. The overall quantity of seed obtained at the end of processing was the largest from the seed with the highest purity value and the smallest from the seed with the lowest purity value and the large amount of weeds. In the seed with high purity value of 85.4%, output of processing was the highest and reached 78.27% with seed losses of 8.39% which was a respectable seed usage. The I lot seed had the smallest losses (6.61%) and the largest in seed with a highest percentage of quarantine weeds (30.34%), which was unacceptable. The acquired data clearly show the influence of initial seed purity, primarily contents of weed, on the processing time, electric power consumption and the quantity of obtained seed.

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Uticaj čistoće naturalnog semena lucerke na potrošnju električne energije u procesu dorade

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Rezime

U radu su prikazani rezultati istraživanja dobijeni u procesu dorade semena lucerke u doradnom centru Instituta za krmno bilje u Kruševcu. Iz tri partije naturalnog semena različite čistoće, na istom sistemu mašina za doradu u tri ponavljanja određivani su sledeći relevantni parametri: vreme dorade, potrošnja električne energije i količina dorađenog semena. Dorađivano seme iz prve partije bilo je prosečne čistoće zrna od 59.0%, sa 39.3% inertnih materije, i 1.7% korova. Seme lucerke iz druge partije je bilo prosečne čistoće od 78.0%, sa 17.9% inertnih materija, i 4.1% korova sa većom količinom karantinskog korova od 35 zrna viline kosice (*Cuscuta spp.*) i 4 zrna štavelja (*Rumex spp.*) u uzorku od 5 g, muhara (*Setaria spp.*) i divljeg sirka (*Sorghum halepense*). Seme iz treće partije imalo je 85.4% čistog zrna, 13.4% inertnih materija i 1.2% korova. Analizom dobijenih rezultata pri doradi svake partije semena različite čistoće na istom sistemu mašina svi navedeni relevantni parametri bili su različiti i zavise od početne čistoće semena koje se dorađuje, kao i od količine i vrste prisutnih korova u naturalnom semenu. Pri procesu dorade najveći problem predstavljalo je prisustvo semena karantinskih korova viline kosice i štavelja, kao i muhara u semenu lucerke.

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APPLICATION OF VIGOR TESTS ON FORAGE CROP SEED

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Review paper

Abstract: Considering that forage plants belong to different families, therefore they differ in genetic, morphological, physiological and other characteristics. Their seed also very differ in morphology, chemical composition, size and other. By area they occupy and yield, the most significant are perennial and annual forage legumes (fam. *Fabaceae*) and perennial and annual forage grasses (fam. *Poaceae*). Therefore, seed of these forage species is the most significant for our seed production. Seed vigor of these species is a group of those characteristics that define activity and behaviour of seed lot that have commercially acceptable germination values of field emergence in different environmental conditions. Aspects that point on seed with high and low vigor are numerous, and among the most important are biochemical processes and reactions during germination, such as level of uniform seed germination and germ growth, level of uniform vigor emergence and field emergence, and analyzing vigor of emergence in adverse environmental conditions. Purpose of vigor tests is to give sum of all seed characteristics that after sowing affect on fast and uniform formation of healthy and powerful seedlings in different conditions. It is possible to apply these tests on forage plants seed: Accelerated ageing test, Electro conductivity test, Cold test and Tetrazolium test.

Key words: field emergence, forage plant, germination, vigor, seed, test

Introduction

Good quality of seed of whatever plant species and also of forage plants is seen in its high vigor, meaning that this seed is more resistant to stress storage and agro ecological conditions (high moisture, low temperature, diseases etc). It is important to determine permitted period for seed storage because of rational usage of every seed lot. During the storage seed vigor is decreasing differently for various plant species, for species with period of dormancy it increases with time. It is very important to determine which seed lots have to be placed on the market and which can be stored for the next season if the low price is present in the market or in case we want to form a seed stock for the next season. Germination percentage is not sufficient information for making such decisions because most of the lots are stored with approximately same germination, but seed do not keep equal germination even in the same conditions in the storage room. It has been noticed that the increase of germination for seed with dormancy is proportional to seed storage duration but in definite period because of the endogenous germination inhibitors *(Harrison, 1966)*. Similar results were obtained by *Hanty et al. (1993)* by analyzing the germination of stored grass and legume seed in which the seed storage increases germination during some time. Legumes keep high germination for the longest time - alfalfa and white clover for 15 years, for the shortest time – English blue grass for 5 – 6 years, but not keeping the vigor. Galega seed (*Galega orientalis* Lam.) even if it has been kept under uncontrolled conditions, kept its germination after 14 years of storage, and seed lots that had high initial germination could be used for sowing *(Djukanović, 2006)*.

Seed vigor is a complex of all characteristics that define activities of seed lot that has commercially acceptable germination in different outdoor conditions *(ISTA, 2005)*. Seed germination is the basic indicator of vigor.

What is vigor?

The idea of vigor was first mentioned in the beginning of the 20^{th} century. Hiltner and Ihssen, from Germany, in 1911 *(Mc Donald, 1993)* used the term "triebkraft" meaning moving force or force of initial growth. Real interest in vigor begins in 1950, at International Seed Testing Association Congress (ISTA Congress) in Washington. The Vigor Committee was then founded with a mission to define what vigor was and to develop and standardize vigor tests.

First definition of vigor gave Isely in 1957 *(Milivojević, 2005)* and from that time, it has endured significant changes. From 1980, we are finding the essential meaning of vigor in ISTA and AOSA handbooks.

Vigor represents overall ability of seed fast and uniform emergence, ability to develop into a plant under various conditions in the field *(AOSA, 2002)*. Seed vigor is a very important indicator of quality. All eventual adverse growing conditions that took place during the vegetation period manifest through seed vigor.

Vigor determinates seed longevity without dangerous consequences *(ISTA, 2005)*. It is known that decreasing of vigor goes with ageing of seed and that it is decreasing more rapidly during the storage than germination *(Dragicević, 2007; Djukanovic, 2003)*.

Vigor tests

Vigor tests are used for analyzing vigor. These tests serve for determination of seed ability to germinate and give normal plant in various field conditions. Development of vigor tests, same as defining the idea of vigor was very long lasting process. *Ching (1973)* says that seed vigor has two components: germination and seedling growth. Most of researchers use development of seedlings for analyzing vigor: whole seedling length or shoot and root length *(Yaklich et al, 1979; Edwards and Sadler, 1992; Djukanović, 1999; Djukanović, 2003)*, seedling mass *(Mc Kersie and Tomes, 1982)* and seedling dry mass *(Anfinrud and Schneiter, 1984; Aschermanu-Koch et al., 1992; Dragicevic, 2007)*. There are certain tests for analyzing of seed vigor that provide more information about physiological seed quality – such as sowing potential that is often not seen in standard germination test *(Djukanović, 2003)*. Vigor test must be repeatable and received results should be in correlation with results received in field conditions. Vigor tests should be reliable, practical, fast and explicit *(Heydeker, 1969)*. Factors that provoke variations of seed vigor are outnumber and in most important are genetic constitution, cultivation method and fertilisation, seed coat characteristics, seed ageing and pathogen presence.

Mc Donald (1975) made three groups of vigor tests:

1. Physical tests determine seed characteristics – size and mass.
2. Physiological tests use germination and growth parameters. There are two types of these tests:
   - standard germination in laboratory conditions and seedling growth test in favourable conditions
   - Cold test, Accelerated ageing test and Hiltner test in adverse conditions.
3. Biochemical tests are indirect methods for evaluating seed value. In this group are Tetrazolium test, Electro conductivity test, enzyme activity and respiration.

Hampton and *Te Krony (1995)* sorted vigor tests in two groups:

1. Recommended tests: electro conductivity test and accelerated ageing test.
2. Suggested tests: these tests are still in development phase and involve cold test, cool germination test, complex stressing vigor test, Hiltner test, seedling growth test and Tetrazolium test.

In AOSA handbook (*2002*) vigor tests are sorted in three groups:

1. Stress tests:

a. Accelerated ageing test

b. Cold test
c. Cool germination test
2. Seedling evaluation and growth:
a. Vigor classification of seedlings
b. Seedling growth test
3. Biochemical tests:
a. Tetrazolium test (TTZ)
b. Electro conductivity test.

Cold test

Cold test is the oldest vigor test and it has been used since 1920. Originally, it was used for analyzing pathogen contamination of young maize plants. However, with chemical industry development and fungicide application in seed treatment pathogen effect was put under control. Cold test simulates adverse conditions in the field: physiological damage of seed that was caused by prolonged and unconditional storage is being evaluated using damages from frost and drought. Advantage of Cold test is that during the analyses seed is being exposed to stress conditions: low temperature, moisture surplus and pathogen presence. Great disadvantage of Cold test is variability of results induced by using different types of soil. This test is widely accepted as maize vigor indicator.

Seedling growth test

It is used for valuation of seedling growth for species that have real shoot (cereals and grasses). Seed lots belonging to the same variety but having higher average length of seedlings have higher vigor *(Hampton and Te Krony, 1995; Djukanović, 1999; Djukanović, 2003)*.

Accelerated ageing test

This test is used for valuation of seed longevity. Seed is exposed to high temperature and high humidity, which leads to its fast deterioration. Seed with high vigor persist these extreme stress conditions and deteriorates slower than seed with low vigor *(Te Krony, 1993; Dragicević, 2007)*. Results of this test correlate with values of field emergence. Accelerated ageing test was introduced with purpose to evaluate storage duration of commercial seed. Accelerated ageing test enables to affirm if the particular seed lot has higher or lower potential to maintain germination and determinate with relative precision safe period for storing the seed in the storage room *(Delonche and Baskin, 1973)*.

Tetrazolium test

Tetrazolium test is used for quick determination of germination. This test is not reliable enough and it is used for getting preliminary results particularly for seed that have long period of dormancy. From 1966, TTZ test is included in ISTA and AOSA rules. In 1942, Lakon developed topographic Tetrazolium test and defined it as a potential germination – viability *(Milivojević, 2005)*. Seed with potential germination are sorted in three categories (high, medium and low vigorous seed) according to the presence of embryo and scutellum necrosis at monocotyle plants and in embryo and cotyledons at dicotyle plants. This test is biochemical analysis and its purpose is to get quick, basic evaluations of seed viability, especially those that express dormancy (ISTA, 2005). Tetrazolium test is based on reduction of colourless solution of 2, 3, 5 – threepheniltetrazolium chloride to insoluble coloured in red 2, 3, 5 – threephenilphormazan. This reaction is passing with activity of dehydrogenase enzyme group in vital seed parts. Preferences of TTZ test are getting quick results, analyzing viability of dormant seed, understanding life process in seed and at the same time determination of vigor and cause of low seed quality. It provides additional information that is significant in experimental programs. Lacks of this test are that it requires specially trained specialists with plenty of experience, it needs more time per sample in comparison with Standard germination test, and it is not possible to determine neither pathogen presence nor phytotoxic effect of fungicides. In plant species without dormancy expression TTZ test and Standard germination test may have equal values *(Milivojević, 2005)*.

Hiltner test

This test is developing in two ways. First is the seed health test that is used to affirm damage caused often by *Fusarium* species. Second is vigor test that reveals damages that inhibit normal seedling growth. Damaged seed or low vigorous seed is incapable of persisting adverse conditions during germination. Lack of this test is that it is expensive and specific (substrate is a ground brick that is sterilized and rinsed). Previously given tests are often used in practice, but there are few more tests that can be sorted in following categories.

Indicators of vigor

Indicators of vigor are:

- Growth rate coefficient (number of grown plants per day) by Heydecker, where middle time of growth is a ratio between number of grown seed until "D" day and number of days since sowing day.

- Middle time of growth is a coalition of growth expressed through number of grown seed per day by Kotowski.
- Vigor index (relative number) that can produced by receiving average values and is potentially possible by receiving maximum values.

Application of vigor tests

Despite great problems in development and standardization of vigor tests, they quickly found their place in practice. In 1988, research has shown that 83% ISTA laboratories consider vigor tests needed and that 65% of these laboratories are using them in practice *(Hampton, 1993)*. Researchers set a goal to determine if the seed lots with high vigor give higher yield. Numerous authors were engaged in this problem on various plant species. Experiments have shown that the effect of faster growth of more vigorous lots do not always demonstrate higher final yield. *Te Krony and Egli (1991)* give the conclusion that vigor in great deal affects on yield on those plant species grown for vegetative mass, but the effect of vigor is not significant on plants grown for seed. Some authors say that vigor indirectly affects on yield in situations when there is tight correlation between vegetative growth and yield (exp. reduced plant density, later sowing). Development of vigor tests took place in several different laboratories that led to appearance of great number of various tests for different plant species. Plenty of vigor tests can be advantage in perceiving seed quality, but that is a major obstacle for standardization in case when one vigor test practiced in different ways (in case of Cold test and Accelerated ageing test). *Milosević et al. (1994), Djukanović et al. (1997) and Djukanović (2003)* applied different methods of analyzing vigor on maize hybrids, and the highest correlation with field emergence had Accelerated ageing test and Cold test. Accelerated ageing test on soybean and Electro conductivity test on peas are only two tests standardized by ISTA rules.

Table 1. Vigor tests practiced on forage in the world

Accelerated ageing test	legumes	clovers	grasses
Electro conductivity test	legumes	clovers	grasses
Cold test	legumes		
TTZ test	legumes		grasses

During germination tests all seedlings that formed basic structures vital for plant development fall into normal seedlings. In vigor handbook, *(Hampton and Te Krony, 1995)* method of measuring shoot length was described. In Seedling growth test, Cold test, Accelerated ageing test as in Standard germination test shoot and root length is measured. Research of *Sautipracha et al. (1997)* had shown that accelerated ageing has less effect on shoot and root length, but more on seedling dry mass. Root and shoot length have tendency of increasing under accelerated ageing treatment *(Milivojević, 2005; Dragicević, 2007)*. Most of the plant species that belong to forage crops expressed dormancy and often weak growth in adverse conditions *(Wang et al, 2001)*. Part of the hard seed of forage galega increased along with the duration of storage *(Djukanović, 2006)*. *Wang et al. (2004)* analyzed different vigor tests (Electro conductivity test, Controlled deterioration test, Field emergence test, germination index, radical length and Standard germination test) to estimate seed lot quality and foresee field emergence. Researches were conducted during two years on four forage crops, two legumes: purple vetch (*Vicia benghalensis* L.) and alfalfa (*Medicago sativa* L.) and two grasses: sudangrass (*Sorghum bicolor* L.) and siberian wild ryegrass (*Elymus sibiricus* L.). Variable field emergence, less than standard germination were expected, so was confirmed in both years of research on alfalfa and siberian wild ryegrass. Research results show that Electro conductivity test on legumes show high correlation with field emergence and standard germination. Electro conductivity test was more precise in determination of quality of alfalfa and *Trifolium pratense* L. Grass seed lots differed in vigor and seed coat density, as seen from the electrolyte leaking results, being the main reason for vigor deterioration. Electro conductivity test on grasses had lower correlation with Standard germination test and field emergence. Received results on grasses are similar with results received from research conducted on perenial ryegrass seed (*Lolium perene* L.) and italian ryegrass (*Lolium multiflorum* L.) (*Marshall and Naylor, 1985*), *Bromus biebersteinii (Hall and Wiesner, 1990)*, *Lotus corniculatus* L. and alfalfa *(Wang et al., 1996)*. Electro conductivity test on *Festuca arundinacea* was in correlation with field emergence and in glasshouse *(Han et al., 1995)*.

Table 2. Correlation coefficients (r) between standard germination or vigor and field emergence of seed lots for two legume species tested in 1999 and 2000 by *Wang et al. (2004)*

	Purple vetch			Alfalfa		
	FE 1999 (n=15)	FE 2000 (n=12)	Total (n=27)	FE 1999 (n=13)	FE 2000 (n=17)	Total (n=30)
SG	0.799**	0.468	0.700**	0.590*	0.494*	0.563**
SGi	0.893**	0.010	0.368	0.601*	0.460	0.511**
GI	0.885**	0.086	0.679**	0.715**	0.303	0.267
RL	9.450**	-0.100	0.285	0.489	-0.242	0.285
CD	0.967**	0.688*	0.745*	0.683**	0.510*	0.568**
EC	-0.808**	-0.610*	-0.757**	-0.758**	-0.540*	-0.645**

* Significant at P<0.05 ** Significant at P<0.01

SG: Standard germination, SGi:Initial count of SG, GI: Germination index, RL: Radical length, CD: Controlled deterioration, EC: Electrical conductivity, FE: Field emergence.

Research results on seed lots with low viability *(Wang et al., 2004)* signify that Electro conductivity test was not in correlation with standard germination. It was noticed that the seed with low germination, so as the dead seed, had similar results received by Electro conductivity test like the seed with high germination. Explanation for correlative relations for grasses is still unknown, so authors believe that research should be continued.

Wang et al. (2004) with application of Control deterioration test show that seed vigor results are better than results received from Standard germination test, with exception it was less precise on alfalfa and not significantly correlated with sigerian wild ryegrass. It happened because of the great number of hard seed and low moisture content in alfalfa seed (in first year of research 16% of hard seed and in second year 10%). Standard germination test has not shown wide range of germination after Accelerated ageing test on alfalfa seed. Authors presume that better results could be received if the seed had higher moisture content. Electro conductivity test on forage legumes and grasses showed great potential in vigor determination and predicting field emergence. *Tomer and Maquire (1990)* point out that high vigorous sees have uniform field emergence, give powerful plants and higher yield. *Ferguson (1993)* says that seed lots that have similar values of germination differ in vigor, which point on faster loss of vigor against the rate of emergence. Comparing standard with vigor method *Djukanovic et al. (2008)* found that there are significant differences for each three maize hybrids between Standard germination test and Cold test, and between TTZ test and Cold test. Same authors point out existence of statistically significant differences between Standard germination test and Accelerated ageing test, same as between TTZ test and TTZ test after Accelerated ageing test.

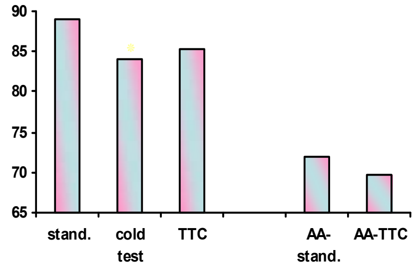

Figure 1. Comparison of different vigor tests with Standard germination test

Conclusion

Vigor test methods are used as additional information about seed physiological quality (sowing potential) and for determination of seed storage conditions. Each of vigor tests has specific preferences and lacks that are necessary to perceive so the received information could be interpreted in a right way. Seed producer can easily associate results received by Cold test with conditions in the field. However, Accelerated ageing test is not easily to correlate with field emergence, although it is better indicator than Standard germination test. Vigor tests should have certain characteristics so they could be acceptable for seed producer as final seed user. Tests cannot predict field emergence (number of plants grown in the field) because of different types of soil and variability of soil temperature and moisture. Seed germination rate is considered for seed vigor indicator.High quality seed is capable to germinate in a high rate and uniformly in different environmental conditions. In favourable conditions for germination, differences between seed lots cannot be noticed, while in adverse conditions are easily noticed. Vigor test (Cold test, Accelerated ageing test) is better field emergence indicator than Standard germination test.

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Primena vigor testova na semenu krmnih biljaka

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Rezime

Imajući u vidu da su krmne biljke iz različitih familija samim tim se i veoma razlikuju po genetičkim, morfološkim, fiziološkim i drugim osobinama. Takođe i njihova semena se veoma razlikuju po građi, hemijskom sastavu, krupnoći i dr. Po površinama koje zauzimaju i prinosu koje daju najznačajnije su višegodišnje i jednogodišnje leguminoze (fam. *Fabaceae*) i višegodišnje i jednogodišnje krmne trave (fam. *Poaceae*). Samim tim i seme ovih krmnih vrsta je najznačajnije za naše semenarstvo. Vigor semena ovih vrsta je skup onih osobina koje određuju aktivnosti i ponašanje partije semena, koja ima komercijalno prihvatljive vrednosti klijavosti u polju u različitim uslovima spoljne sredine. Aspekti koji ukazuju na seme sa visokim i niskim vigorom su brojni, a među najvažnije se ubrajaju biohemijski procesi i reakcije tokom klijanja, kao što su stepen uniformnosti klijanja semena i porasta klice, stepen uniformnosti energije klijanja i porasta u polju, kao i ispitivanje energije klijanja pod nepovoljnim uslovima spoljne sredine Cilj vigor testova je da daju sumu svih karaktera semena

koji posle setve dovode do brzog, ujednačenog stvaranja zdravog i snažnog ponika u različitim uslovima. Kod semena krmnih biljaka je moguće primeniti sledeće testove: test ubrzanog starenja; test elektroprovodljivosti; hladni test i tetrazolium test.

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TESTING OF FIELD PEA SEED VIABILITY

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Original scientific paper

Abstract: Field pea is an important plant species in the diet of all domestic animals due to its high content of crude protein and stimulating substances such as vitamin C. Achievement of high yield depends on genotype, environmental factors and seed quality. An important component of seed quality is seed viability and seed vigor, which represent a set of those characteristics that determine the activity and behavior of a seed lot, commercially acceptable seed germination in different environmental conditions. Seed viability testing was done on three commercial field pea varieties (NS Junior, Javor and Jezero). Standard laboratory method, vigor tests (Cold test, Accelerated aging tests, and Hiltner test), and field emergence were applied. Seed germination obtained by standard laboratory test ranged from 75 to 88%, and by vigor tests from 50 to 87%.

Key words: germination, field pea, seed viability

Introduction

Field pea is an important plant species in the diet of all domestic animals due to its high content of crude protein and stimulating substances such as vitamin C. Due to its high protein content it can partially replace soybean meal in the meal of ruminants and non-ruminants *(Mikić et al., 2003)*. Due to high nutritive value and modest investments in the production process, a significant financial effect can be achieved. Seed production today is an important link in the process of meeting the ever growing demand for sufficient quantity of healthy and safe food. The seed marks the beginning of each plant production, and therefore ensuring its quality is the priority of modern seed science, and prerequisite for achieving high yields of all cultures *(Milošević at al., 2007)*.

Determination of seed quality and its viability indicates what seed lots can be found on the market, and for that reason the control of methods and tests used for testing of seed lot quality, i.e. seed vigor must be very good. Seed viability or seed vigor are the set of characteristics that determine the activity and behavior of the seed lots of commercially acceptable seed germination in different environmental conditions. In addition to the above mentioned, longevity of seed is determined by the seed vigor without adverse consequence *(ISTA, 2009)*. Testing of seed viability using different seed vigor tests is very significant, since vigor tests give results, which are often better correlated with the results of field germination under unfavorable environmental conditions, than the results obtained by application of standard laboratory germination test *(Johansen and Wax, 1978)*. In order to obtain more precise information on seed lot quality different vigor tests such as accelerated aging test, cold test, Hiltner test, etc. are used.

Accelerated aging test is one of the most often used tests for vigor testing today, first of all because it is well correlated with field emergence *(Lovato et al., 2001)*. Accelerated aging test is the most often used test for soybean vigor testing in North America *Ferguson, 1990*). Accelerated aging test is used to estimate if a certain seed lot has lower or greater ability to preserve germination, and to relatively precisely determine the period of safe seed storage. According to *Wotza and TeKrony (2001)* germination results obtained by applied cold test represent the most precise indicators of maize germination in the field. Cold test is the most often used for maize, sorghum and soybean in North America and Europe *(Hampton, 1992)*. Besides estimation of seed germination under the most favorable conditions, *AOSA (2002)* also mentioned other possibilities of usage of this test for estimation of fungicide efficacy, determination of seed lots for early spring sowing, estimation of physiological damages caused by prolonged storage under unfavorable conditions, frost or drought damages, and measurement of the effects of mechanical damages on germination in cold and wet soil. Hiltner test is based on the fact that damaged seeds is often weak (physiological injuries, frost injuries, fungicide treatments etc.) and unable to withstand adverse conditions during germination. Namely, a layer of stones or small brick particles used in this test cause physical stress and prevent germination. Under such unfavorable conditions only high vigor seed is capable of forming typical seedling *(Hampton and TeKrony, 1995)*. The aim of this paper was to determine if there were differences between tested varieties of field pea when different vigor tests were applied.

Materials and Methods

Three commercial varieties of field pea (NS Junior, Javor and Jezero) selected in the Institute of field and vegetable crops in Novi Sad were tested. Germination of seed was tested using standard laboratory test in laboratory condition. Accelerated aging test, cold test and Hiltner test were used to estimate seed vigor. Field germination was determined under field conditions. Four replicates x 100 seeds were used to test standard laboratory germination. Seed was germinated on sand. Incubation period was eight days at 20^{o}C, and relative air humidity of 95% *(ISTA, 2009)*. After that, the number of normal and abnormal seedlings, ungerminated seed and above ground and root system length were determined. Four replicates of 50 seed were used in accelerated aging (AA test). Before the seed was placed under optimal conditions, as in standard laboratory test, the seed was exposed to 42^{o}C, and relative air humidity of 100% for 48 hours *(Hampton and TeKrony, 1995)*. After that the seed germination and growth parameters were determined. When cold test was used, the seed was exposed to low temperature of 10^{o}C for seven days, and then placed under optimal germination for four days, and after that the seed germination and above ground and root system length were estimated. The medium used was a mixture of soil and sand 2:1 *(Hampton and TeKrony, 1995)*. Four replicates of 50 seed were used in Hiltner test. The seed was placed on wet sand (2cm thick) and covered with a 3 cm thick layer of broken bricks *(Hampton and TeKrony, 1995)*. Incubation period was 10 days at 10 20^{o}C, and after that the same parameters were determined as in previous tests. Field germination was determined based on 4 x 100 seeds. After incubation period of 14 days seed germination and above ground and root system length were determined. Obtained data were analyzed using analysis of variance *(Hadživuković, 1991)* and graphically presented.

Results and Discussion

Seed germination of NS Junior ranged from 70 to 79% depending on applied test (Graph. 1). Significantly lower value (P<0.05) was obtained in filed condition. There was no statistically significant difference between applied laboratory test and other vigor tests. Javor variety had the highest seed germination when accelerated aging test was applied (76%) (Figure 1). Statistically significantly the lowest value (P<0.05) in this variety was obtained by applied cold test (50%). When standard laboratory test was applied obtained germination value was 75%, and field germination was 61%, and it was statistically significantly lower (P<0.05) in relation to standard germination. In relation to the above mentioned varieties, Jezero variety had the highest values for seed germination in all applied tests (Graph. 1). In this variety the statistically significant difference (P<0.05) was obtained between standard laboratory germination (88%) and Hiltner tests (87%) on one, and filed germination (81%) on the other hand.

In these investigations the seed germination obtained by standard laboratory test was in good correlation with the values obtained by Hiltner test, and accelerated aging test. According to the results obtained by *Egli and TeKrony*, *(1996)* and *Lovato et al. (2001)* higher correlation was found between the accelerated aging test and emergence values in the field, than the results of standard germination. Hiltner test is applied in some laboratories in Europe, and the basic reason for that is correlation variability between obtained results and field emergence. This test also sometimes fails to give additional information on seed quality in relation to standard laboratory test *(Hampton and TeKrony, 1995)*.

In our investigations the field germination was positively correlated with germination obtained by cold test, which was also confirmed by *Milošević et al. (1994)* and *Lovato et al. (2001, 2005)*. Data obtained by *Gutormsona (1995)* proved that there was a good correlation between standard germination and field germination under favorable field condition, and a positive correlation was obtained between field germination and cold test under stress conditions.

Length of the above ground part ranged from 183.0 mm in Junior variety (Hiltner test) to 51.0 (cold test) (Figure 2).

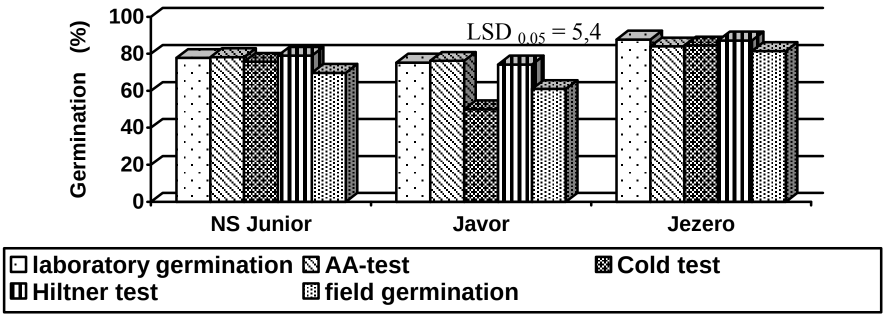

Figure 1. Germination of field pea seed obtained by application of different vigor tests

Statistically significant differences (P<0.05) were found between all applied tests, except between length obtained by standard laboratory test and accelerated aging test; root system length of the same variety, also depended on applied tests and ranged between 140.3 mm (Hiltner test) to 58.75 mm (cold test) (Graph. 3). As in the above ground length, statistically significant differences (P<0.05) were also found in the root system length between all applied tests.

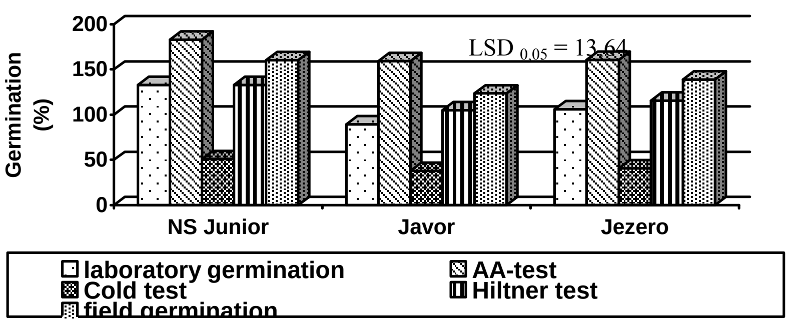

Figure 2. Above ground part length of field pea seedling obtained by application of different vigor tests

Significantly lower values (P<0.05) of above ground length were obtained for Javor and Jezero in relation to NS Junior with all applied tests. The above ground length of Javor variety ranged from 159.3 mm (Hiltner test) to 37.75 mm (cold test). The length of tested parameter of Jezero variety ranged from 160.8 mm (Hiltner test) to 40.5 mm (cold test) (Graph. 2). The length of the root system in Javor and Jezero varieties had the same relationship between applied tests as in NS Junior variety (Figure 3). In all tested varieties statistically the lowest values for the length of aboveground part and root system were obtained using cold test, which indicates that low temperature had inhibitory effect on seedling growth. The speed at which seed germinated and seedling growth intensity may depend on genetic differences in seed size, coat characteristics and chemical composition *(Ferguson, 1995)*. By applying the seedling growth test, the comparisons should be made only within one variety not between varieties, because the varieties may have inherently different seedling growth intensity, which is not a vigor seed characteristic. When comparisons between lots of the same variety are made, the lots that have longer seedlings usually have greater vigor *(Hampton and TeKrony, 1995)*.

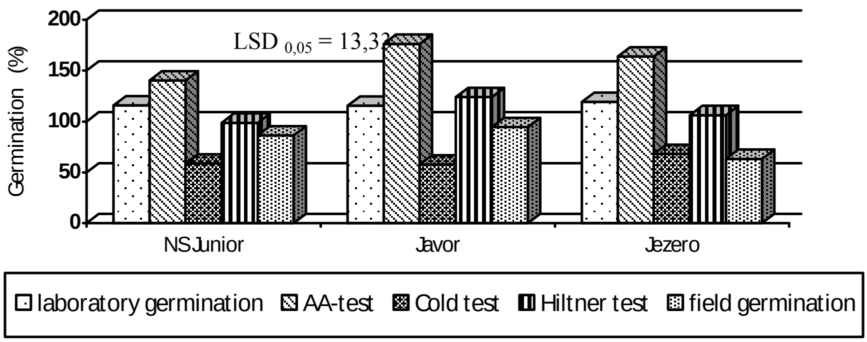

Figure 3. Root system length of field pea seedling obtained by application of different vigor tests

Conclusion

Based on the obtained results the following can be concluded: Seed germination obtained using standard laboratory test was in a good correlation with the values obtained using Hiltner test and accelerated aging test. Field germination was in positive correlation with that obtained using cold test. Cold test could be used as a supplementary test for obtaining reliable results on seed germination. Variety Jezero had the highest values for seed germination in all applied tests.

In all tested varieties statistically the lowest values for the length of aboveground parts and root system were obtained using cold test.

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Ispitivanje životne sposobnosti semena stočnog graška

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Rezime

Stočni grašak je značajna biljna vrsta u ishrani svih vrsta domaćih životinja zbog visokog sadržaja sirovih proteina i stimulirajućih materija poput vitamina C. Ostvarivanje visokog prinosa semena zavisi od genotipa, faktora spoljne sredine i kvaliteta semena. Značajna komponenta kvaliteta semena je životna sposobnost ili vigor koja predstavlja skup onih osobina koje određuju aktivnost i ponašanje partije semena komercijalno prihvatljive klijavosti u različitim uslovima spoljašnje sredine.

Ispitivanje životne sposobnosti semena izvršeno je na tri komercijalne sorte stočnog graška (NS Junior, Javor i Jezero), primenom standardnog laboratorijskog metoda, vigor testova (hladni test, test ubrzanog starenja i Hiltner test) i poljske klijavosti. Klijavost semena dobijena standardnom laboratorijskom metodom bila je od 75 do 88%, a primenom vigor testova od 50 do 87%.

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YIELD AND SEED QUALITY OF ALFALFA SEED (*Medicago sativa L.*) IN AGRO-ECOLOGICAL CONDITIONS OF BANJA LUKA REGION

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Original scientific paper

Abstract: This paper shows the results of yield and quality testing of 18 alfalfa genotypes in agro-ecological conditions of Banja Luka region. The testing was conducted at the trial field of the Agricultural Institute in Banja Luka in three-year period, form 2004-2006. The trial was conducted on alluvial meadow soil. The highest seed yield, for all three years of trial, was obtained by the genotype 62 exp. (502.1 kg ha^{-1}). During the triennial trial alfalfa genotypes gave 341.9 kg ha^{-1} of seed in average. Alfalfa seed germination varied from 74.0% to 88.0%. Portion of alfalfa hard seeds ranged between 3% to 12%, while the 1000-seed weight was in a range between 1,60 g to 1,85 g. The results of this testing indicated the possibility of selecting the genotypes that gave the highest yield and the best seed quality and other traits which presents the ultimate goal in alfalfa breeding.

Key words: genotype, alfalfa, yield and quality of seed.

Introduction

In the Republic of Srpska, alfalfa is produced on 24,428 ha, so that in the structure of sown area it occupied approximately 10% in 2008. The largest part of our need for providing the alfalfa seed is imported, and a small quantity of seeds is produced in our area. Quality seed is a complex category and it is determined by a number of indicators: cleanliness, germination energy and germination, the proportion of hard seeds, 1000-seed weight, etc., which are influenced by various factors of the environment. The local variety of alfalfa Banjalučanka has a high potential for forage and seed yields, but it is being produced on small areas. *Dosen (1989)* reported lower alfalfa seed yield, in three-year average of 81.61 kg ha^{-1}, in Banja Luka. According to the same author, the highest alfalfa seed yield was achieved with sowing variant of 30 x 30 cm, 115.4 kg ha^{-1} of seed was produced. Seed yields range from 100-1500 kg ha^{-1} depending on growing conditions and applied technology *(Vuckovic et al., 2002)*. Also, *Vuckovic (1991)* reported that it is possible to produce 600 kg ha^{-1} of alfalfa seed from the first cutting with spring sowing in the first year of life. The aim of this paper is the analysis of realized yields and seed quality of 18 alfalfa genotypes, which are in the process of selection, in agro-ecological conditions of Banja Luka region.

Materials and Methods

The tests were conducted on the experimental field of Agricultural Institute of Banja Luka, in the three-year period, from 2004-2006. During testing, 18 alfalfa genotypes were tested that are indicated with following numbers: 62, 60, 59, 58, 56, 55, 54, 53, 52, 51, 50, 49, 34, 29, 57, 61, 47 and 33. The standard was alfalfa variety Banjalučanka. The size of the basic plot was to 5 m^2, the distance between rows 20 cm. For sowing, 15 kg ha^{-1} of seed was used. Chemical properties of soil are determined with standard agrochemical analysis. While performing experiments the basic meteorological elements relevant to this study were monitored. Data were obtained from the Meteorological Station Banja Luka.

Agro-ecological conditions. Results of chemical analysis of the alluvial-meadow soil properties showed that this soil was favourable for alfalfa growing (Table 1).

Table 1. Chemical properties of soil

Depth (cm)	pH		Humus (%)	Content in mg/100g of soil	
	H_2O	KCl		P_2O_5	K_2O
0-20	6.6	5.9	3.7	9.5	10.6
20-40	6.7	5.9	3.2	9.8	14.9
40-60	6.9	6.1	3.0	20.6	16.9

Table 2. Precipitation and mean monthly temperatures for vegetation period (2004-2006) and perennial average (1961-2004)

Month	Precipitation (l/m^2)				Temperatures (^{0}C)			
	2004	2005	2006	1961-2004	2004	2005	2006	1961-2004
IV	166.4	80.5	151.6	90.9	11.9	11.8	12,4	10,9
V	86.1	79.2	85.0	95.4	14.8	16,3	15,9	15,9
VI	104.3	135.6	126.7	111.6	19.6	19,4	20,1	19,4
VII	129.6	129.7	90.0	94.5	21.6	22,0	22,9	20,9
VIII	45.0	124.9	220.0	82.8	21.4	19,4	19,6	20,6
IX	63.0	79.9	47.4	94.3	16.0	17,0	17,4	16,1
Total	594.4	529.8	720.7	569.5				
Average					17.6	17.7	18.1	17.3

The climate conditions were analyzed on the basis of monthly precipitations and mean monthly temperatures (Table 2).

Results and Discussion

Seed yield of the tested alfalfa genotypes. The results of the seed yield varied within the trial year, as well as during the total period 2004-2006. In the first year, the average seed yield of 198.8 kg ha^{-1} was obtained. Variation within the genotypes ranged from 129.7 kg ha^{-1} with genotype 53 exp. the maximum yield of 404.7 kg ha^{-1} with alfalfa genotype 62 exp. (Table 3).

Table 3. Yields of tested genotypes of alfalfa during the period 2004/2006 (kg ha^{-1})

No.	Genotypes	Year			Average	Relative yield
		2004	2005	2006		
1	62 exp.	404.7	900.0	201.6	502.1	146.82
2	60 exp.	156.2	303.0	181.3	213.5	62.43
3	59 exp.	189.1	296.0	206.0	230.4	67.36
4	58 exp.	154.7	488.0	285.9	309.5	90.52
5	56 exp.	148.4	380.0	312.5	280.3	81.97
6	55 exp.	139.1	457.0	229.7	275.3	80.49
7	54 exp.	160.9	477.0	314.4	317.4	92.83
8	53 exp.	129.7	407.0	203.1	246.6	72.11
9	52 exp.	215.6	750.0	259.4	408.3	119.41
10	51 exp.	228.1	958.0	225.0	470.4	137.55
11	50 exp.	231.3	651.0	237.5	373.3	109.15
12	49 exp.	176.6	620.0	278.1	358.2	104.75
13	34 exp.	212.5	652.0	250.0	371.5	108.64
14	29 exp.	143.8	557.0	267.2	322.7	94.35
15	57 exp.	168.8	625.0	332.8	375.5	109.81
16	61exp.	129.7	347.0	284.3	253.7	74.18
17	47exp.	204.7	582.0	398.4	395.0	115.52
18	33exp.	385.9	690.0	279.7	451.9	132.14
Average		198.8	563.3	263.7	341.9	100
LSD - 0,05		36,6	39,1	94,3		
LSD - 0,01		50,2	53,7	129,6		

In the second year, the highest alfalfa seed yield of 563.3 kg ha^{-1} was obtained. Statistically, this is a very significant variation within the tested genotypes, from the lowest seed yield of 296.0 kg ha^{-1} with genotype 59 exp., to the maximum yields of 958.0 kg ha^{-1} with genotype alfalfa 51 exp. In the third year, a lower average seed yield was obtained, compared to the previous year (263.7 kg ha^{-1}). Based on the results of *Djukic and Eric (1995)*, the meteorological elements with its complex effect have a decisive influence on the quantity of seed yield. This is especially pronounced in forage plants, both because of their specific morphological structure, and because of the impact of weather on pollination and fertilization processes. Influence of air temperature, amount and distribution of precipitation is the most pronounced. Warm, dry and sunny years are suitable for successful alfalfa seed production as reported by *Vuckovic et al. (2003)*. Based on these results, it is obvious that the seed yield varied among genotypes within one year, as well as for every year of testing depending on weather conditions and other factors. In the second year of testing, weather conditions were favorable for seed production, where the maximum seed yields were achieved. Genotype 62 exp. achieved the highest yield of 502.1 kg ha^{-1} of seed, while the lowest yield had genotype 60 exp (213.5 kg ha^{-1}). Alfalfa genotypes produced 341.9 kg ha^{-1} of seed in average. *Dosen (1989)* reported somewhat lower yields of seed, with a variant of sowing 30 x 30 cm, was 115.4 kg ha^{-1} of alfalfa seed obtained.

Seed quality of the tested alfalfa genotypes. Quality characteristics of seeds: germination energy, germination, hard seed and 1000-seed weight were made in the first year of testing, and the average value of the analysis results are summarized in Table 4.

Table 4. Seed quality of the tested alfalfa genotypes

No.	Genotypes	Germination energy%	Seed germination%	Hard seed%	1000-seed weight in g
1	62 exp.	57.0	75.0	5.0	1.73
2	60 exp.	58.0	82.0	7.0	1.74
3	59 exp.	57.0	88.0	9.0	1.74
4	58 exp.	60.0	82.0	8.0	1.70
5	56 exp.	57.0	80.0	5.0	1.65
6	55 exp.	60.0	85.0	12.0	1.67
7	54 exp.	63.0	85.0	6.0	1.70
8	53 exp.	61.0	79.0	4.0	1.70
9	52 exp.	57.0	80.0	9.0	1.77
10	51 exp.	57.0	78.0	7.0	1.60
11	50 exp.	59.0	84.0	6.0	1.72
12	49 exp.	59.0	77.0	4.0	1.70
13	34 exp.	61.0	84.0	8.0	1.72
14	29 exp.	57.0	74.0	3,0	1.80
15	57 exp.	57.0	75.0	7.0	1.85
16	61exp.	56.0	78.0	11.0	1.84
17	47 exp.	63.0	85.0	7.0	1.74
18	33exp.	59.0	84.0	10.0	1,83
Average		58.8	80.9	7.1	1,73

Seed germination energy - The average energy of germination was 58.8%. The highest energy of germination of seeds had the genotype 47exp. (63.0%), while the lowest energy of germination achieved genotype 61 exp. (56.0%). Good germination of seeds in addition includes a high percentage of germination and germination speed or energy. Weather conditions have major impact on energy of germination of alfalfa seeds. According to research by *Dosen (1989)* germination energy of alfalfa seed in Banja Luka varied on average by 54.2% to 89.9% in the first year of research.

Germination of seeds - The average germination of alfalfa genotypes seed was 80.9%. Germination of seeds varied with the genotypes from 74.0% to 88.0%. This is a very significant biological characteristics and indicators of its environmental capacity and economic value. It depends on weather conditions during the development and maturing of seeds, cultural methods applied and other factors. *Dosen (1989)* noted data for germination ranges from 79.6% to 95.4% at Banja Luka and Roman Šančevi near Novi Sad localities. The average energy of sprouting and germination of cultivars NS Banat ZMS II and NS-Medina ZMS V and all of the amounts to 77% and 82% as reported by *Karagić et al. (2000)*.

The proportion of hard seeds - The proportion of hard seeds varied within the tested genotypes from 3.0% (genotype 29 exp.) to 12.0% (genotype 55 exp.), while the average for all genotypes was 7.1% of hard seeds. Hard seeds are common in alfalfa and usually have good germination, but seedling enclosure prevents the adoption of water necessary for the germination process. The percentage of hard seeds is conditioned edaphic and climatic factors during and after ripening of seeds, and also genetic factors. Some cultivars formed more hard seed than others. According to research by *Došen (1989)*, the presence of the alfalfa hard seeds for the year and the period of research in Banja Luka was in the interval from 12.7% to 20.3%. *Karagić et al. (2000)* reported that the proportion of hard seeds varied among years from 1% to 14%, and the average was low 5%.

1000-seed weight - Is an indication seed size and filling. The average 1000-seed weight for all alfalfa genotypes was 1.73 g. The alfalfa 1000-seed weight varied depending on the weather conditions in the range from 1.60 g to 1.85 g.

Similar results of the 1000-seed weight reported *Dosen (1989)* with alfalfa from 1.72 g to 1.90 g at the site in Banja Luka. According to *Karagić et al. (2000)* the variations of 1000-seed weight amounted to 2.0 to 2.3 g.

Conclusion

Based on the results of the three-year trial on seed yield and quality of alfalfa genotypes in the agro-ecological conditions in the Banja Luka region, we can conclude the following:

Alfalfa genotypes achieved 341.9 kg ha^{-1} of seed in average. The largest seed yield of 502.1 kg ha^{-1} achieved genotype 62 exp. in all the years o testing. The tested genotypes showed significant differences in germination energy. Maximum energy of seed germination had the genotype 47exp. (63.0%), while the lowest germination energy had the genotype 61exp. (56.0%). Germination of seeds varied with the tested genotypes from 74.0% to 88.0%, while the proportion of hard seeds alfalfa ranged from 3% to 12%. Alfalfa 1000-seed weight varied ranging from 1.60 g to 1.85 g.

The results of these studies suggest the possibility of selecting genotypes that have achieved the highest yield and best quality seeds, which represents the ultimate goal of the selection process and breeding.

Prinos i kvalitet semena lucerke (*Medicago sativa L.*) u agroekološkim uslovima banjalučke regije

S. Vojin, Ž. Lakić

Rezime

U radu su prikazani rezultati ispitivanja prinosa i kvaliteta semena 18 genotipova lucerke u agroekološkim uslovima banjalučke regije. Ispitivanja su obavljena na oglednom polju Poljoprivrednog instituta u Banja Luci u trogodišnjem periodu, od 2004. do 2006. godine. Ogled je izveden na aluvijalno-livadskom zemljištu. Najveći prinos semena, za sve godine ispitivanja, ostvario je genotip 62 exp. (502,1 kg ha^{-1}). U trogodišnjim isptivanjima genotipovi lucreke ostvarili su prosečno 341,9 kg ha-1 semena. Klijavost semena ispitivanih genotipova varirala je od 74,0% do 88,0%. Udeo tvrdog semena lucerke bio je od 3% do 12%, dok je masa 1000 semena bila u rasponu od 1,60 g do 1,85 g. Rezultati ovih ispitivanja ukazuju na mogućnost izdvajanja genotipova koji su postigli najveći prinos i najbolji kvalitet semena i druge osobine što predstavlja krajnji cilj u oplemnjivanju lucerke.

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EFFECT OF FOLIAR TREATMENT WITH PHOSPHORUS, POTASSIUM, BORON AND COBALT ON SEED YIELD AND YIELD COMPONENTS OF RED CLOVER (*Trifolium pratense* L.)

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Communication

Abstract: Red clover stands are generally established on acid soils where certain macro- and micronutrients may not be readily available to plants. The objective of this study was to evaluate the effect of foliar treatment with cobalt (Co), boron (B), and phosphorus and potassium (PK) on seed yield and yield components of red clover cultivars K-39, K-17, Una and Viola on the above soils. The foliar treatment with Co resulted in a significant increase in the number of stems per plant in cv. K-17, number of inflorescences per plant in cv. K-39, K-17 and Viola, and number of seeds per inflorescence and fertility in all cultivars. The foliar application of B caused a significant increase in the number of inflorescences per plant in cv. K-17 and in the number of inflorescences per stem, number of seeds per inflorescence and fertility in all cultivars. The foliar application of PK significantly increased the number of stems per plant in cv. K-39 and Viola, as well as the number of inflorescences both per stem and plant, number of seeds per inflorescence and fertility in all cultivars. The actual seed yield, as calculated from yield components, was substantially higher upon foliar treatments with Co, B and PK.

Key words: red clover, cultivar, foliar treatment, seed yield, yield components

Introduction

Seed yield of red clover (*Trifolium pratense* L.) is mostly dependent upon the genetic background of a cultivar, environmental conditions, first cut date, presence of insect pollinators and genotype/environment interaction (*Steiner et al. 1995)*. Moreover, adequate mineral nutrition, i.e. the levels of certain macro- and micronutrients in the plant can also contribute to seed yield of red clover, particularly so in case of seed production on acid soils. *Taylor and Quesenrberry*

(1996) reported that acid soils are rich in readily available forms of aluminium, iron and manganese but deficient in readily available phosphorus, calcium and molybdenum. Phosphorus is a constituent of all major classes of macromolecular and physiologically active compounds in the plant. It plays an important role in organogenesis, particularly in the development of generative organs *(Petrović and Kastori, 1992)*. The low mobility of phosphorus within the plant *(Koontz and Biddulph, 1957)* and its high levels in the generative organs indicate the importance of the nutrient supply to plants. Foliar or soil application of cobalt has a favourable effect on symbiotic nitrogen fixation in red clover *(Vrany, 1978)*, plant growth, dry matter yield and seed yield of soybean *(Reddy and Raj, 1975)*. As reported by *(Lewis, 1978; Wilczek and Ćwintal 2008)*, a sufficient boron supply enhances pollen germination, fertilization and, hence, fertility i.e. seed number per inflorescence in red clover.

Red clover seed production in Serbia is generally undertaken as combined production for both forage and seed, with the seed being produced from the second growth of the second year stand or, at times, from the second growth of the third-year stand, in order to improve reliability and profitability of production *(Lugić et al., 1996)*. Red clover stands are generally established on acid soils where certain macro- and micronutrients may not be readily available for plants. The objective of this study was to evaluate the effect of foliar treatment with micro- and macronutrients on seed yield and yield components of red clover.

Materials and Methods

This experiment was set up on 4 June 2009 as an on-field trial at the Veterinary Extension Service in Čačak (43°54'39.06" N, 20°19'10.21" E, 246m a.s.l.). The soil was alluvial soil acid in reaction (pH_{H2O} 4.8), poor in nutrients and low in organic matter. Primary tillage was coupled with incorporation of 300 kg ha^{-1} $N_{15}P_{15}K_{15}$ into the soil. The factorial trial was established as a completely randomized block design in four replications. Each elementary plot was 5 m (5x1m). The red clover cultivars were used as follows: K-17, K-39 (Institute of Forage Crops, Kruševac), Una (Institute of Field and Vegetable Crops, Novi Sad) and Viola (a Polish cultivar). Sowing was conducted at a row spacing of 20 cm and a seeding rate of 18 kg ha^{-1}, in line with the recommendations regarding combined production *(Lugić et al., 1996)*. Foliar treatments included the following: cobalt (as $Co(NO_3)_2$) at a rate of 0.033 g l), applied, first, during the intensive growth stage and, then, prior to the onset of flowering, boron (Boron-feed, Haifa, Izrael, at a rate of 0.1%) used during the same stages as cobalt, and phosphorus and potassium ($P_{52}K_{34}$, Haifa, Israel, at a rate of 1%) during the intensive growth stage.

Mean annual air temperature in 2009 was 13.3°C (1.33°C above the ten-year mean), and the total sum of precipitation was 645 l m^{-2} (35l m^{-2} below the ten-year mean). Mechanical weed control treatments were performed twice. No irrigation was employed.

The yield components evaluated in this study included the following: number of stems per plant and number of inflorescences per stem (by counting them on a sample of ten randomly selected plants collected from the elementary plot) and number of inflorescences per square metre (by counting fertile inflorescences covering an area of $0.2m^2$ per elementary plot). The following laboratory determinations were made: the number of flowers per inflorescence (in a sample of ten inflorescences per elementary plot), fertility (number of fertile flowers at seed maturity in a sample of 100 pods separated from 15 inflorescences in an elementary plot) and thousand seed weight (based on the weight of 5x100 seeds taken from the sample as in case of pods). The number of seeds per inflorescence was calculated from the number of fertile flowers and total number of flowers per inflorescence. The actual seed yield was estimated using yield components (number of inflorescences per unit area, number of seeds per inflorescence, and thousand seed weight) and calculated as seed yield in kg ha^{-1}.

The obtained data were subjected to a two-factor (cultivar x foliar treatment) analysis of variance. An LSD test was used to analyze the significance of differences between cultivar means, foliar treatments and interaction means.

Results and Discussion

Irrespective of foliar treatment, cv. Viola produced a significantly higher number of stems per plant compared to cv. K-17 and K-39, whereas the trait in cv. Una was not significantly different from that of the other cultivars (Tab. 1). The foliar treatment with phosphorus and potassium (PK) and cobalt (Co) resulted in a significant increase in stem number per plant, as compared to the control, irrespective of cultivar. However, the significance of the cultivar/foliar treatment interaction suggested that the cultivars responded differently to the foliar treatments (Figure 1). A significantly higher number of stems as compared to the control in cv. K-39 and Viola were obtained only upon treatments with PK, whereas the increase in cv. K-17 resulted from Co treatment only. In contrast, the foliar treatments used for cv. Una did not have a significant effect on stem number per plant. *Vrany et al. (1978), Powrie (1964), Ozanne et al. (1963)* and *Reddy and Raj (1975)* reported that foliarly applied Co caused a significant increase in root nodule number and fixed nitrogen, resulting in a more intensified growth of red clover. *Schon and Blevins (1990)* showed that the foliar application of boron in soybean induced a significant increase in branch number per plant. The favourable effect of foliar treatment with PK can be related to its lower availability in acid soils *(Taylor and Quesenrberry, 1996)*, low mobility within the plant *(Koontz and Biddulph 1957)*, and treatments used at the onset of the intensive growth period.

Table 1. Mean values for seed yield components: number of stems per plant – NSP, number of inflorescences per stem – NIS, number of inflorescences per plant – NIP, number of flowers per inflorescence – NFI, number of seeds per inflorescence – NSI, fertility – F (%), thousand seed weight – TSW (g) and seed yield - SY (kg ha^{-1}) in red clover cultivars (A) as affected by foliar treatment (B) (control - 0, cobalt – Co, boron – B, phosphorus and potassium - PK)

	NSP	NIS	NIP	NFI	NSI	F	TSW	SY
A								
K-39	3.06b	2.93b	9.36b	103.7b	91.53b	88.5	1.86a	946b
K-17	2.98b	3.25b	9.72b	103.5b	91.63b	87.87	1.82ab	992.2b
UNA	3.21ab	3.86a	12.5a	112.7a	99.55a	88.37	1.78b	1020ab
VIOLA	3.36a	3.78a	12.24a	110.3ab	98.67a	89.56	1.77b	1208.7a
B								
0	2.94c	3.02c	9.03c	104.54	88.63b	85.06b	1.82	855.6b
Co	3.23ab	3.37bc	11.02b	108.36	96.52a	88.62a	1.81	1134a
B	3.12bc	3.6ab	10.56b	107.52	97.00a	90.31a	1.79	1063.4a
PK	3.37a	3.88a	13.2a	109.85	99.23a	90.31a	1.8	1114.6a
A	**	**	**	*	**	ns	*	*
B	**	**	**	ns	**	**	ns	*
AxB	**	ns	**	ns	ns	ns	ns	ns

Values followed by different small letters within columns are significantly different (P<0.05) according to the LSD test

[*]F test significant at P≤0.05; [**]F test significant at P≤0.01; ns – non significant

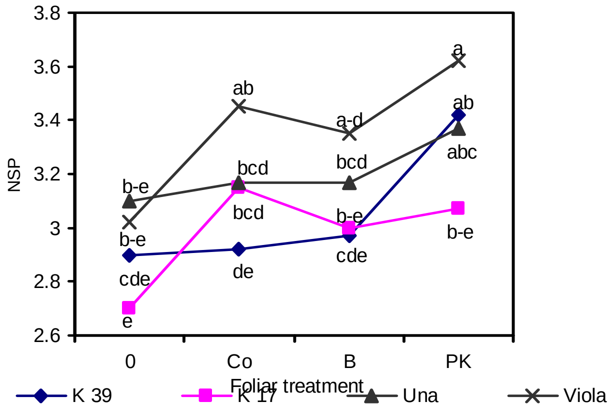

Figure 1. Number of stems per plant (NSP) as affected by foliar treatment with mineral nutrients (control-0, cobalt-Co, boron-B, phosphorus and potassium-PK) in red clover cultivars (K-39, K-17, Una, Viola)

The number of inflorescences per stem was significantly higher in cv. Una and Viola than in cv. K-39 and K-17. All cultivars showed an identical response to the foliar treatment with micro- and macronutrients, with the significantly higher number of inflorescences per plant being obtained with boron (B) and PK treatments, as compared to the control. The favourable effect of foliarly applied boron can be associated with a physiological antagonism between aluminium toxicity and boron deficiency *(Yang and Zhang 1998; Matsumoto 2000; Haby et al. 1998). Schon and Blevins (1989)* reported that the foliar treatment with boron in soybean resulted in a significant increase in pod number per branch.

Irrespective of foliar treatment, cv. Una and Viola developed a significantly higher number of inflorescences per plant compared to cv. K-39 and K-17. The number of inflorescences per plant was significantly higher in treatments with PK than in those with B and Co, regardless of cultivar, with the values obtained for all foliar treatments substantially surpassing those of the control. The significance of the cultivar/foliar treatment interaction for number of inflorescences per plant indicated different responses of the tested cultivars to the foliar treatment with particular nutrients. As opposed to cv. K-39 and Una, cv. K-17 and Viola had a significantly higher number of inflorescences per plant upon treatments with Co compared to the control. The foliarly applied boron resulted in a significant increase in the number of inflorescences per plant only in cv. K-17, whereas the other cultivars showed considerably higher values for the trait at treatments with PK than in the control.

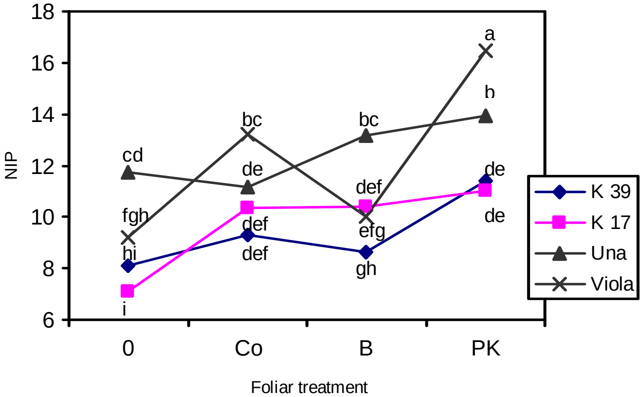

Figure 2. Number of inflorescences per plant (NIP) as affected by foliar treatments with mineral nutrients (control-0, cobalt-Co, boron-B, phosphorus and potassium-PK) in red clover cultivars (K-39, K-17, Una, Viola); Values followed by different small letters are significantly different (P<0.05) according to the LSD test

The number of flowers per inflorescence was significantly higher in cv. Una than in cv. K-39 and K-17, whereas the trait in cv. Viola was not significantly different from that of the other cultivars. The tested cultivars showed that the trait was not significantly affected by the foliar treatments.

A significantly higher number of seeds per inflorescence were developed by cvs. Una and Viola, compared to cvs. K-39 and K-17. The foliar treatment with Co, B and PK resulted in an increase in seed number per inflorescence ($P < 0.05$) in all cultivars equally. *Wilczek and Ćwintal (2008)* reported that foliar application of boron caused a significant increase in seed number per inflorescence i.e. an increase in flower fertility, whereas the number of inflorescences per unit area remained unchanged.

No significant differences between the cultivars were observed in terms of fertility, whereas the foliar treatments with Co, B and PK had an identical effect in increasing the values of the trait ($P < 0.05$) in all cultivars. *Wilczek and Ćwintal (2008)* suggest that boron causes higher pollen viability, increases pollen germination intensity, leads to larger presence of insect pollinators, which is, as reported by *Smith and Johnson (1969)*, associated with increasing levels of total sugars in nectar, primarily glucose and sucrose. *Lewis (1980)* also reported favourable effects of boron on pollination, fertilization and seed and fruit growth.

Thousand-seed weight was significantly higher in cv. K-39 than in cv. Una and Viola, whereas cv. K-17 was not significantly different from the other cultivars. The foliar treatments applied did not have a significant effect on the trait in the tested cultivars. The obtained data were consistent with those given by *Wilczek and Ćwintal (2008)* who likewise reported that foliar treatment with boron did not significantly affect thousand-seed weight.

Seed yield was significantly higher in cv. Viola than in cv. K-39 and K-17, whereas the yield of cv. Una was not significantly different from that of the other cultivars. Foliar treatments with Co, B and PK resulted in a significant increase in seed yield in all cultivars. *Wilczek and Ćwintal (2008)* report that boron fertilization leads to an increase in seed harvest yield and actual yield (as calculated from yield components), suggesting that the increase results from increased flower fertility i.e. seed number per inflorescence. A study by *Santos et al. (2004)* reveals that boron deficiency in alfalfa reduces the root system size and causes death of meristematic tissue, indirectly resulting in decreasing seed yields. *Reddy and Raj (1978)* report that foliar treatment with cobalt in soybean enhances nodulation and grain yield.

The values obtained for the number of inflorescences per plant and number of seeds per inflorescence were in agreement with seed yield, across cultivars and foliar treatments. *Montardo et al. (2003)* indicate a positive correlation between the number of inflorescences per plant and seed yield in red clover. Moreover, they underline the importance of increasing the number of inflorescences per plant as a basis for increased seed yield potential.

Conclusion

The foliar treatment with Co resulted in a significant increase in stem number per plant in cv. K-17, number of inflorescences per plant in cv. K-39, K-17 and Viola, and number of seeds per inflorescence and fertility in all cultivars.

The foliar treatment with B caused a significant increase in the number of inflorescences per plant in cv. K-17 and in the number of inflorescences per stem, number of seeds per inflorescence and fertility in all cultivars.

The foliar PK application significantly increased the number of stems per plant in cv. K-39 and Viola, as well as the number of inflorescences both per stem and plant, number of seeds per inflorescence and fertility in all cultivars.

Consistently with the favourable effect of foliar treatments with micro- and macronutrients on seed yield components, the actual seed yield was found to be significantly higher upon foliar treatments with cobalt, boron, and phosphorus and potassium.

Efekat folijarne primene fosfora, kalijuma, bora i kobalta na prinos i komponente prinosa semena crvene deteline (*Trifolium pratense* L)

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Rezime

Usevi crvene deteline se uglavnom zasnivaju na kiselim zemljištima na kojima su pojedini makro i mikroelementi teže pristupačni biljkama. Cilj istraživanja je da se na takvim zemljištima oceni uticaj folijarne prihrane kobaltom (Co), borom (B) i fosforom i kalijumom (PK) na prinos i komponente prinosa semena sorti crvene deteline: K-39, K-17, Una i Viola. Folijarna primena Co uticala je na značajno povećanje broja izdanaka po biljci kod sorte K-17, broja cvasti po biljci kod sorti K-39, K-17 i Viole, kao i broja zrna po cvasti i fertilnosti kod svih sorti. Značajno povećanje broja cvasti po biljci kod sorte K-17, kao i broja cvasti po izdanku, broja zrna po cvasti i fertilnosti kod svih sorti je zabeleženo na tretmanima sa folijarnom primenom B. Folijarna primena PK je uticala na značajno povećanje broja izdanaka po biljci kod sorti K-39 i Viola kao i broja cvasti po izdanku odnosno biljci, broja zrna po cvasti i fertilnosti kod svih sorti. Stvarni prinos semena, izračunat na osnovu komponenti prinosa, bio je značajno veći na tretmanima sa folijarnom prihranom Co, B i PK.

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THE INFLUENCE OF RAINFALL AMOUNT, POLLINATORS AND FACILITATION OF POLLINATION ON THE YIELD AND ALFALFA SEED QUALITY

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Original scientific paper

Abstract: In this study, the impact of amount of rainfall, the number of pollinators and the usage measures to facilitate alfalfa pollination (sugar syrup application, mechanical facilitation) on the yield and quality components of alfalfa seed was observed. In the first (rainy) year, it was determined that there was larger number of other pollinators than the number of honey bees (ratio 1.5:1). In the second year, there were more honey bees (7:1) than all other pollinators. The best yield was obtained in the treatments with facilitated the process of pollination, and the worst was in the isolated treatment. The largest grains were achieved in isolated treatment with the honey bees. The best germination energy and seed germination were determined in seed from the treatments with the sugar syrup.

Key words: alfalfa pollination, rainfall, honey bee, seed quality

Introduction

Great role of alfalfa in the nutrition of livestock has contributed to its primary importance in the cultivation of forage crops. Problems arising during pollination have led many authors to pay particular attention to the pollination of this crop. The agro-meteorological conditions have the great impact on yield of alfalfa seeds (year) and production technology than genetic potential *(Erić, 1995, Mihajlović et al. 1999)*. When it comes to pollination of alfalfa most comprehensive data is given by *Mc Gregor (1976)*. In addition to the increased use of insects in pollination of alfalfa *Pharis and Unrau (1953)* used many mechanical aids (brushes, wire mesh, air flow, etc.) in their research in order to increase the opening of alfalfa flowers. *Erić (1995)* and many other authors before him suggested spraying alfalfa seed crop with sugar syrup in order to increase honey bee visits. *Marković (1997)* notes that besides cutting the quality of seeds is also affected by meteorological factors, especially precipitation, temperature and light.

The aim of this study was to determine how pollination and its facilitation affect the alfalfa seed yield and seed quality.

Materials and Methods

The experiment was conducted in the experimental field of the Institute for forage crops, Krusevac, during two years period. The domestic alfalfa cultivar K-22, created in the Institute for forage crops–Krusevac, was used. Crop was in the fourth and fifth years of age and for all researches the second cut was used. Alfalfa is sown in rows, and the distance between the rows was 12.5 cm, with the sowing norm 18kgha^{-1} of seed. The experiment was set up by the method of random block system. The size of the basic plot was 20 a, while the sample plot was 2 m^2. At 200 meters from the experimental plots bee colonies were set up of which bees were involved in free pollination of alfalfa.

A total of five treatments were studied: I - Mechanical facilitation of pollination (with the stretched string); II - isolation of the two nuclei; III - isolation without pollinators; IV - spraying sugar syrup; V - control (free pollination). During the experiment, counting of the pollinators in the control plot was done for ten days, three times a day in different time slots of 30 minutes: morning (from 8:30 to 9:00), midday (13:30 to 14:00) and afternoon (17:00 to 17:30). The amount of rainfall was determined by pluviometer and measurements were carried out during periods when pollinators were recorded. After ripening seed was harvested, processed and measured. The total mass (mass of 1000 seeds), germination energy and germination were determined in the laboratory. The analysis of variance was done for the impact of various treatments on yield and seed quality components. The significance of differences between mean values of studied traits was tested and the least significant difference (LSD test) at the level of significance of 1% and 5% was calculated.

Results and Discussion

The first year was a rainy; rain was falling for almost all the period of alfalfa flowering and total amount was 85l/m2, (Table 1). Rain was falling mostly during the night and early morning hours and it hindered pollinator activity. In the measurement in 1998, rain has fallen only once, but very heavy (45 l m^{-2}). Rainfall has favourable influence on the humidity of air and land, but negatively affects the activity of pollinators. In addition, the heavy rain washes off and dilutes nectar for which the honey bees visit alfalfa.

Table 1. Amount of rainfall (mm) in the period of alfalfa flowering

Year	Term	Morning	Noon	Afternoon	Total
I	Average	7,41	1,08	-	7,49
	min	-	-	-	
	max	30	10,8	-	
II	Average	4,5	-	-	4,5
	min	-	-	-	
	max	45	-	-	

In the first year, a total number of 45.6 honey bees and 80.8 other pollinators were recorded (Table 2). In this year, the relationship between pollinators was 1:1.5 for the other pollinators, and it was almost identical by terms. In the second year, 139.5 honey bees and 19.7 other pollinators were counted. In the second year, the number of honey bees has been significantly increased, so in comparison to other pollinators the proportion was 7:1. This relationship between honey bees and other pollinators in the second year of research, confirmed its importance of the honey bees the pollination of alfalfa. During the first year, the highest number of honey bees was determined in the morning (17.4), and the lowest in the afternoon (11.4). In the following year the most honey bees were recorded in the midday period (52.3), while the difference between morning and afternoon term (43.5 and 43.7) was unremarkable.

Table 2. The number of honey bees and other pollinators in the different counting terms

Year	Pollinators							
	Honey bee				Other pollinators			
	Terms			Total	Terms			Total
	I	II	III		I	II	III	
I	17,4	16,8	11,4	45,6	26,6	32,8	21,4	80,8
min	2	2	5		10	14	6	
max	37	33	34		45	49	49	
II	43,5	52,3	43,7	139,5	9,3	5,1	5,3	19,7
min	5	24	18		3	2	0	
max	72	69	68		18	11	13	
Average	30,4	34,5	27,5	185,1	17,9	18,9	13,3	100,5

Considering the terms of counting, the highest number of the other alfalfa pollinators in the first year was in the noon time, and then in the morning, and the least was in the afternoon. It was quite different in the second year when the highest number of other pollinators was in the morning, and much less in the noon and the afternoon.

The seed yield differed between studied years and between treatments. Significantly higher yield was obtained in the second year (Table 3). In the first year the highest yield was achieved in the treatment with the mechanical facilitation process, less seed was obtained in treatment with sugar syrup, and at the least was in isolated treatment without bees. During the second year the effect of treatments on the variation of yield was much higher. The best results were achieved in treatment with sugar syrup, and worst in isolated treatment without pollinators. The differences that arose between treatments with facilitation of pollination and the control treatment were not statistically significant. The differences between the isolated treatments (with and without honey bees) are very significant both between them and in comparison to other treatments. *Stjepanović and Bošnjak (1979)* found that alfalfa do not develops or under develops seed (0.22-0.36 kg ha^{-1}). In their studies, in the presence of natural pollinators the 83-118 kg ha^{-1} of seed was achieved and under the influence of honey bees 198-215 kg ha^{-1} seed was achieved.

Table 3. Yield and quality components of alfalfa seed depending on treatments in the studied years

Year	Treatment / Trait	I	II	III	IV	V	MS	Lsd 0,05	Lsd 0,01
I	Seed yield (g/m^2)	25,25	12,65	1,82	22,37	19,90	15,75	7,17	11,89
	Weight of 1000 seeds (g)	2,04	2,28	2,11	1,98	2,24	2,13	9,74	14,99
	Germination energy (%)	93,0	89,0	84,67	90,0	87,33	88,80	0,23	0,22
	Germination (%)	98,33	98,67	95,67	100,0	98,67	98,27	0,32	0,30
II	Seed yield (g/m^2)	60,03	26,98	2,83	67,42	40,92	36,14	6,88	6,58
	Weight of 1000 seeds (g)	2,28	2,48	2,34	2,15	2,28	2,31	9,35	9,24
	Germination energy (%)	89,0	93,67	88,33	93,67	95,0	91,93	2,64	2,57
	Germination (%)	95,33	97,67	96,0	98,33	98,0	97,01	3,59	3,38

In the first year the average weight of 1000 seeds was 2.13 g. In the second year of study the average weight of 1000 seeds was 2.31 g. The largest seed was obtained in the treatment with honey bee (2.48g), and the smallest in the treatment with sugar syrup. In the first year there was a significant difference between the best and worst treatment, and in the second year the differences were not significant. The weight of 1000 seeds, which was obtained in our study, was similar to the results of many authors. In the research of *Mihailović et al. (1999)* the weight of 1000 seeds ranged from 1.80-3.30g, while in the researches of *Stanisavljević (2006)* the average weight of 1000 seeds was 1.9 g, and the differences that have arisen under the influence of density of crops and cultivars were not statistically significant.

The average value for germination energy in the studied period was very high. In the first year, the germination energy was 88.80%, and 91.93% in the second. The difference in germination energy in the first year was significant between treatments with mechanical facilitation (93.0%) and isolated treatment without bees (84.67%), while the significant difference between the control and isolated treatment without bees was determined in the second year. The obtained values for the germination energy are similar with the results of *Tomić et al. (1998)*. In these studies germination energy of alfalfa cultivar K-22, in the year of harvest was 88%.

The obtained average values of alfalfa seed germination for both years were very high and were 99.00% in the first year and 97.22% in the second year. This is the only studied trait that had higher value in the first year than in the second. The difference was 1.78%. In both years, the best germination was in the treatment with sugar syrup and the worst in the isolated treatment without bees. Seed germination in 1998 in research of *Mihajlović et al. (1999)* ranged from 86-95%. Total seed germination decreases with storage and ranges from 91% in the year of harvest to 75% in the fifth year of storage *(Tomić et al., 1998)*. *Stanisavljević (2006)* states that the average value for was 83.7% and that the seed germination is significantly affected by the density of crops.

Conclusion

Based on the presented results on the impact of rainfall and pollinators on yield and seed quality components, it can be concluded that the number of alfalfa pollinators was significantly influenced by year as a factor. Year with plenty of rainfall is favourable for other alfalfa pollinators, while dry years are ideal for honey bee. Alfalfa pollinators were significantly more active in the morning and noon than in the afternoon terms. Facilitation of pollination gave positive results on yield and seed quality. There are significant differences between treatments and years for the observed components of seed quality. The quality of seeds obtained in an isolated treatment without bees was the worst and statistically significantly different from the quality of seeds from other treatments.

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Uticaj količine padavina, oprašivača i pospešivanja oprašivanja na prinos i kvalitet semena lucerke

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Rezime

U radu je praćen uticaj količine padavina, broja oprašivača i mera koje pospešuju oprašivanje lucerke (upotreba šećernog sirupa, mehaničko pospešivanje)

na prinos i komponente kvaliteta semena lucerke. U prvoj (kišnoj) godini na luceríštu je evidentiran veći broj ostalih oprašivača, nego medonosnih pčela (odnos 1.5:1). U drugoj godini bilo je znatno više medonosnih pčela (7:1) nego svih ostalih oprašivača. Najbolji prinos je ostvaren u tretmanima sa pospešivanjem oprašivanja, a najlošiji u izolovanom tretmanu. Najkrupnije seme je dobijeno u izolovanom tretmanu sa pčelama. Najbolju energiju klijanja i klijavost imalo je seme kod koga je oprašivanje pospešivano šećernim sirupom.

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OCCURRENCE OF ALFALFA MOSAIC VIRUS (AMV) IN ALFALFA SEEDS

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Original scientific paper

Abstract: The paper discusses the percentage of seeds infected with the alfalfa mosaic virus (AMV) in different alfalfa fields. Alfalfa seed samples were collected in 2007 and 2008 from a large number of different sites in Serbia, Romania and Ukraine. Five cultivars were involved in the study: NS Banat ZMS II, NS Mediana ZMS V, Banat VS, NS Alfa and Niagara. The seeds were tested for the presence of the virus using the DAS-ELISA test. The incidence of AMV in samples of commercial alfalfa seeds ranged between 0 and 11%, which was within the expected range. The percentage of infected seeds depended on location and stand age.

Key words: alfalfa seeds, AMV virus, DAS ELISA

Introduction

Alfalfa mosaic virus (AMV) is a widespread pathogen which was first described in 1931. The virus is highly variable and is comprised of many strains differing in virulence and symptoms they cause in alfalfa plants. The high variability of the strains makes it impossible to breed alfalfa for resistance to the disease, which is why there are no AMV-resistant varieties of this crop anywhere in the world *(Elgin et al., 1988)*.

Some alfalfa plants are resistant to most but not all AMV strains. Symptoms of AMV range from being completely invisible, where the infected plants show no symptoms of the disease, to being very severe, leading to root necrosis and deterioration of the whole plant. No varieties resistant to AMV have therefore been developed through breeding. Due to a large number of hosts (300 plant species from 73 genera) and vectors, AMV is present throughout the world *(http://www.ncbi.nlm.nig.gov/ICTVdb/ICTVdB/)*.

Studies conducted in controlled conditions (laboratories and greenhouses) have shown that AMV may cause damage to individual alfalfa plants. However, there are no recorded cases in agricultural practice of an alfalfa stand being reducedas a result of AMV, let alone completely destroyed *(http://cals.arizona.edu/PLP/plpext/diseases/agronomic/alfalfa/alfamv.htm)*.

Damages caused by AMV in alfalfa production are not economically significant, despite the presence of infected plants *(Leath et al., 1988)*.

It is not uncommon for an alfalfa field in the second year of growth to have as many as 80 % of infected plants, which show no symptoms of the disease *(www.ipmcenters.org/cropprofiles/docs/alfalfa)*. *Malvic (2002)* reported that AMV was found widely in alfalfa fields in Wisconsin but that this caused no major problems in the production of the crop. In alfalfa fields with a high plant population, AMV-infected plants may be covered by healthy, tolerant plants and thus hidden from view. The healthy plants eliminate the diseased ones and in that way obtain more room for them selves to grow. They can thus develop better and produce more shoots, so the plant density and biomass yield remain the same *(www.ipmcenters.org/cropprofiles/docs/alfalfa)*. For this reason, plants with pronounced symptoms of AMV infection are difficult to spot in alfalfa fields. The AMV virus is transferred by sap, and the most common vector by which the pathogen spreads in alfalfa fields are aphids. AMV can also be spread by seeds. Seed crops can have up to 10% of infected plants, while infection levels of 3-8% *(Elgin et al., 1988)* or 1-3% *(Graham et al., 1998)* are common in commercial alfalfa crops. There are no control measures against this virus in existence *(http://cals.arizona.edu/PLP/plpext/diseases/agronomic/alfalfa/alfamv.htm)*.

Alfalfa is an extremely important forage crop. In Serbia, it is grown on about 200,000 ha and is the cornerstone of cattle production. In view of this, we decided to investigate the occurrence of the AMV virus in alfalfa seed samples.

Materials and Methods

During 2007 and 2008, alfalfa seed samples were collected from a number of sites in Serbia. Six samples of the commercial variety Banat ZMS II were taken in 2007 and 17 in 2008. In 2007 and 2008, two samples were collected in Romania (Arad and Timisoara) and one in Ukraine (Kharkov). Seed samples of the cultivar Mediana ZMS V were collected in Serbia from five locations in 2007 and eight locations in 2008. One sample was brought in from Ukraine in 2008 (Herson). Samples of the cultivars Banat VS, NS Alfa and Niagara were taken from a single locality in 2007 and 2008. The alfalfa fields from which the samples were taken were anywhere from one to five years old. The seeds came from the second cut in 56 of the locations and from the third cutting in four localities.

Serological testing was carried out by the DAS ELISA technique using Nunc-96 96-well polystyrene micro titer plates and a commercial AMV antiserum kit manufactured by LOEWE Biochemica GmbH, Germany. The kit consists of IgG antibodies, phosphatase-conjugated IgG antibodies, and positive and negative controls. The DAS ELISA test was performed following the standard procedure for this kind of testing *(Clark and Adams, 1977)* according to the instructions provided by the manufacturer of the antibody kit (LOEWE Biochemica GmbH, Germany). The ELISA results were read on the Multiskan Ascent microplate reader at 405 nm. Extinction values of the isolates that were at least twice as high as those of the negative controls (LOEWE Biochemica GmbH) were considered positive.

Results and Discussion

In the variety Banat ZMS II, there was no significant difference in the percentage of seeds infected with AMV between 2007 (4.3%) and 2008 (3.8%). The standard deviation was 2.2%. The proportion of AMV-infected seeds in different locations ranged from 1 to 6% in 2007 and from 0 to 9% in 2008, which might reflect the importance of locality for the incidence of the virus (Table 1).

Table 1. Incidence of AMV in seeds of the alfalfa cultivar NS Banat ZMS II in 2007 and 2008

Year	Location	Stand age (yr)	Cut	Percentage of infected seed (%)
2007	Gakovo	II	II	5
	Čenej	III	II	4
	Kelebija	III	II	6
	Romania – Timisoara	III	II	2
	Romania - Arad	III	II	4
	Ukraine - Kharkov	III	II	1
	Titel	III	III	5
	Vrbas	IV	II	3
	Bašaid	IV	II	3
	Average	-	-	4.33
	σ			1.58
2008	Vrbas	II	II	5
	Sivac	II	II	2
	Krivaja	II	III	5
	Kovilj	III	III	1
	Titel	III	III	3
	Prigrevica	III	II	3
	Krivaja	III	II	4
	Feketić	III	II	9
	Novi Bečej	III	II	0
	Kelebija	III	II	3
	Đurđin	III	II	1
	Sojan	III	II	1
	Kikinda	III	II	4
	Čenej	IV	II	4

This incidence was the highest at the Kelebija, Feketić, Ada, and Dolova sites, all of which have lighter, drier soils and are characterized by arid conditions. The incidence of the virus varied very little between seeds of the variety NS Banat ZMS II obtained from the second cut and those produced in the third (Table 1).

In the cultivar NS Mediana ZMS V, the average contribution of infected seeds was 4.2% in 2007 and 7.0% in 2008 (Table 2). The average for the two years was 5.6% and the standard deviation was 3.2%, showing that the differences between the two years were not significant. The percentage of infected seed varied little in 2007, while in 2008 there was much more variation.

Table 2. Incidence of AMV in alfalfa seeds of the variety NS Mediana ZMS V in the second cut in 2007 and 2008

Year	Location	Stand age (yr)	Percentage of infected seed (%)
2007	Kikinda	III	5
	Sremska Mitrovica	III	5
	Zrenjanin	IV	3
	Bačka Topola	IV	4
	Rimski Šančevi	IV	4
	Average	-	4.2
	σ		0.83
2008	Dobanovci	II	3
	Sivac	III	11
	Kikinda	III	8
	Bačko Gradište	III	9
	Ukraine-Kharkov	III	0
	Kikinda	IV	9
	Lazarevo	IV	10
	Taraš	V	8
	Rimski Šančevi	V	5
	Average	-	7.0
	σ		3.60
	Overall average	-	5.6
	σ		3.18

In 2007 and 2008 at the Rimski Šančevi site, AMV incidence in the cultivars Banat VS, NS Alfa and Niagara varied significantly, ranging from 0 to 8% (Table 3). The percentage of AMV-infected seeds increased with stand age, but the differences were minimal and within one standard deviation of the mean. According to the results, the percentage of AMV-infected seeds ranged between 0 and 11%. Our findings are in agreement with those available at the website http://www.ncbi.nlm.nig.gov/ICTVdb/ICTVdB/, according to which AMV infections of up to 10% can be expected in commercial alfalfa seed samples. *Pesic and Hiruki (1986)* report AMV infection levels of up to 20% for Canada and note that the incidence of the pathogen may be about three times lower (7.3%) in the seedlings. Similarly, *Baillis and Offei (1990)* observed significantly lower levels of infection by AMV compared to the frequency of AMV antigens revealed by the ELISA test.

Table 3. AMV incidence (%) depending on cultivar, year and stand age

Cultivar	Year	Stand age (yr)				
		I	II	III	IV	V
NS Banat ZMS II	2007	-	5	3.6	3	-
	2008	-	4	2.8	4.8	6.5
	Average	-	4.5	6.2	3.9	6.5
NS Mediana ZMS V	2007	-	-	5	3.7	-
	2008	-	3	7	9.5	8
	Average	-	3.0	3.4	6.6	8.0
Banat VS	2007	2	-	-	-	-
	2008	-	8	-	-	-
NS Alfa	2007	3	-	-	-	-
	2008	-	7	-	-	-
Niagara	2007	5	-	-	-	-
	2008	-	0	-	-	-
Average		3.3	4.5	3.3	5.2	7.2
σ		2.39				

According to *Pecetti et al. (2001),* luxuriant, erect alfalfa genotypes are more AMV-susceptible than prostrate. In a study by *Latham and Jones (2001),* the percentage of AMV-infected alfalfa seeds ranged from 0.4 to 19%, while *Zeynaddini et al. (2005)* reported an incidence of 53% in Iran with no significant differences among six sites. AMV is widespread in alfalfa fields in Wisconsin *(http://www.uwex.edu/ces/forage/wfc/proceedings2002/rots_and-viruses).*

Our present results are in agreement with the findings from the USA, Australia, Canada and Iran indicating that the virus is widespread in alfalfa fields. In California it is thought that all alfalfa fields are infected by AMV *(www.ipm.ucdavis.edu/PMG/r604101011.html).* Researchers from Arizona are of the opinion that there are no control measures against this pathogen and that the only prevention available is to plant susceptible species (pepper, potato) at a safe distance from alfalfa *(http://cals.arizona.edu/PLP/plpext/diseases/agronomic/alfalfa/alfamv.htm).*

The percentage of AMV-infected seeds in our samples was small and within the expected range. The occurrence of AMV in alfalfa seeds should be further studied from the aspect of location, stand age, and agricultural practices used. Since the incidence of the virus was found to be small, no forage yield losses are expected, but this should be double-checked by sowing different infected seeds in a field trial.

Conclusion

The incidence of AMV in samples of commercial alfalfa seeds ranged between 0 and 11%, which was within the expected range. The percentage of infected seeds depended on location and stand age.

The occurrence of AMV in alfalfa needs to be studied more in order to determine the method by which commercial seeds of this crop should be tested for the presence of the virus.

Pojava virusa mozaika lucerke (AMV) u semenu lucerke

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Rezime

U radu je ispitivan udeo zaraženih biljaka virusom mozaika lucerke (AMV) u luceništima na većem broju lokaliteta. Uzorci semena lucerke su prikupljani tokom 2007. i 2008. godine, na području Srbije, Rumunije i Ukrajine. U istraživanju je bilo uključeno 5 sorti lucerke: NS Banat ZMS II, NS Mediana ZMS V, Banat VS, NS Alfa i Niagara. Utvrđivanje prisustva virusa AMV u semenu lucerke rađeno je DAS-ELISA testom. Pojava AMV u uzorcima komercijalnog semena lucerke bila je od 0-11 %, što su očekivane vrednosti. Postoje razlike u pojavi zaraze zavisno od lokaliteta i starosti useva.

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SEED QUALITY TESTING OF MEADOW FESCUE (*Festuca pratensis* L.) WITH STANDARD GERMINATION METHOD DURING POST-HARVEST MATURING PERIOD

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Original scientific paper

Abstract: In seed market of perennial forage plants, meadow fescue has significant place. Because of that, seed quality testing of meadow fescue is very important. According to quality norms, seed testing include first breaking of dormancy in 7 days period. In practice, there are examples of seed testing without pre chill. Purpose is to determine differences between lots and between properly used Standard germination test with pre chill and Standard germination test without pre chill. Obtained results show statistically significant differences between lots and during post-harvest seed maturing period for each lot. Results of total germination show statistically significant differences between seed lots.

Key words: meadow fescue seed, dormancy germination test, total germination.

Introduction

Meadow fescue is widespread species in Europe. It is found as a frequent component of meadows, pastures and grasslands for combine usage. On natural meadows, it is found in several plant associations. In stock feed production, meadow fescue represents important species for providing quality feed, on artificial meadows and pastures and in natural plant associations. In seed production it shows the best results in the second and the third year *(Vucković, 2004)*. Same author indicates that seed germination was the highest when crops were sown on row to row distance of 50 cm and applied seed rate of 4 kg ha^{-1}. Forage grass seed right after harvest have very low germination energy and less germination percentage because of dormancy and must go through maturing period, in other words post-harvest dormancy period to improve this characteristic (energy).

Grass seed dormancy can depend on tissues that surround embryo. Those are permeable membranes that inhibit water absorption or gas exchange, then mechanical barriers that block embryo growth or those that inhibit germination *(Adkins et al., 2002)*.

As the seed mature, the germination is increasing, and that can be useful for avoiding unfavourable agro-ecological conditions when plants are underdeveloped *(Marić, 1987)*.

Seed is very important factor in forage crops, perennial grasses and legumes production *(Tomić et al., 1998)*. Seed germination testing in laboratory is conducted in optimal, controlled conditions (substrate, moisture, temperature and light). Such results can be good indicator for seed growth in favourable field conditions. Germination values obtained in laboratory conditions are not adequate indicators of field emergence in case of unfavourable agro ecological factors. High germination energy indicates a uniform germination under field conditions. On two-year results basis *Stanisavljević et al. (2007)* have shown that meadow fescue seed had lower germination energy if it has been taken from upper branch of panicle (60.93%) than if taken from middle branch (64.70 %) and low branch (68.44 %). Total germination from the middle branch was 89.01 %, while from upper and lower branches had been slightly lower (79.72 % and 79.22 %).

Stanisavljević et al. (2009) by analyzing seed of timothy and tall oat grass showed that there was strong positive correlation between germination energy and total germination. Germination energy of meadow fescue decreased in the third year of storage from 85 % to 60 %, such as 38 % and 34 % in the fourth and fifth year. Variation of germination energy and total germination in all three analyzed years was statistically significant *(Tomić et al., 1998)*.

Post-harvest maturing of seed and forage grass germination define possibility of sowing in autumn term and in the year of harvest *(Stanisavljević et al., 2008)*. By analyzing seed of *Festuca* species during post-harvest maturing period it was determined that germination of meadow fescue 120 days after harvest did not satisfy technological request and seed legislation for putting seed on the market. Seed of tall fescue and red fescue had enough germination, so the authors recommend sowing in autumn after harvest in June that year *(Stanisavljević et al., 2010)*.

Materials and Methods

Four seed lots (P1, P2, P3 and P4) of meadow fescue of same cultivar K 21, received in the same year from conventional production were used in this research. Two seed lots (P1 and P2) were produced in Zaječar region, one lot (P3) in Knjaževac region and one lot (P4) in Kruševac region. After harvest all seed material was dried in floor store to 13 % of seed moisture. Material was processed in the Institute for forage crops, and bagged.

Seed material was analyzed on every 60^{th} day from the end of seed procession in Institute for forage crops Kruševac and in Seed Testing Laboratory "Sirmium-Seme" Sremska Mitrovica. In Institute for forage crops, seed was analyzed with Standard germination test without pre chill and in Seed Testing Laboratory "Sirmium-Seme" with Standard germination test with pre chill.

For analyses of germination energy and total germination with Standard germination test, filter paper was used as substrate. Analyze of every seed lot was performed in four repetitions. Temperature was constant during Standard germination test without pre chill 23°C, and during Standard germination test with pre chill was alternate (20 °C-16 h without light, 30 °C-8 h with light). Seed pre chill was performed on temperature of 5 °C during 4 days. Germination energy was evaluated after 7 days and total germination after 14 days, by separating normal seedlings from abnormal and dead seed.

Statistical analysis was performed by using Analysis of variance (ANOVA) for two factorial trials. Significance of difference was determined by LSD test for 0.05 significance level. Trend was performed as tendency of development observed during certain period that appeared as a result of existing factors.

Results and Discussion

Seed of meadow fescue immediately after harvest and drying had low germination energy and total germination (Table 1). After large research of seed dormancy during post-harvest maturing, still there are not found complete explanations about process that takes place not allowing germination in this period *(Bewley, 1997)*.

Tab.1 Average values of germination energy and total germination obtained by Standard germination test

Lot	Standard method	storage period (days)							
		0		60		120		180	
		GE	TG	GE	TG	GE	TG	GE	TG
P_1	without pre chill	26	60	33	69	48	76	76	87
	with pre chill	58	63	64	72	71	86	81	90
P_2	without pre chill	32	60	41	61	51	78	67	84
	with pre chill	60	65	64	69	77	85	85	90
P_3	without pre chill	41	59	43	64	48	79	66	86
	with pre chill	61	66	60	69	67	86	85	91
P_4	without pre chill	37	66	44	75	48	79	64	88
	with pre chill	62	67	63	77	70	88	86	90
LSD 0.05	lot	4.26	3.37	4.38	3.58	4.01	3.65	4.56	3.98
	method	3.02	2.38	3.1	2.63	2.96	2.89	2.87	2.56

GE- germination energy
TG- total germination

After harvest and drying, the large ratio of seed was dormant. This led to the low germination, which was 51% (P_3) - 66% (P_4) without chill and 59% (P_3) - 67% (P_4) with chill.

The optimal sowing period of forage grasses in Serbia is August to September *(Vučković 2004)*. The harvest of the meadow fescue is mostly done in June, so 60 days afterwards is the period of autumn sowing. Due to this, the legislation for putting the seed on the market *(sl. list 47)* and the technologic requirements for high seed germination are of great significance.

In this period, of four studied lots, with seed chill, two lots met the requests for sowing, while one lot met those requests in the treatment without chill (Table 1).

In the period of 120 days after processing, the germination was increased. The seed of all studied lots passed the standards of quality. This period is in October when the agro-ecological conditions are unfavourable. After 180 days after processing germination was high and uniform, but without chill was lower in each lot for 2-5%. The influence of the lot and the treatment with chill on the germination energy and total germination had statistically significant differences during the research period ($P \geq 0.05$; Table 1).

Linear trend for the germination energy of lots P_1 (Figure 1) and P_2 (Figure 3) without pre chill shows an increase from the first term of examination to the fourth. In this case, the increase is very rapid with larger intervals. Germination energy with pre chill of lots P1 and P2 shows increasing linear trend, but growth is slower with smaller intervals of increase.

The energy of germination of the lots P_3 (Figure 5) and P_4 (Figure 7) without pre chill shows an increasing linear trend. In this case, growth is moderate with smaller intervals. Germination energy of lots P_3 and P_4 with pre chill shows an increasing linear trend, but moderate growth with intervals. Total germination of lots P_1 (Figure 2) and P_2 (Figure 4) without pre chill shows increasing linear trend depending on the term of testing. In this case, the increase is with smaller intervals. Overall values of the total germination at the beginning and the end of the analyzed period are similar. Total germination of lots P_3 (Figure 6) and P_4 (Figure 8) without pre chill shows an increasing linear trend. Values of total germination of lot P_3 at the beginning and the end of the period of testing differ by 5 %. Overall values of the total germination of lot P_4 at the beginning and the end of the analyzed period are similar.

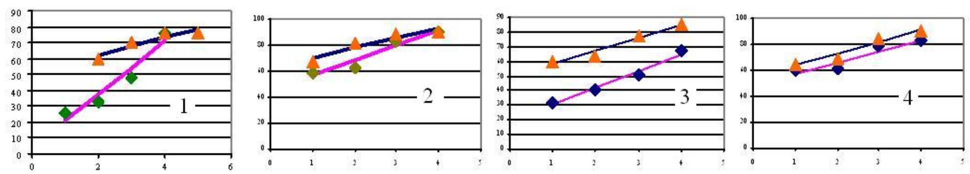

Figure 1. Germination energy for P1; Figure 2. Total germination for P1; Figure 3. Germination energy for P2; Figure 4. Total germination for P2, (♦ Germination energy and total energy without pre chill; ▲ Germination energy and total energy with pre chill)

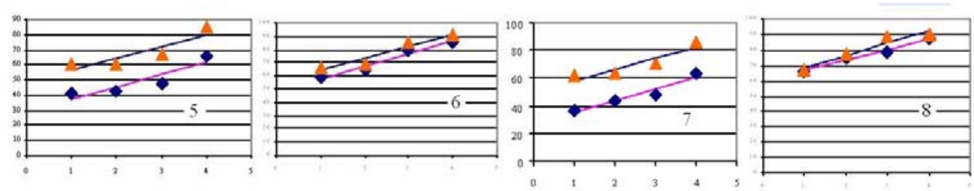

Figure 5. Germination energy for P3; Figure 6. Total germination for P3; Figure 7. Germination energy for P4; Figure 8. Total germination for P4, (♦ Germination energy and total energy without pre chill; ▲ Germination energy and total energy with pre chill)

Conclusion

Based on the results of testing of the meadow fescue (*Festuca pratensis* L.) seed quality using standard germination method during post-harvest maturing period, the following can be concluded:

- after harvesting and drying meadow fescue shows high seed dormancy and low germination energy and total germination
- 60 days after processing the seed germination show limit values for the placing on the market
- throughout the period of testing the impact of the seed lot as well as seed chill demonstrated a significant impact on the germination energy and total germination.

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Analiza kvaliteta semena livadskog vijuka (*Festuca pratensis* L.) standardom metodom ispitivanja tokom posle žetvenog perioda

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Rezime

U prometu semena višegodišnjih krmnih trava livadski vijuk zauzima značajno mesto. Samim tim i ispitivanje kvaliteta semena ove vrste je veoma značajno. Prema normama kvaliteta ispitivanje semena podrazumeva prvo prekidanje mirovanja (hlađenje na temperaturi 5°C u vremenskom periodu do sedam dana) za rod iz tekuće godine proizvodnje. U praksi ima primera da se ispitivanje kvaliteta radi bez prethodnog hlađenja. Cilj ovog rada je da se utvrde razlike između pravilno primenjene standardne metode ispitivanja sa hlađenjem i standardne metode bez hlađenja na četiri partije semena. Dobijeni rezultati pokazuju statistički vrlo značajne razlike u energiji klijanja i ukupnoj klijavosti, kako za partije tako i za uticaj rashlađivanja, tokom perioda posležetvenog dozrevanja semena.

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EFFECTS OF DIFFERENT ALFALFA SEED EQUIPMENT ON PROCESSING PARAMETERS

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Original scientific paper

Abstract: This paper presents the results of a qualitative and quantitative analysis of natural alfalfa seed processing of the same purity, processed by two different sets of machines. One set consisted of a fine cleaner, trier and magnetic cleaner. In addition to these, the other set included a specific gravity separator which preceded the magnetic cleaner. The relevant parameters analyzed (the processing time, electric energy consumption and the quantity of processed seed), were different and depended on the set of machines that had been used. According to these indicators it is possible to decide on adequate processing machinery for alfalfa seed, i.e. to optimize and rationalize its production. By making the right choice it is likely to achieve considerable energy savings, which was the primary purpose of this study.

Key words: seed, alfalfa, processing, equipment, purity, electric energy consumption

Introduction

Owing to its great significance as a forage crop, in a ten-year time, from 1999 to 2008, alfalfa *(Medicago sativa* L.*)* was cultivated in Serbia on an average of 189.904 ha (*SGS 1999 - 2008*). Throughout the world, alfalfa as a perennial forage legume covers about 35 million hectares *(Barnes et al., 1988)*.

By growing this plant for seed and using adequate technology, it is likely to achieve high yields as well as great profit *(Stanisavljević, 2006)*. For producing alfalfa seed second cutting is the best since in that period the soil moisture content is the lowest, alfalfa is less lodged, the yields are higher and the seed quality is much better *(Vučković, 2003; Vučković et al., 2004)*.

In standard production, harvesting alfalfa seed is being done from mid-August *(Karagić et al., 2003; Stanisavljević et al., 2007)*. The material obtained by harvesting is a mixture of seed of a cultivated plant, other plants and weeds, as well as waste of organic and inorganic origin. By adaptation of header, the thresher, separators and by adequate adjustment of operational regime for certain combine elements, a quality alfalfa seed threshing can be carried out, which implies a high level of thrashed seeds out of pods and high purity value with minor losses *(Krmpotić et al., 1990; Vučković, 2003).*

There are several technological schemes of alfalfa seed processing used in practice. Processing should satisfy legally recognized seed quality. According to The Law *(Official Journal of RS, 2005)*, processed alfalfa seed must be of at least 95% purity value with not more than 2% seed of other species, 0.5% of weeds (with no quarantine weeds), 2.5% of inert matter, germination of 70% and 13% of moisture.

Materials and Methods

The research was carried out at the processing centre of The Institute for Forage Crops in Kruševac. In three repetitions, natural alfalfa seed of 85.4% purity was processed by two different sets of equipment. The quantity of seed in each turn was 300 kg, i.e.900 kg for each purity value (1800 kg for both sets of equipment).

The first processing set (A1) consisted of the following machines:

- intake pit with belt conveyor,
- belt conveyors,
- bucket elevators,
- fine cleaning machine by a Danish manufacturer Damas – type Alfa – 4,
- trier by a Danish manufacturer Damas (with three rollers) - type Hotyp,
- magnetic cleaner by a German manufacturer Emceka Gompper type 4.

The second processing set (A2), in addition to all above mentioned~~, and~~ had a pre-cleaner before fine cleaning machine Vibam - Uni. The cleaning of waste seed by magnetic cleaner was done after processing on a specific gravity separator "Oliver - 240" by a German manufacturer Emceka Gompper, which separates two or more ingredients of the same size and different specific weight *(Copeland and McDonald, 2004).*

By using a specific gravity separator in processing two lots of different purity value seed, better results were achieved with seed of higher initial purity value compared to seed of lower purity value *(Djokić et al., 2009)*.

The purity value of the processing naturalized seed was 85.43%. There was no other species in a sample of naturalized seed while the contents of inert matter consisted of 13.4% of sickly grain and harvest residue. An average amount of weeds was small 1.17%, while the amount of dodder (*Cuscuta spp.*), a harmful quarantine weed, in a sample of 5 g was three grains. Apart from dodder, the sample contained a certain number of redroot pigweed grains (*Amaranthus retroflexus* L.), wild carrot (*Daucus carota*), 6 grains of curly dock (*Rumex spp.*) in 5 g, as well as perennial ryegrass (*Lolium perenne* L.).

The analysis of contents of traces in seed samples of 5 g and 50 g was performed in a laboratory. The following parameters were measured: clean seed (%), seed of other species (%), inert matter (%), weed seed (%), the quantity of processed seed (kg), seed processing time (h), active electric energy consumption (kWh) and reactive energy consumption (kVArh), high - processing output (%) and seed losses on processing equipment (%). The readings of power consumption were done by multifunctional digital three - phase power meter DMG2. Time was measured by a stopwatch. According to these indicators and by comparing average results, an optimal processing set would be determined, i.e. which set would give seed of better quality and greater quantity in a shortest time and with lowest energy consumption.

Results and Discussion

Table 1 shows the processing of naturalized seed of 85.4% purity value. Due to its high purity value, this seed went through processing set once and after being processed by magnetic cleaner increased its purity value to 95.7%, which is legally recognized value for alfalfa seed. An average processed quantity of seed was 220.67 kg.

Table. 1. Seed processing - A1 The first processing set

Seed structure	%	Weed species
Natural alfalfa seed (sample from the intake pit)		
Pure seed	85.4	
Other species	0	
Inert matter	13.4	sickly grains, harvest residues
Weed	1.2	*Amaranthus retroflexus* L., *Daucus carota*, 3 *Cuscuta spp.* in 5 g, 6 *Rumex spp.* in 5 g, *Lolium perenne* L.
Seed purity after first pass through machine set A1 (sample from mixer)		
Pure seed	90.8	
Other species	0	
Inert matter	9.2	sickly grains, damaged seed
Weed	0	1 *Cuscuta spp.* in 5 g
Seed purity after first pass through magnetic cleaner (sample from bag)		
Pure seed	95.7	
Other species	0	
Inert matter	4.3	sickly grains, damaged seed
Weed	0	2 *Rumex spp.* in 5 g, (3 *Rumex spp.* in 50 g)

The waste seed which was gathered from the trier, lower sieves of fine cleaner and magnetic cleaner had an average of 75.0% pure seed and 25.0% of inert matter with sickly and ruined grains. The waste seed was processed in the same way and by the same equipment as the naturalized seed out of which the waste was obtained. The seed from the waste was then returned to the intake pit and after one pass through machines and magnetic cleaner it had 97.0% purity value with 3.0% of sickly grains. On average, there was 12.33 kg of processed seed.

As for the second processing equipment (set A2), the seed of 85.43% purity value, after going through machines and magnetic cleaner once, at the end of processing reached the purity value of 95.53% with 4.47% of inert matter containing sickly and ruined grains (Table 2).

Table. 2. Seed processing – A2 The second processing set

Seed structure	%	Weed species
Natural alfalfa seed (sample from the intake pit)		
Pure seed	85.43	
Other species	0	
Inert matter	13.4	sickly grains, harvest residues
Weed	1.17	*Amaranthus retroflexus* L, *Daucus carota*, 3 *Cuscuta spp.* in 5 g, 6 *Rumex spp.* in 5 g, *Lolium perenne* L.
Seed purity after first pass through machine set A2 (sample from bulk hopper)		
Pure seed	92.53	
Other species	0	
Inert matter	7.47	sickly grains
Weed	0	5 *Rumex spp.* in 5 g, 5 *Cuscuta spp.* in 5 g
Seed purity after first pass through magnetic cleaner (sample from bag)		
Pure seed	95.53	
Other species	0	
Inert matter	4.47	sickly grains, damaged seed
Weed	0	4 *Rumex spp.* in 50 g

The waste seed gathered after the first pass through the equipment set had an average purity of 60% with 40% of inert matter containing sickly grains. Such seed was then processed by specific gravity separator which separated high quality from low quality seeds. High quality seed was then processed on magnetic cleaner while low quality seeds were discarded as waste. At the end of processing, the seed was of 96.67% purity with 3.3% of sickly grain and 4 grains of dock in a sample of 50 g. Out of waste, after processing, an average of 15.33 kg of quality seed was obtained.

Table 3 shows the data gathered through analysis of natural 85.4% purity seed processed by seed processing equipment set A1 and A2.

Table 3. The average time of the processing, power consumption, quantity of processed seed, output of processing and seed loss during the alfalfa seed processing for A1 and A2

Equipment	Processing time (min)	Power consumption		Seed quantity (kg)		Processing output (%)	Grain loss (%)
		Active (kWh)	Reactive (kVArh)	From processing	From waste		
A1	159.7	56.54	80.57	220.67	12.33	77.7	9.09
A2	126.7	59.7	68.97	222.0	15.33	79.1	7.41
LSD 0.05	9.706	4.182	5.206*	12.03	3.069	3.69	4.301
0.01	16.09**	6.935	8.634	19.951	5.09	6.12	7.132

* Statistically significant, **statistically very significant

The analysis indicates that using a specific gravity separator shortens the processing time for 33 min, increases the quantity of seed for 4.33 kg with higher processing output and reduces the seed losses. Active electric energy consumption is lower in A1 set compared to A2 set for 3.15 kWh, as well as reactive energy consumption for 11.6 kVArh. Based on LSD test, the processing equipment sets have different processing times on the level of 99%, and reactive energy consumption on the level of 95%. There is no other statistically significant difference.

Conclusion

The processing of 85.4% purity value seed with a small amount of inert matter and weeds was also done by two different equipment sets. By using the second processing set, which, in addition, had a pre-cleaner Vibam-Uni and a specific gravity separator, the processing time was reduced for 33 min compared to the first processing equipment set. There was, also, a statistically considerable impact on reactive energy consumption. The analysis of the waste seed in the first equipment set shows the purity value of 75% with 25% of inert matter, while in the second processing set the purity value was 60% with 40% of inert matter. Consequently, the cleaning quality with the use of pre-cleaner was much better since more inert matters were singled out by processing.

The use of a specific gravity separator in processing seed from waste reduces the processing time and electric energy consumption, due to its large capacity.

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Uticaj različitih sistema mašina za doradu semena lucerke na parametre dorade

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Rezime

U radu su prikazani rezultati kvalitativne i kvantitativne analize pri doradi naturalnog semena lucerke iste čistoće, dorađene na dva različita sistema mašina za doradu semena. Prvi sistem mašina za doradu se sastojao od mašine za finu doradu semena, trijera i trifolin mašine – dekuskutora. Kod drugog sistema mašina za doradu semena iz otpada se pre trifolin mašine koristio gravitacioni sto čiji rad se zasniva na razlici specifičnih masa pojedinih komponenata semenskog materijala. U procesu dorade semena iste čistoće, koje se dorađuje na različitim sistemima mašina, relevantni parametri koji su se ispitivali kao što su: vreme dorade, utrošak električne energije i količina dorađenog semena bili su različiti. Na osnovu ovih pokazatelja moguće je izvršiti izbor odgovarajućeg sistema mašina za doradu semena lucerke, odnosno optimizaciju i racionalizaciju u procesu njene proizvodnje. Izborom odgovarajućeg sistema mašina za doradu semena lucerke moguće je ostvariti značajnu uštedu u potrošnji električne energije, što je bio primarni cilj ovog istraživanja.

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ALFALFA RESPONSE TO LOW SOIL pH AND LIMING

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Abstract: Low soil pH is a constraint to alfalfa production and a major cause of low stand persistence of alfalfa. Field trials were conducted on pseudogley (pH_{nKCl} 4.08) over a period of three years to estimate the dry matter yield and quality of five alfalfa cultivars (Banat, Medijana, K-28, Sinskaja, OS 66) under lime treatments (control, 0.5, 1.5 and 3 t ha^{-1} CaO). Irrespective of liming rate, cv. K-28 had a significantly higher dry matter yield during the three-year period as compared to the other cultivars used. Regardless of cultivars, dry matter yields were substantially higher at a treatment rate of 3 t ha^{-1} CaO in all years of observation. The significance of the cultivar/liming interaction in all years indicated that the cultivars showed different responses at different CaO application rates. Lime treatments induced a significant increase in protein content, the highest being produced at 3 t ha^{-1}CaO in all cultivars. Acid tolerance efficiency, as a ratio of dry matter yield in no lime treatment to dry matter yield in lime treatment can be used as an indicator of cultivar tolerance to low pH. The absence of correlation between acid tolerance efficiency and dry matter yield potential suggests that an increased response to acidity and yield potential are not mutually exclusive, i. e. that breeding for both traits is justified.

Key words: alfalfa, cultivar, liming, soil acidity, dry matter yield, quality

Introduction

Acid soils constrain agricultural production in more than 1.5 Gha worldwide *(Edwards et al., 1991)*. The scope of the problem is likely to increase as the result of acid rain, long-term N fertilization and legume N-fixation *(von Wexeüll and Mutert, 1995; Graham and Vance, 2000)*. Alfalfa (*M. sativa*) is the most common perennial forage legume in the Republic of Serbia, grown on about 200,000 ha. However, the value and utilization of alfalfa as a forage legume have been constrained by its low tolerance to soil acidity *(Bouton, 1996; Cooks, 2001)*. Soil acidity adversely affects crop emergence, early seedling growth and total biomass production i.e. root and top dry matter yield *(Zhang et al, 2004; Zhang et al, 2006)*. The H^+ ion concentration per se, as well as the related Al and Mn toxicity problems and Mo, Ca and P deficiencies can further aggravate the problem *(Graham, 1992)*. Furthermore, acidity limits both survival and persistence of nodule bacteria in sol, nodule formation and nitrogen fixation.

The basic cultural operation commercially available, as described by *Toma and Saigusa (1997), Koopman et al. (1997)* and *Grewal and Williams (2003)* is reclamation of acid soils through the application of lime and other material as top dressings to reach the target pH at which available Al and Mn levels are non-toxic. *Glenn et al. (1998), del Papa et al., (1999), Dilworth et al. (2001)* recommended selection *Rhizobium meliloti* strains that show effectiveness in nitrogen fixation, competitiveness with indigenous strains in the nodules and tolerance to low pH of soil may be used as a means to solve the problem. Additionally, *Dall´Agnol et al. (1996), Petcu et al. (2006)* and *Pan et al. (2008)* report the possibility of identifying and selecting acid-tolerant alfalfa germplasm. A major goal of alfalfa breeding efforts is to increase tolerance to soil acidity, directly focusing on ensuring cost-effective production and increasing the contribution of this legume to "sustainable" agricultural systems. The objective of this study was to estimate the effect of liming low pH soils on the yield and quality of dry matter in alfalfa cultivars with diverse genetic backgrounds developed at different breeding institutions, which may be used as an indicator of their tolerance to acidity.

Material and Methods

This experiment was conducted as an on-farm trial at the Secondary School of Agriculture and Food Engineering in Kraljevo (43^0 43' 53'' N, 20^0 40' 39'' E). The soil was very acid pseudogley having a pH in KCl of 4.08, a low content of humus (2.56%), moderate levels of nitrogen, phosphorus and potassium (0.135% N, 7.20 mg100 g^{-1} P_2O_5 and 11.0 mg100 g^{-1} K_2O) and a mobile aluminium level of 4.4 mg100 g^{-1} soil. The experiment was set up in a split-plot design with lime treatments in main plots. Hydrated lime composed of 70% CaO and 1.8% MgO was used as a liming fertilizer at 0 (control), 0.5, 1.5 and 3 t ha^{-1} CaO, as calculated from the hydrated lime content of CaO. The lime material was uniformly broadcast onto the main plots before primary tillage. The alfalfa cultivars Banat, MS Mediana, K-28, Sinskaja and OS-66 were planted in subplots in three replications. Each elementary plot was 5 x 1 m. Alfalfa sowing was carried out using a hand-operated drill during the last ten days of April. The row spacing was 20 cm and the seeding rate was18 kgha^{-1}. The three-year study was designed to evaluate the following: total dry matter yield (t ha^{-1}) and quality (the contents of crude proteins - CP, crude ash - CA, crude fats - CFs, crude fibre – CF and the proportion of nitrogen-free extract – NFE). The obtained data were subjected to a two-factor (cultivar x liming) analysis of variance (ANOVA). Individual and interaction differences among the treatments were assessed by t-test.

Results and Discussion

The generally low dry matter (DM) yield of all cultivars in the establishment year was due to a somewhat late sowing date and unfavourable distribution of precipitation during the growing season (data not presented).

Table 1. Dry matter yield (t ha^{-1}) of alfalfa cultivars at different CaO rates (t ha^{-1}) during period 2006-2008

	2006	2007	2008
Cultivar (A)			
Banat	1.50c	6.64b	9.72c
Medijana	1.67bc	6.34bc	10.27ab
K-28	2.26a	8.49a	10.59a
Sinskaja	1.78b	6.75b	9.79bc
OS-66	1.84b	6.02c	8.77d
Liming (B)			
No lime	1.06c	3.12c	5.94c
0.5	1.06c	3.28c	6.44c
1.5	2.18b	9.77b	12.77b
3	2.94a	11.22a	13.56a
Banat No lime	1.23gh	3.21ij	5.05i
0.5	0.81i	3.09ijk	7.73g
1.5	1.59fg	9.37ef	13.04cde
3.0	2.37cd	10.88cd	13.05cde
Medijana No lime	0.86hi	3.40ij	7.47g
0.5	1.05hi	2.95jk	7.60g
1.5	1.93fg	8.61fg	12.80cdef
3.0	2.82b	10.40d	13.21cd
K-28 No lime	1.25gh	3.97i	7.03gh
0.5	0.89hi	5.00h	7.89g
1.5	2.98fg	12.23ab	12.49def
3.0	3.93b	12.57a	14.85a
Sinskaja No lime	1.01hi	2.17k	5.24i
0.5	0.99hi	2.66jk	5.69ij
1.5	2.24de	10.41d	13.68bc
3.0	2.90b	11.77bc	14.57ab
K-28 No lime	0.94hi	2.85jk	4.83i
0.5	1.57fg	2.71jk	6.29hi
1.5	2.15de	8.23g	11.84f
3.0	2.68bc	10.30de	12.14ef
ANOVA			
A	**	**	**
B	**	**	**
AB	**	**	**

Values followed by different small letters within columns are significantly different ($P<0.05$) according to the LSD test

*F test significant at $P\leq0.05$; **F test significant at $P\leq0.01$; ns – non significant

Lower amounts and unfavourable distribution of precipitation during the growing seasons of the first and second years induced a stronger cultivar response to increasing rates of CaO. DM yield was 2.77- and 3.60-fold higher under treatments with 3 tha^{-1}CaO in the first and second years, respectively, than in the control (Table 1). The yield of all cultivars in the third year, when a higher amount and more favourable distribution of precipitation were recorded, was 2.28-fold higher at a rate of 3 t ha^{-1} CaO than in the control.

Irrespective of liming rate, cv. K-28 produced a significantly higher DM yield in 2006 and 2007 as compared to the other cultivars, whereas the yield of cv. Medijana in the third year was comparable to that reported for the above cultivar (Table 1). However, the DM yield of Medijana in the first and second years was significantly lower than that of K-28, i.e. it was on the level with the cultivars that produced lowest yields (Banat and OS-66). The response pattern exhibited by cv. Medijana can be related to a specific trait that allowed it to achieve maximum yield potential under favourable distribution of precipitation over the year. A substantially lower yield in the third year was observed for cv. OS-66, as compared to the other cultivars. Irrespective of cultivars, significantly higher DM yields were obtained upon treatments with 3 t ha^{-1} CaO in all years. The control and the treatments with 0.5 t ha^{-1} resulted in significantly lower yields as compared to the treatments with higher CaO rates (Table 1). A significant increase in DM yield of alfalfa on acid soils treated with CaO was also reported by *Stout et al. (1997)* and. *Koopman et al. (1997)*. *Grewal and Williams (2003)* also reported a significant increase in DM yield in ten alfalfa cultivars as induced by treatments with 2 t ha^{-1} CaO on a soil having a pH_{CaCl2} 4.2. At the end of the third year, soil pH under treatments with 3 t ha^{-1} CaO was somewhat higher (4.41) as compared to the control (4.08). Mobile aluminium levels declined steadily with increasing CaO rate (from 4.4 to 1.1 mg $100gr^{-1}$ soil in the control and at 3 tha^{-1} CaO, respectively). A study by *Rechcigl et al. (1985)* revealed that Ca applications broadcast without incorporation can also lead to an increase in pH and a significant decline in Al levels in the top 15cm-layer of soil. The same authors reported a 2-3-fold increase in DM yield of alfalfa as induced by the above application (without tillage and 6-18 months before sowing). The significance of cultivar/liming interaction observed in all years suggests that the cultivars responded differently to different CaO rates (Table 1). The increase in CaO rate to 1.5 t ha^{-1} and 3 t ha^{-1} resulted in an increase in DM yield of all cultivars, the increase being significantly higher at the highest CaO rate. The strongest response to increasing CaO rates was exhibited by cv. K-28. In the second year, the lowest CaO rate of 0.5 t ha^{-1} only resulted in a significant increase in DM yield in K-28. This cultivar also showed the strongest response to increasing CaO application rates (Table 1). The lowest response to increasing CaO rates was exhibited by cvs. OS-66 and Medijana. In the third year, a significant increase in DM yield was observed only in cv. K-28 at CaO rates increasing from 1.5 to 3 t ha^{-1}. The lowest response of cv. OS 66 in this study can be due to its lower DM yield potential, given all liming rates and years. The favourable distribution of precipitation during the growing season in the third year, as opposed to the previous two years, likely contributed to achieving maximum DM yield potential in cv. Medijana in that its yield was not significantly different (except at the highest CaO rate) from that of cv. K-28. No significant differences in DM yield at higher CaO rates were recorded for cv. Sinskaja relative to cv. K-28, which also suggests that the achievement of its yield potential was largely dependent on environmental conditions. *Grewal and Williams (2003)* report that acid tolerance efficiency, as a ratio of dry matter yield in no lime treatment to dry matter yield in lime treatment (expressed as percentage), can be used as an indicator of cultivar tolerance to low pH. As observed by the authors, there is no correlation between this indicator and DM yield potential. Acid tolerance efficiency was 44.4%, 39.2%, 36.1%, 34.3% and 28.9% in cvs. Medijana, K 28, Banat, OS 66 and Sinskaja, respectively, as expressed through the average DM yield produced over the three-year period in both the control and the plots treated with 3 t ha^{-1} CaO. The results obtained in this study confirm the reports of the above authors and the suggestion provided by *Dall´Agnol et al. (1996)* that increased stress response and yield potential are not mutually exclusive. In addition, *Petcu et al. (2006)* report that the tested alfalfa germplasm show sufficient genetic variability for tolerance to soil acidity. Conversely, *Bouton (1996)* and *Stout et al (1997)* indicate a rather narrow range of genetic variability for alfalfa tolerance to soil acidity.

Table 2. The average contents (%) of crude proteins (CP), crude fibre (CF), crude ash (CA), crude fats (CFs) and nitrogen-free extracts (NFE) in the dry matter of the tested cultivars at different liming rates

	CP	CF	CA	CFs	NFE
Cultivar (A)					
Banat	15.13ab	30.63a	10.59a	2.63a	41.02a
Medijana	14.40b	31.30a	10.35a	2.28a	41.66a
K-28	13.96b	31.03a	10.15ab	2.45a	42.42a
Sinskaja	14.28b	31.05a	10.35a	2.07a	42.24a
OS-66	16.22a	31.10a	9.48b	1.96a	41.24a
Liming (B)					
No lime	12.86c	29.09b	10.73a	3.38a	43.95a
0.5	12.66c	29.84b	10.50ab	2.70a	44.31a
1.5	15.39b	33.88a	9.57c	1.52b	39.64b
3	18.29a	31.29ab	9.95bc	1.51b	38.97b

Values followed by different small letters within columns are significantly different ($P<0.05$) according to the LSD test

Crude protein content was significantly higher in cv. OS-66 than in the other cultivars (excepting cv. Banat) (Table 2). The cultivars showed no significant differences in the other quality indicators, excepting those in crude ash content. Increasing CaO rates (1.5 and 3 t ha^{-1}) caused a significant increase in protein content. *Stout et al. (1997)* reported an increase in crude protein content (from 9.2 to 16.3 %) upon treatment with 5 t ha^{-1} lime on a soil having a pH_{H2O} 5.1. *Gomes et al. (2002)* obtained a significant increase in Ca, Mg and N levels in DM by applying increasing CaO rates. The considerably higher fibre content in the treatment with higher CaO rates can be associated with more intensified growth and more rapid alternation of phenological stages. Consistently with the increase in fibre content, the other quality indicators (CA, CFs) had significantly lower values.

Conclusion

Irrespective of liming rate, cv. K-28 had a significantly higher dry matter yield in 2006 and 2007 as compared to the other cultivars. Regardless of cultivars, dry matter yields were substantially lower in the control and at 0.5 t ha^{-1} CaO than in the treatments with higher CaO rates in all years. The significance of the cultivar/liming interaction in all years indicated that the cultivars showed different responses at different CaO application rates.

Increased liming rates induced a significant increase in protein content in all cultivars. Acid tolerance efficiency, as a ratio of dry matter yield in no lime treatment to dry matter yield in lime treatment, can be used as an indicator of cultivar tolerance to low pH. The absence of correlation between acid tolerance efficiency and dry matter yield potential suggests that an increased response to acidity and yield potential are not mutually exclusive, i. e. that breeding for both traits is justified.

Reakcija lucerke na niske pH vrednosti zemljišta i kalcizaciju

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Rezime

Niska pH vrednost zemljišta je ograničavajući faktor u gajenju lucerke i osnovni uzrok slabe trajnosti lucerišta. U poljskim ogledima na zemljištu tipa pseudoglej (pH$_{nKCl}$ 4,08), u trogodišnjem periodu analiziran je prinos i kvalitet suve materije pet sorti lucerke (Banat, Medijana, K-28, Sinskaja, OS 66) uz primenu kalcizacije (kontrola, 0.5, 1.5 i 3 t ha^{-1} CaO). Nezavisno od nivoa kalcizacije, sorta K-28 je u trogodišnjem periodu imala značajno veći prinos suve materije u odnosu na ostale sorte. Ne uzimajući u obzir sorte, u svim godinama značajno veći prinosi suve materije su postignuti na tretmanima 3 t ha^{-1} CaO. Značajnost interakcije sorta/kalcizacija u svim godinama ukazuje na različitu reakciju sorti pri unošenju različitih količina CaO. Kalcizacija je uticala na značajno povećanje sadržaja proteina, pri čemu je najveći sadržaj kod svih sorti na varijanti 3 t ha^{-1} CaO. Stepen tolerantnosti na kiselost, kao odnos prinosa suve materije na kontrolnim tretmanima i tretmanima sa CaO može biti jedan od indikatora tolerantnosti sorte na niske pH vrednosti. Odsustvo korelacije između stepena tolerantnosti na kiselost i potencijala za prinos suve materije ukazuje da se pojačana reakcija na kiselost i

potencijal za prinos međusobno ne isključuju, odnosno da je selekcija na obe osobine opravdana.

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PRELIMINARY REPORT ON FORAGE YIELDS IN MID-TO LATE SPRING-SOWN ANNUAL LEGUME INTERCROPS

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Original research paper

Abstract: A small-plot trial was carried in 2009 at Rimski Šančevi and Zemun Polje, including pure stands of several mid- to late spring annual legume crops and their intercrops, where one component was prone to lodging (supported crop) and another one with good standing ability (supporting crop) and with mung bean intercropped with soybean 00 and white lupin and lablab bean with soybean I and pigeon pea. In average, Land Equivalent Ratio (LER) for green forage yield varied from 1.01 in the intercrop of mung bean and soybean 00 to 1.12 in the intercrop of lablab bean and pigeon pea. At the same time, the highest LER value for forage dry matter yield was in the intercrop of lablab bean and pigeon pea (1.10).

Key words: annual legumes, forage dry matter yield, green forage yield, intercropping, LER, soybean.

Introduction

Annual legumes have a prominent place in supplying animal husbandry with both voluminous and concentrated feed *(Mikić et al., 2006)*. The most important annual forage legumes in Serbia are pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.).

One of the strategic goals of a concerted action between the Institute of field and Vegetable Crops and the Novi Sad Faculty of Agriculture is the re-introduction of neglected and the introduction of the novel annual legume crops in the agriculture of Serbia. The evaluation of the potential for forage production in diverse annual legumes such as white lupin (*Lupinus albus* L.), mung bean (*Vigna radiata* (L.) R. Wilczek), pigeon pea (*Cajanus cajan* (L.) Millsp.) and lablab bean

(*Lablab purpureus* (L.) Sweet) has been carried out with promising results *(Mihailović et al., 2006a)*.

The cultivation of legumes in mixtures may have many advantages in comparison to pure stands, especially in a more efficient exploitation of environmental resources. Intercrops with annual legumes, such as soybean mung bean, white lupin, pigeon pea or lablab bean, may improve soil characteristics *(Ghosh et al., 2006)*, increase availability of nutrients such as nitrogen *(Abu-Gyamfi et al., 2007)* and phosphorus *(Cu et al., 2005)*, facilitate nodulation *(Li et al., 2009)* and improve yield itself *(Roy et al, 2003)*. Legume intercropping may be carried out in the form of a mixture of one perennial and one annual legume crop, where the latter plays a role of a bio-herbicide in the establishment of the former and contributes to the total yield *(Ćupina et al., 2010)*, as well as a mixture of one annual legume prone to lodging and another that has a good standing ability.

The aim of the research was to examine the potential of annual legume mixtures for forage yields.

Materials and Methods

A small-plot trial was carried in 2009 at two locations in Serbia, namely Rimski Šančevi near Novi Sad and Zemun Polje near Belgrade. The trial included pure stands and intercrops of several mid- to late spring annual legume crops (Table 1). In each intercrop, one component was prone to lodging and was designed as supported crop, while another had good standing ability and was referred to as supporting crop. At the same time, all components were intercropped according to the times of their maturity, with soybean of 00 maturity group, white lupin and mung bean in one, earlier, group and soybean of I maturity group, pigeon pea and lablab bean in another, later, group.

Table 1. Intercrops of mid- to late spring-sown annual legumes in the trial at Rimski Šančevi and Zemun Polje in 2009

Group	Supported crop			Supporting crop		
	Species	Genotype	Country of origin	Species	Genotype and status	Country of origin
Earlier	Mung bean	MM 06/18	China	Soybean 00	Gracia	Serbia
				White lupin	Vesna	Serbia
Later	Lablab bean	MM 08/01	Serbia	Soybean I	Lana	Serbia
				Pigeon pea	ICPL 88020	Australia

The trial was established on April 23 at Zemun Polje and on April 28 at Rimski Šančevi. In pure stands, the densities were 60 plants m^{-2} for soybean, 80 plants m^{-2} for white lupin, 50 plants m^{-2} for mung bean and 20 plants m^{-2} for pigeon pea and lablab bean. In all intercrops, both supporting and supported crops had a proportion of 50 % of their pure stand density. The prevailing weather and soil conditions during the trial period are given in Tables 2 and 3.

Table 2. Average monthly temperatures and monthly precipitation sums at Rimski Šančevi and Zemun Polje during the trial in 2009

Location	Year	April	May	June	July	Average/sum
Average monthly temperature (°C)						
Rimski Šančevi	2009	15	18	20	23	19
	Long-term	11	17	20	21	17
Zemun Polje	2009					
	Long-term	13	18	22	23	19
Total monthly precipitation (mm)						
Rimski Šančevi	2009	2	47	123	57	229
	Long-term	47	59	85	70	261
Zemun Polje	2009	7	27	72	31	137
	Long-term	43	41	76	56	216

Table 3. Chemical properties of the surface soil layers at Rimski Šančevi and Zemun Polje during the trial in 2009

Location	pH (H_2O)	N (%)	P_2O_5 (mg 100^{-1} g^{-1})	K_2O (mg 100^{-1} g^{-1})	$CaCO_3$ (%)	Humus (%)
Rimski Šančevi	7.89	0.195	51.13	40.00	8.64	2.95
Zemun Polje	7.97	0.177	26.80	24.90	1.90	2.80

Each pure stand was cut in the stages of full bloom and first pods forming, while each intercrop was cut when one of its components reached the same stages, although in all cases they were more or less concurrent.

Since the preliminary character of the whole trial, there were monitored green forage yield (t ha^{-1}) and forage dry matter yield (t ha^{-1}) in each pure stand and in each component of all intercrops. Green forage yield was measured immediately after the cutting, while the forage dry matter yield was calculated on the basis of the ratio between the masses of small green forage samples before and after the drying at the room temperature until constant values were achieved.

The reliability of the intercrops was determined upon their Land Equivalent Ratio (LER) values, calculated using the following formula:

$$LER = Sd(IC) / Sd(PS) + Sg(IC) / Sg(PS),$$

with Sd(IC) being supported crop yield in intercropping, Sd(PS) supported crop yield in pure stand, Sg(IC) supporting crop yield in intercropping and Sg(PS) supporting crop yield in pure stand. The LER values were calculated separately for green forage and forage dry matter yields.

The results were processed by the method of analysis of variance (ANOVA) using the LSD test.

Results and Discussion

There were significant differences at the levels of both 0.05 and 0.01 in green forage yields among the pure stands and among the intercrops (Table 4).

The average green forage yields in pure stands ranged between 14.5 t ha^{-1} in pigeon pea and 42.0 t ha^{-1} in soybean 00 and 42.8 t ha^{-1} in soybean I. The intercrops of mung bean with soybean 00 and lablab bean with soybean I had the highest average total green forage yield (37.9 t ha^{-1} and 38.3 t ha^{-1}), while the intercrop of lablab bean and pigeon pea had the lowest average total green forage yield (26.8 t ha^{-1}). In comparison to the results of previous research in the same conditions *(Mihailović et al., 2006b)*, the pigeon pea cultivar had much higher green forage yields, while lablab bean had similar performance.

The LER values for green forage yield varied from 1.01 in the intercrop of mung bean and soybean 00 to 1.12 in the intercrop of mung bean and white lupin at Rimski Šančevi and from 1.01 in the intercrop of mung bean and soybean 00 to 1.16 in the intercrop of lablab bean and pigeon pea. In average, LER for green forage yield varied from 1.01 in the intercrop of mung bean and soybean 00 to 1.12 in the intercrop of lablab bean and pigeon pea.

Table 4. Green forage yields (t ha^{-1}) in the pure stands and the intercrops of mid- to late spring-sown annual legumes in the trial at Rimski Šančevi and Zemun Polje in 2009

Location	Supported crop (Sd)	Supporting crop (Sg)	Pure stand		Intercropping			LER
			Sd	Sg	Sd	Sg	Sd +Sg	
Rimski Šančevi	Mung bean	Soybean 00	26.2	55.0	13.0	28.0	41.0	1.01
		White lupin		34.7	15.0	19.0	34.0	1.12
	Lablab bean	Soybean I	24.0	48.0	13.0	25.0	38.0	1.06
		Pigeon pea		13.0	14.7	6.0	20.7	1.07
Zemun Polje	Mung bean	Soybean 00	39.3	30.7	17.3	17.3	34.6	1.01
		White lupin		17.0	25.3	7.0	32.3	1.06
	Lablab bean	Soybean I	36.0	36.0	22.7	16.0	38.7	1.05
		Pigeon pea		16.0	26.0	7.0	33.0	1.16
Average	Mung bean	Soybean 00	32.8	42.8	15.2	22.7	37.9	1.01
		White lupin		25.8	20.2	13.0	33.2	1.09
	Lablab bean	Soybean I	30.8	42.0	17.8	20.5	38.3	1.05
		Pigeon pea		14.5	20.3	6.5	26.8	1.12
		$LSD_{0.05}$	5.7					0.05
		$LSD_{0.01}$	8.2					0.08

With significant differences in both pure stands and their intercrops (Table 5), the average forage dry matter yields in pure stands ranged between 4.4 t ha^{-1} in pigeon pea and 12.2 in soybean I and from 7.7 t ha^{-1} in both intercrops of both mung bean and white lupin and lablab bean and pigeon pea to 10.9 t ha^{-1} in the intercrop of lablab bean and soybean I. Mung bean confirmed its potential for the forage dry matter production *(Mikić et al., 2009)*.

The highest LER values for forage dry matter yield were 1.12 in the intercrop of mung bean and white lupin at Rimski Šančevi and 1.13 in the intercrop of lablab bean and pigeon pea at Zemun Polje. In average, the highest LER value for forage dry matter yield was in the intercrop of lablab bean and pigeon pea (1.10).

Table 5. Forage dry matter yields (t ha^{-1}) in the pure stands and the intercrops of mid- to late spring-sown annual legumes in the trial at Rimski Šančevi and Zemun Polje in 2009

Location	Supported crop (Sd)	Supporting crop (Sg)	Pure stand		Intercropping			LER
			Sd	Sg	Sd	Sg	Sd +Sg	
Rimski Šančevi	Mung bean	Soybean 00	6.3	13.8	3.1	7.0	10.1	1.01
		White lupin		7.6	3.6	4.2	7.8	1.12
	Lablab bean	Soybean I	6.7	13.9	3.6	7.3	10.9	1.06
		Pigeon pea		3.9	4.1	1.8	5.9	1.07
Zemun Polje	Mung bean	Soybean 00	9.4	7.7	4.2	4.3	8.5	1.01
		White lupin		3.7	6.1	1.5	7.6	1.06
	Lablab bean	Soybean I	10.5	10.4	6.3	4.6	10.9	1.05
		Pigeon pea		4.8	7.3	2.1	9.3	1.13
Average	Mung bean	Soybean 00	7.9	10.7	3.6	5.7	9.3	0.99
		White lupin		5.7	4.8	2.9	7.7	1.09
	Lablab bean	Soybean I	8.6	12.2	5.0	5.9	10.9	1.05
		Pigeon pea		4.4	5.7	2.0	7.7	1.10
		$LSD_{0.05}$	1.4					0.05
		$LSD_{0.01}$	1.9					0.07

Conclusions

Although still preliminary, the obtained results offer a solid basis for the possibility of intercropping diverse species of mid- to late spring-sowing annual legumes for forage production in the agro-ecological conditions of Serbia.

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Preliminarni izveštaj o prinosima krme u združenoj setvi srednje i kasno prolećnih jednogodišnjih mahunarki

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Rezime

Mikro ogled je izveden tokom 2009. godine na Rimskim Šančevima i Zemun Polju, koji je uključivao čiste i združene useve nekoliko srednje i kasno prolećnih jednogodišnjih mahunarki, gde je jedna komponenta bila osetljiva na poleganje (nošeni usev) a druga otprorna (noseći usev) i sa mungom združenim sa sojom 00 grupe zrenja i belom lupinom i lablabom sa sojom I grupe zrenja i kajanusom. U proseku, LER za prinos zelene krme kretao se u rasponu od 1.01 u združenom usevu munga i soje 00 grupe zrenja do 1.12 u združenom usevu lablaba i kajanusa. Istovremeno, najviša vrednost LER za suvu materiju krme bila je u združenom usevu lablaba i kajanusa (1.10).

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IMPORTANCE OF WINTER FORAGE PEA IN HIGH-QUALITY FORAGE PRODUCTION

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Abstract: Winter forage pea becomes more important forage crop for haylage production. Even in unfavourable conditions (drought) this crop provides good yields of high-quality fodder. It is usually sown as a mixture with cereals. Dry matter yield of forage pea is being increased with plant height but protein yield is being decreased due to leaves falling off of lower nodes. Researches conducted on Agricultural Institute Osijek, on commercial farming crops have shown the need for denser pea stand in mixtures with late-ripening wheat without awns. Crude protein yield of pure stand pea was 1603 kg ha^{-1} (when seeded 110 seeds per m^2), and of mixture with wheat (110 seeds of pea and 100 seeds of wheat per m^2) was 1174 kg ha^{-1}. Protein yield of pure stand pea was sufficient for production of 26716 litres of milk per ha, of pea/wheat mixture was sufficient for 18916 litres of milk and of pea/barley mixture for 19474 litres per ha. Good yields of silage maize were achieved when pea/wheat mixture was cut till 10th May and maize (OSSK 430) subsequently seeded. Such obtained dry matter yield of maize silage in 2008 year was 7.5 t ha-1and in 2009 6.3 t/ha. Winter pea/wheat mixture gave 7.5 t ha-1of dry matter in 2008 and 7.0 t ha-1in 2009 year. Haylage quality was affected by dry matter content during ensiling. Pea/wheat mixture should be cured to at least 35% dry matter prior to ensiling.

Key words: winter pea, mixture, forage, quality, yield

Introduction

Crop area of forage pea in Croatia is being enlarged each year. The most often use is for ensiling (haylage), enabling intensive use of arable land in this way. When forage pea is harvested in time, it enables subsequent maize growing for grain or silage production. Winter forage pea variety *Osječki zeleni* is 170 to 200 cm tall at the end of flowering what falls at the end of May and beginning of Jun in East Slavonia. This is too late for cutting because the late sowing of subsequent maize crop will considerably decrease silage yield and because the pea/cereal mixture will lose its quality (protein yield and digestibility). Forage pea gives a high quality fodder *(Uher 2007, Rodino et al. 2009)*. Forage pea contains 17 to 19 % of protein in dry matter *(DLG 1997)*. According to *Fraser et al. (2001)*, dry matter concentration increases in pea with plant maturation but protein yield decreases after the 1st cutting term. Forage pea is not grown as a pure stand crop but rather as a mixture with winter cereals to support against lodging. *Uher (2007)* has obtained average herbage yield of pea/wheat mixture of 80.05 t ha^{-1} in two years of research. *Stjepanović et al. (2007)* have obtained the highest protein yield at pea height about 112 cm. According to *Borreani et al. (2006)* and *Cavallarin et al. (2006)* pea/wheat mixture provides very good ensiled forage. According to *Salawu et al. (2001)* it can be better to adjust the cutting time with regard to wheat ripening than to pea, because wheat looses its quality with maturation much faster than pea. Since pea has capability to intake nutrients of less accessible forms and to withstand lower soil pH, it is suitable for achieving good yields in less favourable soils.

Materials and Methods

Research of pea/cereal mixtures was conducted on Agricultural Institute Osijek in two trials: "1st trial" with 3 cutting terms in 2007 and "2nd trial" with 1 cutting term and ensiling in 2008, and on dairy farm Krndija d.o.o. 2008 and 2009 with subsequent silage maize cropping, both in East Croatia. Seedings of pea and pea/wheat mixtures were done in October. Wheat varieties used were *Srpanjka* (early ripening) on Agricultural Institute and *Ludwig* (late ripening) on Krndija d.o.o. Seeding rate in the 1st trial was 110 kg of pea and 150 kg of wheat per ha, and seeding density in the 2nd trial was 110 seeds of pea per m^2 (P-110), and 100 and 200 seeds of wheat or barley per m^2 (W- or B- 100 or 200). Silage quality regarding the acids content was analyzed in the 2nd trial. Herbage was ensiled in PE ensiling bags, hand compressed and closed. Silage was assessed according to *Fliege*. Silage samples of 1 kg were taken of the mid of bags. Subsequent maize (hybrid OSSK 430, FAO 400) was sown on 20th May 2008 and 15th May 2009, 3 to 5 days after cutting pea/wheat mixture.

Results and Discussion

Investigation of cutting term effects has shown that delay in cutting increases forage yield. Pea herbage yield in the first cut (24th April 2007) was 20.3 t ha-1and it was growing to 29.1 tha^{-1} in the last cut at 21st May 2007, (Table 1).

Table 1. Average herbage yield, pea partition in mixture herbage and leaf partition in dry matter yield, depending on cutting term

Cutting term	Average herbage yield (t ha^{-1})			Pea partition in mixture herbage (%)	Leaf partition in dry matter yield (%)	
	Pea	Wheat	Mixture		Pea	Wheat
24th April	20.3	28.1	48.4	41.9	55.0	40.7
8th May	29.0	27.9	56.9	51.0	47.4	30.4
21st May	29.1	28.2	57.3	50.8	40.8	25.3
LSD 0.05	7.6	9.7	10.6		3.8	5.7

Wheat herbage yield varied between 27.9 and 28.2 t ha^{-1}. Partition of pea in mixture herbage yield was highest (51%) in the middle cut (Table 1). Leaf partition in dry matter yield of pea and wheat was highest in the 1st cut and it was decreasing to the last cut. The highest leaf partition (55%) was in pea in the 1st cut (Table 1). Protein concentration in herbage depends on leaf partition in herbage yield. *Stjepanović et al. (2007)* have obtained protein concentration in dry matter of pea green leaves about 37%, in yellow leaves about 20% and in stem between 10.15 and 15.34%. Protein concentration in pea and wheat was highest in the 1st cut. Decrease in protein concentration was faster in pea than in wheat. Protein concentration in pea was decreased 28.3% in 26 days (1.1% daily), and in wheat 24.8% (0.95% daily). Dry matter concentration has been increasing with cutting term. Dry matter and protein yield were highest in the last cut (Table 2).

Table 2. Dry matter (DM) and protein concentration and yield

Cutting term	Protein concentration in dry matter (%)		Dry matter concentration (%)		Yield of mixture DM (t ha^{-1})	Protein yield (kg ha^{-1})
	Pea	Wheat	Pea	Wheat		
24th April	22.10	13.30	13.6	18.5	8.0	1310
8th May	20.71	10.00	17.0	22.0	10.3	1489
21st May	15.84	10.00	18.9	25.4	12.6	1581
LSD 0.05			3.4	2.5	2.3	
LSD 0,01			4.7	3.5	3.1	

In the 2nd trial (Agricultural Institute Osijek, 2008), there were grown pure stand pea and pea/wheat and pea/barley mixtures. Pea plant height varied between 81 and 90 cm, average wheat height was 75 cm and barley 80 cm. Pure stand pea had protein concentration 183.53 g/kg of dry matter (Table 3), while the addition of wheat and barley caused lowering of protein concentration. Pure stand pea herbage had 14 % dry matter, and addition of wheat and barley particularly has increased the dry matter concentration (Table 3).

Table 3. Concentration of crude nutrients, energetic value and dry matter concentration (DM)

Variant (seeds/m^2)	Protein (g/kg)	Fibre (g/kg)	Fat (g/kg)	N.F.E.* (g/kg)	ME (MJ/kg)	NEL (MJ/kg)	DM (%)
P-110	183.53	163.94	44.95	176.92	9.693	5.485	14.0
W-100,P-110	167.76	145.80	50.41	206.59	9.812	5.578	15.1
W-200,P-110	149.53	148.72	26.66	252.20	9.313	5.284	16.0
B-100,P-110	119.68	155.68	20.96	278.35	9.065	5.142	19.0
B-200,P-110	112.74	171.67	20.71	270.89	9.048	5.121	18.6
Wheat							16.5
Barley							18.6
LSD 0,05	13.1	17.4	10.8	10.8	n.s	n.s	1.65
LSD 0,01	20.0	n.s.	n.s	n.s.	n.s	n.s	2.40

*N.F.E. = nitrogen free extract

The highest metabolic energy concentration (ME) was achieved in pea/wheat mixture with thinner wheat stand (9.812 MJ/kg), following was pea/wheat mixture with denser what stand, and the least in pea/barley mixtures (Table 3). Pure stand pea had ME of 9.69 MJ/kg. Net lactation energy concentration (NEL) had similar pattern (Table 3). Average dry matter yield of pea/barley mixture was 10545 kg ha^{-1}, of pure stand pea was 8737 kg ha^{-1} and of pea/wheat mixture was 6834 kg ha^{-1}. The highest yield of mixtures with barley was due to much taller barley variety compared to wheat, and earlier ripening of barley. Obtained protein yield varied between 997 kg ha^{-1} (denser stand of wheat + pea) and 1603 kg ha^{-1} (pure stand pea). Mixtures protein production could suffice for milk production between 16616 and 21009 l ha^{-1}, and NEL production for 11107 to 17249 l ha^{-1} (Table 4).

Table 4. Yield of dry matter (DM), crude protein (CP) and energy (ME and NEL) per ha, and potential milk yield (P.M.Y. in litres per ha) based on protein and NEL yield

Variant	DM (kg ha^{-1})	CP (kg ha^{-1})	ME (MJha^{-1})	NEL (MJ ha^{-1})	P.M.Y. based on protein (l ha^{-1})	P.M.Y. based on NEL (l ha^{-1})
P-110	8737	1603	84688	47921	26716	15117
W-100,P-110	7000	1174	68667	39058	19566	12321
W-200,P-110	6669	997	62085	35211	16616	11107
B-100,P-110	11587	1266	95915	54415	21099	17249
B-200,P-110	9504	1071	86010	48660	17850	15350
W + P	6834	1085	65376	37234	18916	12346
B + P	10545	1168	90962	51537	19474	16299
LSD: 5%	1800	2097	2655	18268	1033	3257
LSD:1 %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Winter forage pea/cereal mixture should be cut in earlier terms and used for ensiling as haylage. Earlier cutting gives lower DM yield but just slightly lower protein yield. When deciding on cutting term, the yield of subsequent crop should be taken into consideration too. There were obtained good silage maize yields in 2008 and 2009 year (100 ha each year) on the farm Krndija when maize was sown till 15th May, despite not so favourable rainfall in these years. Obtained dry matter yield of pea/wheat mixture (late ripening tall wheat *Ludwig* was used) on the farm Krndija was 7.5 t ha^{-1}and subsequent silage maze also 7.5 t ha-1in the year 2008, while the yields in 2009 were 7.0 t ha^{-1} and 6.3 t ha^{-1} , respectively. Pea/wheat mixture was used for haylage preparation each year. Haylage quality appraisal according to *Fliege* was very good. Protein concentration in dry matter was 13% in 2008 and 14% in 2009. The lower protein concentration in 2008 was due to lower pea content in the mixture. Pea partition in 2009 was 2.2 times greater than in 2008. The effect of dry matter concentration in haylage on its quality was investigated on the Agricultural Institute Osijek. The higher dry matter concentration was associated with higher quality of haylage (Table 5). In mixtures with wheat, dry matter concentration was slightly above 26% what was too low and affected the unfavourable acids content and low *Fliege* appraisal.

Table 5. Dry matter and acids concentration in haylage (%) and quality appraisal

Mixture variant	DM (%)	Lactic acid (%)	Acetic acid (%)	Butiric acid (%)	Appraisal (*Fliege* points)
W-100,P-110	26.19	3.43	0.25	1.17	60
W-200,P-110	26.21	3.10	0.65	0.69	68
B-100,P-110	31.87	4.27	0.60	0.19	85
B-200,P-110	31.81	3.05	0.45	0.26	80

The higher dry matter (near 32%) in mixtures with barley caused the better quality of haylage. Therefore, ensiling should be performed when dry matter concentration is at least 35% to achieve the high quality of haylage.

Conclusion

Winter forage pea is very valuable crop in high quality fodder production and intensive use of arable land. Winter pea/cereal mixture yields 6.6 to 11.6 t ha^{-1} of dry matter and subsequent maize silage about 7 t ha^{-1} dry matter what together give considerable amount of ensiled forage per hectare. Protein yield of pea/wheat mixture was about 1180 kg ha^{-1}. Potential milk yield based on protein production per ha of pea/wheat mixture was 18916 l ha t ha^{-1}, of pea/barley mixture was 19474 l ha^{-1} and of pure stand pea was 26716 l ha^{-1}. Pea and late-ripening wheat mixture should be sown in wheat-sowing term, with density of 100 seeds of pea and 110 seeds of wheat per m^2. Increase in wheat partition decreases protein concentration and protein yield. Pea/cereal mixture should be cut at pea height of about 110 cm and in heading of cereal. In East Croatian conditions this term is about 10th May. Pea/wheat mixture is suitable for ensiling but it should have at least 35% of DM.

Značaj ozimog graška u proizvodnji kvalitetne krme

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Rezime

Ozimi grašak postaje u Hrvatskoj sve značajnija kultura za proizvodnju krme u obliku senaže. Ova kultura i u nepovoljnim klimatskim uslovima (suša) daje dobre prinose kvalitetne krme. U proizvodnji krme seje se u smeši sa žitaricama. Sa povećavanjem visine biljke raste prinos suve materije ali pada prinos belančevina. Lišće s donjih etaža stabljike opada, stabljika postaje gola. Ispitivanja sprovedena na Poljoprivrednom institutu u Osijeku i široka proizvodnja pokazuju potrebu gušćeg sklopa graška u smeši sa kasnim sortama pšenice bez osja. Prinos sirovih belančevina graška bez žitarica je bio 1.603 kg ha^{-1} pri setvi 110 zrna po m^2, a smeša graška (110 zrna/m^2) i pšenice (100 zrna/m^2) dala je prinos belančevina od 1.174 kg ha^{-1}. Graškom bez žitarice proizvedeno je belančevina za 26.716 l ha^{-1} mleka, smešom s pšenicom 18.916 l ha^{-1}, a s ječmom 19.474 l ha^{-1}.

Košenjem smeše graška i pšenice do 10. maja i brzom naknadnom setvom kukuruza (OSSK 430) postižu se dobri prinosi kukuruza za silažu. U 2008. godini postignut je prinos suve materije silo mase kukuruza od 7,5 t ha^{-1}, a u 2009. godini prinos od 6,3 t ha^{-1}. Prinos suve materije osnovnog useva (smeše) bio je 2008. godine 7,5 t ha^{-1}, a 2009. 7,0 t ha^{-1}. Kvalitet senaže uslovljen je sadržajem suve materije u silo masi pri konzerviranju. Smeša graška i pšenice mora se prosušiti da se dobije suva materija od barem 35%.

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EFFECT OF GENOTYPE AND CROP DENSITY ON SAINFOIN (*Onobrychis vicifolia* Scop.) FORAGE YIELD

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Abstract: Sainfoin has a small potential for seed yields and that often limits its wider cultivation. Sainfoin is, also, a highly competitive species in both mixtures and pure stand and that makes the research on different stand densities especially important. Four year (2004-2008) field trial was carried out as a three–factor random block design. The Factor A was cultivar, genotype (Makedonka and EG Norm), the Factor B row spacing (15, 25 and 30 cm) and the Factor C seed rate (80, 120 i 160 kg ha^{-1}). The paper presents the total annual forage yields (t ha^{-1}) during all four trial years. The achieved forage yields are in accordance with the environment and sainfoin life. The influence of cultivar is not crucial for satisfactory green forage yields in sainfoin. In all four trial years, regardless of cultivar and seeding rates, the highest green forage yields were achieved in dense stand (15 cm), followed by 25 cm and finally by 35 cm. the highest green forage yields were at a seed rate of 120 kg ha^{-1}. For the widely present dual-purpose cultivation, that is, for forage and seed, the most reliable production of both forage and seed production is at a seed rate of 80 kg ha^{-1} and at a row spacing of 25 cm.

Key words: sainfoin, crop density, seeding rate, forage yield

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) may be a potential replacement for lucerne (*Medicago sativa* L.) in the agro-ecological conditions of Vojvodina due to its productive and quality traits. Being cultivated mainly in non-irrigated farming systems, lucerne often produces yields much lower than its biological potential. In the conditions of Vojvodina, there is a lack of precipitations of about 250 mm for the successful forage production *(Bošnjak, 1991)*. The research so far points that sainfoin is well tolerant to drought, as well as that it has a considerable growth rate in arid conditions too *(Ćupina et al., 1993; Ćupina et al., 1999)*. Sainfoin has a small potential for seed yields and that often limits its wider cultivation. Along with this, sainfoin is a highly competitive species in both mixtures and pure stand and that makes the research on different stand densities especially important. The results of various trials in semi-arid conditions, with sainfoin grown for both forage and seed, reveal that, in all trial years and in most cuts, sainfoin produces the highest forage yield at the lowest row spacing, while the highest seed yields are at a seed rate of 100 do 150 kg ha^{-1} *(Vučković et al., 1996)*. Depending on the cultivation purpose, forage or seed, there is row spacing from 12.5 cm to 35 cm and seed rates from 70 kg ha^{-1} to even 360 kg ha^{-1}. It should be remembered that the main reason for high seed rates in sainfoin is the sowing of a one-seed pod *(Ivanova-Bandžo, 1973)*. *Spedding and Dikmans (1972)*, *Ćupina et al. (1993)* and *Ivanovski et al.,* (*1998*) emphasize that the highest forage yields in sainfoin are produced in a dense stand, that is, at small row spacing. By decreasing seed rate, by regulating seed quantity and row spacing, yields of both forage and seed are affected. The seeding rates higher than 160 kg ha^{-1} may lead to a decrease in forage yields as a consequence of higher competitiveness between plants, as well as to a decreased stand density. *Ćupina and Erić (1999)* point out that the highest green forage yields are achieved at a seed rate of 140 kg ha^{-1} and row spacing of 25 cm. They also point that seed rate does not significantly affect the yields of both forage and seed, meaning that satisfactory yields are possible at a lower seed rate and that the seed rates needs to be adjusted according to the genetic potential for seed yield and thus contribute to the production reliability. Being aware of all that was said, this paper is aimed at assessing the impact of genotype, row spacing and seed rate to forage yields in sainfoin, since the sainfoin production could be more reliable if the adequate genotype is assessed for the agro-ecological conditions of Vojvodina and if the optimal seed rates are determined.

Material and Methods

A field trial was carried out at Čenej, near Novi Sad, in the unit of *Agricultural Production – Matica*, the section of *Agriculture*, of the company of Neoplanta Meat Industry, Novi Sad, as a three–factor random block design and with four replicates. The Factor A was cultivar, that is genotype (Makedonka and EG Norm), the Factor B row spacing (15, 25 and 30 cm) and the Factor C seed rate (80, 120 i 160 kg ha^{-1}). The trial was established on 23 April 2004, with wheat as a preceding crop. In the first year (2004), sainfoin was cut twice, on 10 July and 31 August. In the second year (2005), it was cut four times, on 25 May, 27 June, 11 August and 18 October. In the third year (2006), sainfoin was cut also four times, on 12 June, 11 July, 16 August and 29 September. In the fourth year (2007), it was cut four times again, on 5 May, 13 June, 25 July and 10 October. The paper presents the total annual forage yields (t ha^{-1}) during all four trial years. Each time, the stand was cut in its stage of the technological maturity for forage production.

Soil conditions. The trial was conducted on a slightly carbonated chernozem soil, with its chemical properties shown in Table 1. The reaction of this soil is between neutral and acid, with a high $CaCO_3$ in the sowing layer. The $CaCO_3$ content increases together with the depth, unlike humus and total nitrogen. The soil was well provided with nitrogen in its active layer, that is, humus-accumulative part, while the content of both phosphorus (P_2O_5) and potassium (K_2O) was low, decreasing as the depth increases.

Table 1. Soil properties (Čenej)

Depth	$CaCO_3$	pH in H_2O	pH in KCl	Humus (%)	N (%)	mg/100 g soil	
						P_2O_5	K_2O
0-30	17.34	8.23	7.63	3.08	0.153	5.7	14.0
30-60	24.45	8.34	7.67	2.01	0.101	2.6	10.0
60-90	26.12	8.42	7.78	1.46	0.073	1.8	9.0
Pros. 0-90	22.64	8.33	7.69	2.18	0.109	3.37	11.0

Rainfall amount and distribution as well as monthly temperatures are presented in table 2. There were favourable weather conditions for crop establishment in the sowing year. In the second and fourth year of sainfoin life i.e. 2005 and 2007, deficit of precipitations in April effected yield in the first cutting.

Table 2. Amount of precipitation (mm) and mean monthly temperatures (^oC) for hydrological years (2004-2007)

Rainfall (mm)												
Year/ month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2004	46.5	22.6	20.5	100.2	105.6	123.4	70.5	47.0	42.7	70.5	117.0	35.5
2005	30.2	41.2	33.7	23.7	52.0	143.2	160.7	120.1	57.0	0	19.8	52.9
2006	44.0	12.3	68.7	73.9	89.8	106.7	15.2	129.6	9.0	13.4	9.3	27.2
2007	26.0	48.1	82.9	0	66.1	53.1	31.2	82.7	58.1	117.4	56.5	0
Long term avarage	36.7	31.1	51.5	49.5	78.4	106.6	69.4	94.9	41.7	50.3	50.7	28.9
Temperature (^oC)												
Year/ month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2004	-1	2.5	6.7	12.5	15.2	19.8	22	21.7	16.3	14.2	7	2.7
2005	0.3	-3.3	4.7	12.1	17	19.3	21.7	20.3	18	12.7	5.3	2.3
2006	-1.0	1.7	6.0	13.3	16.3	19.3	23.3	20.3	18.7	11.6	6	1.2
2007	6.2	5.8	8.9	13.4	18.6	22.0	23.2	22.6	14.6	10.9	3.9	0.1
Long term avarage	-0.6	1.6	6.2	11.4	16.8	19.9	21.2	21	16.9	10.4	8.4	2.5

Results and Discussion

The achieved forage yields are in accordance with the environment (Table 3a and 3b) and years of life of sainfoin. The highest average forage yield was in the second year (62.0 t ha^{-1}). The average forage yield in the fourth year was 54.1 t ha^{-1}, in the third year it was 43.7 t ha^{-1}, while in the first year it was lowest (27.7 t ha^{-1}). It is indicative that that precipitation sum in the summer months in 2005 was above the long-term average, especially in June, July and September, while the temperatures in these months were either average ones or slightly below the long-term average. In 2007, the precipitation sum was the same as that of the long-term average and it is a good distribution of the precipitations that resulted in high green forage yields in this year. Regarding the cultivar, that is, the Factor A, in 2004 the cultivar EG Norm had higher green forage yield for 2.2 t ha^{-1}, while in the second year (2005) the cultivar Makedonka had higher green forage yield for 0.8 t ha^{-1}. In the third year, the difference in green forage yields between the two cultivars of 1.7 t ha^{-1} was not significant. In the fourth year, the cultivar Makedonka had higher green forage yield for 2.0 t ha^{-1} with no significance. These results show that the influence of cultivar is not crucial for high green forage yields in sainfoin. In all four trial years, regardless of cultivar and seeding rates, the highest green forage yields were achieved in dense stand (15 cm), followed by 25 cm and finally by 35 cm. The lowest green forage yield at a row spacing of 15 cm was produced in the first year (2004) in the cultivar EG Norm at a seed rate of 80 kg ha^{-1} (29.8 t ha^{-1}). The highest average green forage yield was in the second year (2005), at a row spacing f 15 cm and in the cultivar Makedonka, what was higher for 13.9 t ha^{-1} in comparison to the yield at a row spacing of 25 cm. This difference was significant and offers the basis for recommending a dense stand for green forage production. In all trial years, the highest green forage yields were at a seed rate of 120 kg ha^{-1}. In the first year (2004), regardless of cultivar, the highest green forage yield (28.5 t ha^{-1}) was at a seed rate of 120 kg ha^{-1} and was not significantly higher in comparison to the other seed rates. The difference between this seed rate and the seed rate of 160 kg ha^{-1} was only 0.6 t ha^{-1}. Somewhat higher difference (1.9 t ha^{-1}) was in comparison to the seed rate of 80 kg ha^{-1}, although it was not significant as well. In the second year (2005), the highest green forage yield (63.7 t ha^{-1}) was at a seed rate of 120 kg ha^{-1}, what was only 0.2 tha^{-1} higher in comparison to the green forage yield at a seed rate of 80 kg ha^{-1}, with no significant difference in both this and the comparison to the green forage yield at a seed rate of 160 kg ha^{-1}. In the third year (2006), the highest green forage yield (46.7 t ha^{-1}) was achieved at a seed rate of 120 kg ha^{-1}, not significantly higher in comparison to the green forage yield at a seed rate of 80 kg ha^{-1}, but significantly higher than the green forage yield at a seed rate of 160 kg ha^{-1}. The same trend in both cultivars was obvious in the fourth year (2007): the highest green forage yield (57.7 t ha^{-1}) was at a seed rate of 120 kg ha^{-1}, significantly higher in comparison to the green forage yield at a seed rate of 160 kg ha^{-1} and not significantly higher than the green forage yield at a seed rate of 80 kg ha^{-1}, with a difference of 3.4 t ha^{-1}.

Table 3a Effect of genotype, row spacing and seeding rate on sainfoin forage yield

Year		2004				2005			
		Seeding rate							
Variety	Row spacing (cm)	80	120	160	Average	80	120	160	Average
Makedonka	15	30.6	29.7	30.6	30.3	79.2	75.8	67.1	74.1
	25	27.6	26.5	26.6	26.9	60.1	60.7	60	60.2
	35	19.1	23.1	25.4	22.6	49.1	55.1	54.7	53
	Average	25.8	26.5	27.5	26.6	62.8	63.8	60.6	62.4
EG Norm	15	29.8	33.2	30.9	31.3	74.4	73.4	65.5	71.1
	25	28.6	30.3	28.1	29.0	60.6	60.7	56.8	59.4
	35	23.8	28.3	25.8	26.0	57.6	56.5	48.9	54.3
	Average	27.4	30.6	28.2	28.8	64.2	63.5	57.1	61.6
Average		26.6	28.5	27.9	27.7	63.5	63.7	58.8	62.0

LSD $_{0.05}$	A	B	C	AB	AC	BC	ABC
2004	3.673	1.551	2.046	2.86	3.102	3.17	4.692
2005	12.88	4.58	6.11	9.93	10.23	9.44	14.44

Table 3b Effect of genotype, row spacing and seeding rate on sainfoin forage yield

Year		2006				2007			
		Seeding rate							
Variety	Row spacing (cm)	80	120	160	Average	80	120	160	Average
Makedonka	15	49.8	54.8	47.8	50.8	66.8	72.1	60.1	66.3
	25	41.4	44.7	42.2	42.8	51.9	57.0	52.0	53.6
	35	36.7	32.8	35.6	35.0	46.5	47.1	42.4	45.3
	Average	42.6	44.1	41.9	42.9	55.1	58.7	51.5	55.1
EG Norm	15	51.5	58.3	49.8	53.2	66.0	69.7	60.8	65.5
	25	41.4	46.5	38.9	42.3	49.0	51.3	48.6	49.7
	35	37.7	43.2	33.9	38.2	45.3	48.9	38.2	44.1
	Average	43.5	49.3	40.9	44.6	53.5	56.6	49.2	53.1
Average		43.1	46.7	41.4	43.7	54.3	57.7	50.4	54.1

LSD $_{0.05}$	A	B	C	AB	AC	BC	ABC
2006	7.839	4.186	3.829	6.477	6.284	6.506	9.476
2007	16.4	5.56	6.61	12.66	12.56	10.46	16.51

Considering seed rate, regardless of the significant differences in single years and depending on cultivar, the reliability of production should be taken into account. In most cases, the differences in yield are not significant enough to justify higher seed rates than the most reliable ones. Regarding the genetic potential of sainfoin for seed yield, as well as the available quantities on the seed market, it is recommended to apply lower seed rates, that is, 80 kg ha^{-1}.

Conclusion

On the basis of the results obtained during four years, the following conclusions may be drawn:

The influence of cultivar is not crucial for satisfactory green forage yields in sainfoin. The most appropriate row spacing for green forage production in sainfoin is 15 cm, at a seed rate of 120 kg ha^{-1}. For the widely present dual-purpose cultivation, that is, for forage and seed, the most reliable production of both forage and seed production is at a seed rate of 80 kg ha^{-1} and at a row spacing of 25 cm.

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Uticaj genotipa i gustine useva na prinos krme esparzete (*Onobrychis vicifolia* Scop.)

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Rezime

U agroekološkim uslovima Vojvodine izvršeno je četvorogodišnje ispitivanje (2004-2007) uticaja genotipa i gustine useva na prinos krme esparzete. Ispitivan je uticaj dve sorte (Makedonka i EG Norm), međurednog razmaka (15, 25 i 35 cm) i setvene norme (80, 100 i 120 kg ha^{-1}). Najveći prinos krme ostvaren je pri gustorednoj setvi na 15 cm sa količinom semena od 120 kg ha^{-1}. Ova setvena norma, međutim nije dala statistički značajnu razliku u prinosu zelene krme u odnosu na setvu sa normom od 80 kg ha^{-1} semena.

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INVESTIGATION OF THE PRODUCTIVITY OF SOYBEAN AND FIELD BEAN AS SECOND CROPS

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Original scientific paper

Abstract: In this study, results of two-year research of the yield and quality of soybean (*Glycine hispida* Max.) and field bean (*Vicia faba* L.) as second crops are presented. Crops were grown in irrigation conditions. Later sowing in the first research year influenced lower yields of both crop species. Soybean had lower average yield of 4573 kg ha^{-1}, however, in regard to chemical quality, soybean contained higher amount of crude proteins, crude fat, calcium and magnesium in comparison to field bean, whereas field bean had higher content of crude fibre, phosphorus and potassium. Soybean realized higher energy value in MJ kg^{-1} DM in production of milk and meat. Higher yield in kg ha^{-1} of field bean and lower energy value expressed in MJ g kg^{-1} resulted in uniform production of NE_L and NE_M units per surface for both species. Soybean realized higher content of DCP (g kg^{-1}) and higher production (kg ha^{-1}).

Key words: soybean, field bean, second crops, productivity, chemical composition

Introduction

One of major reasons for low livestock production in our country is inadequate nutrition. Use of fodder feeds of high quality would solve numerous problems of nutrition of cows in our country *(Koljajić et al., 1995)*. Research results obtained by *Stošić et al. (1996)*, in low land region of Serbia, on individual agricultural holdings, indicated shortage of protein in diet. This indicates that in the sowing structure of areas under forage plants the share of leguminous plants should be increased. Also, it is expected that in future nitrogen fixating plants will have more important role in sustainable development of agriculture, and are basis of sustainable livestock production in low land region.

Importance of annual forage leguminous plants reflects primarily in realization of high yields of forage plants of excellent quality *(Erić et al., 2007)*. The most important annual forage leguminous plants in Serbia are fodder peas (*Pisum sativum L.*) and common vetches (*Vicia sativa L*) *(Mihailović et al., 2007)*.

Selection of species for second crops is of great importance. *Nenadić (1983), Stjepanović et al. (1988), Ostojić et al. (1996), Zarić (1998),* pointed out that beside the grain also the whole soybean plant has high quality and it is very suitable for production of voluminous forage feed. *Nenadić (1983)*, in the study of the possibility to grow soybean as second crop, stated that high yields of green mass, dry matter, crude proteins and mineral substances are obtained. *Popović et al. (1988)* stated that field beans also can be very important source of proteins, in production of grain as well as biomass.

Objective of this research was to determine the productivity and quality of biomass of soybean and field bean as second crops.

Materials and Methods

Field trial was set on experimental field of the Institute of forage crops, Kruševac, according to random block system in four repetitions. Size of calculation parcel was 16 m^2 (3.2 x 5 m). Sowing for the trial was carried out on 24^{th} of July in the first and on 15^{th} of July in the second year. After pre-sowing preparation, soybean - Danica (*Glycine hispida* Max.) and field bean - Vezuvijana (*Vicia faba* L.) were manually sowed. Sowing was carried out in distance between rows of 20cm. Planed density of both crops was 800 000 plants per hectare. Crops were grown in controlled humidity conditions. In the time of cutting soybean was in the stage of initial maturation and field bean in blooming stage.

All results obtained are expressed in absolute dry matter. For determination of nutritious value of above ground biomass, data on chemical composition of species from our researches was used. Digestibility of protein was determined based on table values, *Obračević (1990)*. Based on obtained results NEL and NEM were calculated.

Results and Discussion

Based on data relating to yield of dry matter (Table 1), it can be concluded that both species realized higher yield of dry matter in the second research year. Realized higher yield in the second year is consequence of earlier sowing compared to preceding year. In both research years, field bean had higher yield (4918 kg ha^{-1}) of dry matter compared to soybean (4573 kg ha-^{1}). The decrease of yield of second crops as result of later sowing is indicated in studies by other authors *(Vučić 1981, Terzić 2001).*

Table 1. Yield of dry above ground biomass of soybean and field bean (kg ha^{-1})

Species	year	Yield kg ha^{-1}	Lsd	
			0.05	0.01
Soybean	I	4454	616	830
	II	4691	522	768
	average	4573	443	594
Field bean	I	4664	616	830
	II	5171	522	768
	average	4918	443	594

Realized average yield of soybean (4573 kg ha^{-1}) was at the level of yield reported by *Nenadić (1983)*, *Stjepanović et al. (1988).* Realized yield of above ground mass of field bean in our research was significantly lower (6.7 t ha^{-1} to 12.2 t ha^{-1}) than yield reported by *Popović et al. (1988)* achieved in regular sowing deadline from March to April.

Biological value of forage is result of share of protein, first of all, as one of the most important parameters of the livestock food quality. Soybean in both research years had higher content of crude proteins (187.7 and 204.0 g kg^{-1}DM) compared to field bean (173.4 and 180.3 g kg^{-1}) (Table 2). Higher crude protein content in g kg^{-1} contributed to higher quantity of crude proteins also per unit of surface in case of soybean (895.9 kg ha^{-1}) compared to field bean (886.7 kg ha^{-1}). Crude protein content in soybean dry matter is in accordance to results obtained by *Kolarski et al. (1988)*, where 20.61% of crude protein was established. Content of crude proteins field bean in this study is almost average value compared to values for crude protein content (14.9-19.5%) obtained in three year research by *Popović et al. (1988)*. Similar results of crude protein content in soybean and field bean dry matter and silage are stated by *Dinić et al. (1999)*.

Table 2. Chemical composition of biomass and soybean and field bean yields (g kg^{-1}DM and kg ha^{-1})

Spe-cies	Research year	Crude protein	Crude fibre	Crude fat	NFE	Ash	P	K	Ca	Mg
Soy bean	I	187.7	263.9	30.2	421.7	96.5	3.6	11.4	12.8	7.1
	II	204.0	261.6	42.6	409.9	81.9	3.1	9.2	10.4	6.4
	Average g kg^{-1}DM	195.9	262.8	36.4	415.8	89.2	3.4	10.3	11.6	6.8
	yield kg ha^{-1}	895.9	1201.8	166.5	1901.5	407.9	15.5	47.1	53.0	31.1
Field bean	I	173.4	370.9	21.3	347.0	87.4	3.9	15.4	4.5	5.1
	II	187.1	363.1	19.6	347.8	82.4	4.1	12.4	4.6	4.4
	Average g kg^{-1}DM	180.3	367.0	20.5	347.4	84.9	4.0	13.9	4.6	4.8
	Yield kg ha^{-1}	886.7	1804.9	100.8	1708.5	417.5	19.7	68.4	22.6	23.6

By analyzing the content of crude fibre it can be concluded that field bean showed significantly higher content and production of crude fibre (367 g kg^{-1} and 1804.9 kg ha^{-1}) compared to average content and production established in soybean (262.8 g kg^{-1} and 1201.8 kg ha-^{1}). Crude fibre content in soybean is in accordance to values stated by *Stjepanović et al.(1988)*. Authors stated that crude fibre content had decreased from 31.09% in the stage of pod forming to 26.93% in the stage of forming of grain. Higher fibre content in biomass of field bean compared to soybean is consequence of higher share of stem in biomass, since when it was cut field bean was in blooming stage whereas soybean was in stage of grain forming.
Soybean formed significantly higher content of crude fat 36.4 g kg^{-1}DM and 166.5 kg ha^{-1} compared to field bean (20.5 g kg^{-1} and 100.8 kg ha^{-1}). Higher average crude fat content in soybean is consequence of higher crude fat content in soybean in both investigation years, especially in the second year (42.6 g kg^{-1}DM). Higher content of crude fat in soybean in second research year is consequence of early sowing, i.e. higher share of grain in time of cutting and higher content of crude fat in soybean grain (170.0 g kg^{-1}SM).

Average content of NFE in field bean was 347.4 g kg^{-1}DM and 1708.5 kg ha^{-1}, whereas soybean had slightly higher NFE content 415.8gkg^{-1}DM and 1901.5 kg ha^{-1}. Quantity of crude ashes is equal and in average of 89.2 g kg^{-1}DM, with production of 407.9 kg ha^{-1} in soybean biomass and 84.9 g kg^{-1}SM and 417.5 kg ha^{-1} in field bean biomass (Table 2). Same tendency was established also for P content. Content of P in soybean biomass was 3,4, and in biomass of field bean 4,0 g kg^{-1}DM. Contrary to calcium and magnesium, lower quantity of potassium (10.3 g kg^{-1}DM) and phosphorus (3.4 g kg^{-1}DM) was concluded in soybean compared to field bean (13.9 g kg^{-1}DM. and 4.0g kg^{-1}DM).

Calcium content in investigated species varied significantly. It was established in limits of 4.6 g kg^{-1}DM in case of field bean to 11.6 g kg^{-1}DM in soybean (Table 2). *Marten (1984)* stated that higher quantity of calcium in leguminous plants was the most critical deviation in animal nutrition. However, surplus of calcium usually is harmless when phosphorus supply is sufficient. With adequate amount of phosphorus in food ruminants can tolerate Ca : P ratio up to 7 : 1, *(NRC, 1985)*. Considering the results of our study we observe that Ca : P ratio even in soybean which contains more calcium, is not over 7:1, but misbalance must be compensated with adequate quantities from concentrated feed, or balancing of diet in regard to P in mineral feeds rich in this element. Calcium and phosphorus content in soybean and field bean was within the limits stated by *Dinić et al. (1999)*.

In order to adequately take into consideration nutritious value of the two species, their net energy values expressed in MJkg^{-1}DM and MJha^{-1} are presented in Table 3 as well as content and quantity of digestible proteins.

In regard to net energy values of soybean in the first and second investigation year, it is noticeable that net energy values in the second year are higher compared to the first year, which is result of higher share of grain in the second year. Soybean and field bean realized higher net energy value in milk production (NE_L 5.70 and 5.30 MJ kg^{-1}DM) compared to meat production (NE_M 5.69 and 5.17 MJ kg^{-1}), which is in accordance with values stated by *Obračević (1990)*.

Table 3. Energy and protein value of soybean and field bean

Species		NE_L MJ kg^{-1}DM	NE_M MJ kg^{-1-} ^{1}DM	DCP G kg^{-1-} ^{1}DM	NE_L MJ ha^{-1}	NE_M MJ ha^{-1}	DCP kg ha^{-1}
Soybean	I	5.60	5.59	145.1	24 937	24 892	646
	II	5.79	5.78	158.1	27 057	27 010	739
	average	5.70	5.69	151.6	25997	25951	693
Field bean	I	5.27	5.15	128.4	24 579	24 020	599
	II	5.32	5.19	138.5	27 509	26 837	716
	average	5.30	5.17	133.5	26044	25429	658

Soybean realized higher net energy value in milk production (5.70 MJ kg^{-1}DM) compared to average values realized by field bean (5.30 MJ kg^{-1}DM). However, per unit of surface, field bean realized higher amount (26044 MJ ha^{-1}) compared to soybean, considering that field bean had higher production of dry matter than soybean. Soybean also realized higher net energy value in meat production (5.69 MJ kg^{-1}) compared to field bean (5.17 MJ kg^{-1}), but at the same time higher amount per unit of surface (25951 MJ ha^{-1}) in comparison to amount realized by field bean (25429 MJ ha^{-1}) even though field bean had higher production of dry matter.

Soybean realized more DCP - 145.1 g kg^{-1}DM, compared to field bean (128.4 g kg^{-1}DM), which contributed to higher quantity of digestible crude proteins per unit of surface in case of soybean (693 kg ha^{-1}) compared to field bean (658 kg ha^{-1})

Conclusion

Based on results of the investigation of soybean and field bean as second crops, the following can be concluded:
Soybean and field bean in the two year study as second crops had equal production of nutritious matters.

Field bean had higher production of above ground biomass in both investigation years.

Soybean had higher contents of crude proteins, fat, NFE, ashes, calcium and magnesium, whereas field bean had higher contents of crude fibre, phosphorus and potassium.

Soybean realized higher net energy values in production of milk and meat expressed in $MJkg^1$. Field bean had higher production of NE_L per unit of surface, and soybean realized higher production of NE_M and digestible crude proteins per unit of surface.

As second crops and in conditions of irrigation both species can produce significant quantities of high quality fodder food, of great importance in nutrition of ruminants.

Ispitivanje produktivnosti soje i stočnog boba u postrnoj setvi

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Rezime

Soja i stočni bob su ispitivani u postrnoj setvi u dvogodišnjim istraživanjima. Usevi su gajeni u uslovima navodnjavanja. Ispitivani usevi su formirali ujednačenu produkciju hranljivih materija. Stočni bob je formirao nešto veću produkciju suve materije po jedinici površine (4918 kg ha^{-1}). Soja je ostvarila veći prosečan sadržaj i veću produkciju sirovih proteina (195.9 g kg^{-1} i 895 kg ha^{-1}) u odnosu na bob (180.3 g kg^{-1} i 886.7 kg ha^{-1}). Soja je ostvarila i veći sadržaj svraljivih proteina i veću energetsku vrednost u proizvodnji mleka i mesa u MJ kg^{-1}. Veća produkcija suve materije stočnog boba doprinela je da stočni bob formira veću produkciju NEL-a po jedinici površine (26044 MJ ha^{-1}) u odnosu na soju (25997 MJ ha^{-1}). Soja je formirala veću produkciju NEM-a i svarljivih sirovih proteina po jedinici površine (25951 MJ ha^{-1} i 693 kg ha^{-1}) u odnosu na bob (25429 MJ ha^{-1} i 658 kg ha^{-1}). Obe vrste u postrnoj setvi u uslovima navodnjavanja mogu da formiraju značajne količine kvalitetne kabaste hrane i njihovo gajenje u postrnoj setvi u uslovima navodnjavanja ima puno opravdanje.

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PRODUCTIVITY OF INTERCROPPED FIELD PEA AND SPRING WHEAT

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Original scientific paper

Abstract. The paper presents the results of researches carried out over 2000-2002 which aim was to compare the yield of mixtures of field pea with spring wheat depending on the proportion of components, in changeable weather conditions of Central-East Poland. The following combinations with field pea were taken into account: (100, 80, 60, 40, 20, 0%) and with spring wheat (0, 20, 40, 60, 80, 100%) in the mixture. The participation of plant was normalized relatively to the number of sowed seeds in pure sowing; it means 100 seeds on $1m^2$ of field pea and 400 grains of spring wheat. The mixtures were sown in the first decade of April. Directly before the harvest of mixtures, from every field the weed samples were collected to determine the dry mass. Then the lodging resistance of plants was carried out in 9°scale. The collections of mixtures were taken in the stage of full maturity of grain of spring wheat. During the harvest, on every field the grain yield was determined. In collected samples the content of crude protein was also determined (N*6.25) by Klejdahl method. Weather conditions affect grain yield, crude protein content, weeding and lodging of field pea and spring wheat mixture. The highest grain and straw yield was achieved from the mixture of field pea with spring wheat of components content 60 + 40%, and the highest efficiency of crude protein was achieved from field pea cultivated in pure sowing.

Key words: field pea, mixture, spring wheat, crude protein, yield.

Introduction

The cultivation of leguminous-cereal mixtures for grain is one way of obtaining a concentrate fodder, with a higher nutritive value. The selection of components of mixtures is one of the key factors for good yield *(Ceglarek et al., 2007; Księżak and Magnuszewska, 1999; Michalski, 1994; Rudnicki, 1999)*. Useful for the cultivation on fertile soils are considered the mixtures of spring wheat with field pea *(Ceglarek et al., 2007; Księżak and Magnuszewska, 1999; Podleśny,* *1996; Rudnicki, 1999; Rudnicki and Wenda-Piesik, 2002, Szczygielski, 1993)*. Important and difficult part of agriculture is to determine the optimal quantitative composition of cereals and peas in mixtures. The views of different authors on this subject are divided *(Ceglarek i in., 2002; Michalski, 1994; Rudnicki, 1999)* so this type of research is needed. The hypothesis is that the proportion of field pea will differentiate the yield of mixtures of field pea and spring wheat. This allows selecting such combinations, which irrespectively of weather conditions will issue the high yield of grain. The aim was to compare the yielding of mixtures of field pea and spring wheat depending on the share of components, in changeable weather conditions of Central-East Poland.

Materials and Methods

A field experiment was carried out at the Experimental Farm in Zawady, University of Podlasie in Siedlce, located on 52^o 20'north latitude, and on 22^o 30'east longitude. Researches were carried out on a grey-brown podsoile soil formed from strong loamy sand, with neutral pH and medium available phosphorus, potassium and magnesium contents. Humus content was 1.37%. The experiment was a three-replicate split blocks design. Following combinations with field pea were taken into account: (100, 80, 60, 40, 20, 0%) with spring wheat (0, 20, 40, 60, 80, 100%) in the mixture. The species share in the mixture was established in relation to the number of grains sown in pure stand that is 100 grains of field pea (Kier cultivar) and 400 grains of spring wheat (Banti cultivar) per 1m^2. Phosphorus and potassium fertilizers were applied in the autumn in the amounts adjusted to soil fertility. In the spring a nitrogen fertilizer was applied on all the plots prior to planting at the rate of 30 kg N ha^{-1}. In the phase of shooting of spring wheat, additionally on control object with spring wheat nitrogen fertilization at amount of 50 kg N ha was applied, and on objects with mixtures at amount of 30 kg N ha^{-1}. The mixtures were planted in the first decade of April. Weeds were controlled mechanically by using a harrowing twice before and once after emergence of plants. Directly before the harvest of mixtures, from every plot the weed samples were collected to determine the dry mass. The collection of mixtures was taken in the stage of full maturity of spring wheat grain. During the harvest, on every grain field, yield was determined. In collected samples the content of crude protein was also concerted by Klejdahl method. The experimental data were subjected to statistical analysis. The course of weather conditions in years of studies was significantly different. The most favorable to cultivation mixtures of field pea with spring wheat was year 2002, with the highest amount of rainfalls evenly distributed in each month of growing season. Slightly worse weather conditions appeared in 2001 and the worst in 2000. Distribution of rainfalls, in each month of growing season has been uneven. Small amount of rainfalls was recorded in June (17.0 mm) and May (24.6 mm) and excess in July (155.9 mm).

Results and Discussion

Grain yield of field pea and spring wheat mixtures significantly diversify the course of weather conditions, the components proportion in the mixture and its co-work (Table 1). The highest grain yield was obtained in favourable year 2002. Slightly worse weather conditions appeared in 2001 caused the decrease of grain yield by 5.6%, and unfavourable in 2000 further decrease in grain yield by 20.4%. In own researches the share of components in mixture also significantly differ the grain yield. The highest grain yield was collected from field pea and spring wheat mixtures whose components share was 60+40%. It is supported by *Ceglarek et al. (2007)*, *Księżak and Magnuszewska (1999)*, *Michalski (1994)* and *Rudnicki (1999)* studies, who showed that the plants in mixed sowings generally more appropriate and more accurately yield than in pure sowing. In our researches the mixtures with proportion of components 80+20%, 40+60% i 20+80% yielded significantly lower. But those yields differ significantly from grain yield of field pea and spring wheat cultivated in pure sowing. It is in line with to *Rudnicki and Wendy-Piesik (2002)* sufficient. In conducted experiment the interaction was showed, that the highest grain yield was obtained in 2002 from a mixture of field pea with spring wheat with the share of components 60 +40%, and the lowest in 2000 from a mixture of field pea with spring wheat with the share of components 20 +80% and from spring wheat cultivated in pure sowing. *Ceglarek et al. (2007), Kotecki (1990), Michalski (1994)* and *Rudnicki (1999)* claim that plants cultivated in mixed sowings showed higher resistance to adverse on unfavourable weather course than in pure sowing. In our researches such relation was noted only from mixtures with share components 60 +40%.

Table 1. The grain yield of field pea and spring wheat mixtures, t ha^{-1}

Proportion of components, %		2000	2001	2002	Average
field pea	spring wheat				
100	0	3.56	4.42	4.70	4.23
80	20	3.69	4.59	4.85	4.38
60	40	3.94	4.72	5.12	4.59
40	60	3.62	4.56	4.78	4.32
20	80	3.42	4.38	4.60	4.13
0	100	3.46	4.63	4.85	4.31
Average		3.62	4.55	4.82	-
$LSD_{0.05}$					
Years					0.23
Proportion of components					0.18
Interaction					0.31

The efficiency of crude protein from seed of field pea and spring wheat mixtures was significantly diversified by atmospheric conditions, proportion of components and its interaction (Table 2).

The highest efficiency of crude protein from grain of field pea and spring wheat mixtures was noted in favourable year 2002, significantly lower in 2001, and the lowest in unfavourable year 2000. The highest efficiency of crude protein was achieved from field pea cultivated in pure sowing. The addition of spring wheat to field pea caused the decrease of protein yield in 1 ha. Analogically to those experiments, such as *Ceglarek et al. (2002), Green et al. (1985), Kotecki (1990)* claim that leguminous-cereal mixtures provide more protein from grain than spring cereals.

Table 2. The efficiency of crude protein from grain of field pea and spring wheat mixtures, kg ha^{-1}

Proportion of components, %		2000	2001	2002	Average
field pea	spring wheat				
100	0	860	1093	1151	1034
80	20	810	1033	1081	975
60	40	778	959	1029	922
40	60	636	826	855	772
20	80	525	697	722	648
0	100	456	636	656	583
Average		677	874	916	-
$LSD_{0.05}$					
Years					29
Proportion of components					53
Interaction					61

In our researches the interaction between weather conditions with the share of components in mixtures was showed, from which it follows that the highest efficiency of crude protein was achieved in 2002 from grain of field pea cultivated in pure sowing, and the lowest in 2000 from grain of spring wheat.

Table 3. Dry matter of weeds before harvest of field pea and spring wheat mixtures, g m^{-2}

Proportion of components, %		2000	2001	2002	Average
field pea	spring wheat				
100	0	25.8	30.5	44.9	33.7
80	20	14.7	29.2	39.8	27.9
60	40	12.6	17.4	25.0	18.3
40	60	13.9	18.6	23.2	18.6
20	80	15.4	20.7	36.6	24.2
0	100	28.5	33.2	38.8	33.5
Average		18.5	24.9	34.7	-
$LSD_{0.05}$					
Years					2.9
Proportion of components					3.2
Interaction					3.6

Weed infestation of stand before the harvest of field pea and spring wheat mixture significantly diversity the weather conditions, share of components and its interaction (Table 3). The highest weed infestation recorded in 2002, but the lower number of rainfalls was noted in years 2000 and 2001 it caused significant reduce of weeds presence. The mixtures of field pea and spring wheat with proportion of components 60+40% and 40+60% characterized with the lowest weed infestation. But the highest amount of weeds was noted in field pea and in spring wheat cultivated in pure sowing. Also *Cousens (1996), Creamer et al. (1996), Haugaard-Nelsen and Jensens (2004), Kotecki (1990), Michalski (1994)* and *Sobkowicz and Podgórska (2006)* claim that plants which grow in mixtures usually better use the productive space than the same plants in pure stand sowings, which contributes to reduce weed infestation. In this experiment the interaction was showed that the lowest mass of weeds were noted in 2000 in field pea and spring wheat mixtures with share of components 60+40% and 40+60%, and the highest in 2002 in field pea cultivated in pure sowing.

Conclusion

The course of weather conditions in years of researches significantly diversify the grain yield, the efficiency of true protein, weed infestation of field pea

and spring wheat mixtures. The highest grain yield achieved from field pea and spring wheat mixtures with a share of components 60+40%, and the highest efficiency of true protein from field pea cultivated in pure sowing. Field pea and spring wheat mixtures with a share of components 60+40% and 40+60% characterized with the lowest weed infestation.

Produktivnost stočnog graška i jare pšenice gajenih u smeši

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Rezime

Rad prikazuje rezultate istraživanja sprovedenih u periodu 2000-2002. godine u cilju utvrđivanja uticaja udela komponenti smeše stočnog graška sa jarom pšenicom na prinos u uslovima centralno-istočne Poljske. Korišćene su sledeće kombinacije stočnog graška: (100, 80, 60, 40, 20, 0%) i jare pšenice (0, 20, 40, 60, 80, 100%) u smeši. Udeo biljke je određen u odnosu na broj posejanih semena u čistoj setvi (100 semena na 1 m^2 stočnog graška i 400 zrna jare pšenice). Smeša je sejana u prvoj dekadi aprila. Neposredno pre žetve, korovi su uzorkovani iz svakog polja i određena je njihova suva masa. Ocenjena je i otpornost na poleganje. Kosidba smeše je obavljena u fazi pune zrelosti zrna pšenice. U sakupljenim uzorcima je određen sadržaj sirovih proteina Kjeldahl-ovom metodom. Dobijeni rezultati ukazuju da vremenski uslovi utiču na prinos zrna i sadržaj proteina. Najveći prinos semena i suve mase je dobijen iz smeše stočnog graška i jare pšenice u odnosu 60+40%, a najveći sadržaj proteina je utvrđen kod stočnog graška gajenog u cistoj kulturi.

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THE DRY MATTER YIELD AND QUALITY OF THE MIXTURE OF SOME FORAGE CROPS RESISTANT TO DROUGHT

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Abstract: During 2006-2008, at NARDI Fundulea, researches having as aim the achievement of some forage crop mixtures resistant to drought (sainfoin, smooth brome) have been performed. The researches focused on obtaining of economical fodder yields, with high nutritive value and application of some adequate technologies, under prolonged drought. The effect of sowing (4 sowing variants in autumn vs. classic spring one) and harvesting times on yield of three forage mixtures were tested. In the first year of vegetation, the obtained yield of variants sown in autumn ranged between 12.6 and 13.5 t ha^{-1} dry matter, vs. only 9.3 and 9.9 t ha^{-1} dry matter in spring sowing. On the first – third year vegetation average, the dry matter yields ranged between 10.5 and 12.7 tha^{-1}. There were emphasized the sowing times from first and tenth September and mixtures consisting of: 70 kg ha^{-1} sainfoin + 9 kg ha^{-1} smooth brome + 6 kg ha^{-1} hybrid ryegrass; 70 kg ha^{-1} sainfoin + 9 kg ha^{-1} smooth brome + 9 kg ha^{-1} perennial ryegrass, which yielded between 12.0 and 12.7 t ha^{-1} dry matter, with very significant yield gains (8-19%) vs. variant sown in spring. The harvesting time influenced the yield level, achieving 12.2 and 12.7 t ha^{-1} dry matter, during 7-14 days of harvesting, starting with sainfoin blooming stage.

Key words: sainfoin, smooth brome, forage mixture, sowing time, harvesting time, drought.

Introduction

Forage crop breeding and technology researches emphasize the breeding importance for spring green-up, recovery after cutting and drought stress as well as choice of the suitable crop technology for Romanian weather conditions in relationship to biological request of new genotypes *(Moga and Mateiaş, 2000; Moga and Schitea, 2005)*. Over the past year, the researchers have focused on development of the intensive technology for alfalfa (*Medicago sativa L*) and pasture mixtures, efficiently under favourable conditions of soil moisture *(Moga et al., 1983, 1996, Moga and Schitea, 2005)*. During last years, due to climatic changes the fervency and severity of drought and heat increased in Romania *(Petcu et al., 2009)*. The forage and livestock production will not be excluded from the impact of climate change which consisted in decrease of livestock production and unbalance at the soil and vegetation level. Studies concerning technology measures that can be taken against drought effects have been applied during last years at NARDI Fundulea. In this respect, the sainfoin (*Onobrychis viciifolia* Scop.), perennial forage legume with high hay productivity, nutritive quality, adapted to the limestone soils and drought tolerant was included into forage mixture. In addition to sainfoin also smooth brome (*Bromus inermis* Leyss.) was studied due to spring growth-up and because mixtures with sainfoin gave higher dry matter yield (over 20-25%) than the single crop *(Moga et al., 1983)*.

Material and Methods

The experiment was conducted at experimental field of the National Agricultural Research and Development Institute, located in south eastern part of Romania, with temperate climate and chernozem soil. One sainfoin variety, ssp. bifera (destined for more cuttings/year), Splendin –, earliness and with four years persistence, Olga - smooth brome cultivar, Marta - perennial ray grass and Florentin hybrid rye grass were used. Two trials were performed during three vegetation periods (2006-2007). Experimental design was a randomized complete block (on plots of 8.75 m^2 each) with two factors. For first trial, the factors were: a) sowing time (beginning with August 2nd and end of September 20th, the check was sowing in the spring) and 3 levels of factor b - forage mixtures with different seeding rates (b_1= sainfoin - 70 kg ha^{-1}, smooth brome - 9 kg ha^{-1}, hybrid ryegrass - 6 kg ha^{-1}; b_2 = sainfoin - 70 kg ha^{-1}, perennial rye grass - 9 kg; b_3 = sainfoin - 70 kg ha^{-1}, smooth brome - 9 kg ha^{-1}, perennial rye grass - 6 kg ha^{-1}). For second trial, the factors were: a) forage mixtures with different seeding rates (a_1= sainfoin - 70 kg ha^{-1}, smooth brome - 9 kg ha^{-1}, hybrid rye grass - 6 kg ha^{-1}; a_2 = sainfoin - 70 kg ha^{-1}, perennial rye grass - 9 kg, hybrid rye grass - 6 kg ha^{-1}) and 4 levels of factors b - harvest time (first harvest at sainfoin boot stage and than every seven days). These two experiments were fertilized with only 70 kg ha^{-1} phosphorus. The nutritive units, digestibility coefficient, net energy, digestible protein and dry matter yield were analyzed. The results were subjected to analysis of variance.

Results and Discussion

Total rainfall and its monthly repartition were totally different in the studied years. So, the cumulated rainfall was with 79.8 mm, 125.8 and 178.6 mm below the normal of the zone, suggesting unfavourable conditions for forage crops (table 1). All testing years were characterized by hot summer, the average temperature of summer being over multiannual zone average, up to 2.1°C respectively 3.3°C in July 2007, (Table 1).

Table 1. Average temperature (°C) and monthly distribution of rainfall (mm) during the forage mixtures vegetation period. Fundulea, 2006 -2008

Month	Rainfall (mm)				Temperature (^{0}C)			
	2006	2007	2008	Multiannual average	2006	2007	2008	Multiannual average
I	35.6	30.3	15.0	31.5	-3.6	4.1	-3.1	-1.7
II	13.0	12.0	2.3	30.,8	-0.8	3.3	2.4	0.6
III	41.3	33.4	21.4	37.6	5.2	7.3	8.2	5.7
IV	66.2	4.9	61.6	45.5	13.2	11.5	12.7	11.9
V	50.5	21.3	59.9	59.1	21.1	19.3	16.6	18.0
VI	73.3	18.5	30.6	70.1	20.9	24.1	21.9	21.6
VII	61.1	60.5	57.5	71.7	22.9	26.9	23.3	23.6
VIII	58.1	84.6	1.6	51.4	23.0	23.9	25.0	23.1
IX	49.4	21.7	59.2	50.9	18.0	16.9	16.6	17.4
X	15.1	46.2	25.9	40.4	13.1	11.7	12.6	11.9
XI	19.1	52.7	27.5	43.1	7.0	-3.3	5.8	4.1
XII	11.8	62.4	33.3	42.1	1.8	-0.6	2.5	0.5
Sum Average	494.5	448.5	395.7	574.3	11.8	12.1	12.0	10.0
Differences	-79.8	-125.8	-178.6		+1.8	+ 2.1	+ 2.0	

Under these conditions, the dry matter yield achieved by mixtures of sainfoin with grasses at different planting date ranged between 10.1 and 12.3 t ha^{-1}. Dry matter yields from sowings in spring were between 10.1 and 12.3 t ha^{-1}, being significantly lower than from sowings in autumn (1-10 September) (10.9-12.3 t ha^{-1}). Crude protein concentrations were established between 1856 and 1947 kg ha^{-1} for sowing in spring and 2366 and 2655 kg ha^{-1} for sowing in autumn. Nutritive units were established between 8189-8870 for sowing in spring and 11034-12138 for sowing in autumn (Table 2). The superiority of the forage mixtures sowing in the autumn could be explained by the root system able to uptake the nutrients from the soil. Also, the close interdependency between root and taproot of plant was confirmed by the correlation between the carbo hydrate reserve of roots and plant vegetative resumption *(Habben and Volence, 1990).* Mixing of sainfoin in a grasses mixture increased the dry matter with 8% when was used hybrid rye grass and with 19% if was used perennial rye grass comparatively with pure crop of sainfoin (Table 2). The effect of sowing date on yield of forage mixtures was higher in the first vegetation year, when the dry matter yield produced by mixtures sown according intensive technology were 34-41% higher ($P > 0.01$) than those sowing according traditional technology (Table 3).

Table 2. Effect of sowing time on mixtures of sainfoin with grasses. Fundulea, 2006-2008

Planting date	Seeding rate (kg ha^{-1})				Dry matter yield (t ha^{-1})				Crude protein	Nutritive units
	Sainfoin	Smooth brome	Perennial rye grass	Hybrid rye grass	Year			Average		
					2006	2007	2008	T ha^{-1}	kg ha^{-1}	kg ha^{-1}
20 august	70	9	-	6	12.8	11.8	10.1	11.6	2518	11469
	70	-	9	-	11.8	11.0	9.9	10.9	2366	11103
	70	9	9	-	12.6	11.9	10.3	11.6	2462	10861
1 September	70	9	-	6	13.3	12.8	10.7	12.3	2616	11917
	70	-	9	-	12.8	12.3	10.8	12.0	2566	12043
	70	9	9	-	13.3	12.6	10.6	12.1	2599	11465
10 September	70	9	-	6	13.5	12.2	10.3	12.2	2655	12096
	70	-	9	-	12.9	11.7	10.1	11.6	2586	12138
	70	9	9	-	13.4	11.9	10.5	11.9	2618	11551
20 September	70	9	-	6	12.8	12.3	9.9	11.7	2518	11469
	70	-	9	-	11.9	11.6	9.8	11.1	2386	11197
	70	9	9	-	12.8	12.2	10.2	11.7	2501	11034
Sowing in spring	70	9	-	6	9.9	11.0	10.2	10.4	1947	8870
	70	-	9	-	9.3	10.9	10.0	10.1	1865	8750
	70	9	9	-	9.5	11.5	10.5	10.5	1856	8189
LSD 5%					0.3	0.5	0.3	0.4	76	344

Table 3. Effect of sowing time of sainfoin and grasses mixture, first year of vegetation

Forage mixtures/Seeding rates	Dry matter			
	Sowing autumn	Sowing spring	Differences	
	t ha^{-1}	t ha^{-1}	t ha^{-1}	**%**
1. Sainfoin (70 kg ha^{-1}), smooth brome (9 kg ha^{-1}), hybrid rye grass (6 kg ha^{-1})	13.5	9.9	3.6	136***
2. Sainfoin (70 kg ha^{-1}), perennial ryegrass (9 kg ha^{-1})	12.9	9.3	3.6	134***
3. Sainfoin (70 kg ha^{-1}), smooth brome (9 kg ha^{-1}), perennial rye grass (9 kg ha^{-1})	13.4	9.5	3.9	141***
LSD 5%			0.4	4

Contribution of hybrid rye grass (biennial species) in the second vegetation year was lower than in the first vegetation year. The high yield obtained in the first vegetation year from the variants sown in autumn may be explained by the good development of the plants at the beginning of the winter. So, those plants supported better the great temperature differences between day and night even without snow layer. The average dry matter yield obtained from the trail with different harvest time for the forage mixture consisted by sainfoin, smooth brome, perennial and hybrid rye grasses ranged between 11.4-12.8 tha^{-1} (Table 4).

Table 4. Effect of harvest time on dry mater yield and quality of forage mixtures, Fundulea, 2006 – 2008

Forage mixtures	Harvested time	Dry matter yield (t ha^{-1})				Crude protein		Nutritive units
		Year			Average			
		2006	2007	2008	t ha^{-1}	%	kg ha^{-1}	
Sainfoin (70 kg ha^{-1}), smooth brome (9 kg ha^{-1}), perennial rye grass (6 kg ha^{-1})	Beginning of budding	12.5	11.1	10.5	11.4	100	2459	11200
	After seven days of first harvested	13.6	12.7	11.3	12.5	110	2675	12186
	After fourteen days of first harvested	13.2	12.5	11.1	12.3	108	2596	11021
	After twenty one days of first harvested	12.1	11.8	10.8	11.6	101	2380	10842
Sainfoin (70 kg ha^{-1}), perennial ray grass (9 kg ha^{-1}), hybrid rye grass (6 kg ha^{-1})	Beginning of budding	12.7	11,7	10.9	11.8	100	2546	11949
	After seven days of first harvested	13.9	12.5	11.9	12.8	108	2787	13079
	After fourteen days of first harvested	13.7	12.1	118	12.5	106	2747	12890
	After twenty one days of first harvested	12.3	11.5	11.0	11.6	98	2466	11573
	LSD 5%	0.9	0.4	0.3	0.5	4	105	482

The lowest yield was obtained in the trial harvested at the beginning of the budding stage of sainfoin and the highest was given by the trials harvested after 7-4 days from first harvested, irrespective of forage mixtures composition when the yield gain compared to control were 6-10%, DM and 17%, for nutritive units (Figure 1).

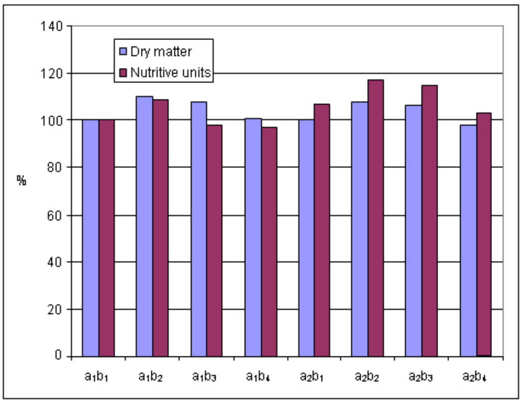

Figure 1. The forage yield and quality of sainfoin and grasses mixtures depending on harvesting time

In the first vegetation year the total dry matter yield (13, 2-13,7 t ha^{-1}) increased up to mid flowering, after this the yield reduction was recorded (12, 1-12,3 t ha^{-1}). This could be explained through the negative effect of late cutting, due to lost of important quantity of leaves and late tillers. Same observations are valid for the crude protein per hectare and nutritive units. The crude protein ranged from 2675 to 2787 kg ha^{-1}, at the beginning mid of flowering and 2380-2466 kg ha^{-1} at full flowering, and nutritive units ranged from 10842 to 11573, at the beginning mid of flowering and 12186-13079 at full flowering. The main indicators of forage quality for ruminants are chemical composition, expressed in digestible elements and equally the consumption. Nutritive values of studied mixtures were influenced by sowing ratio and the chemical composition of the species forage mixtures (Table 5). The content of the crude protein in the first vegetation year (average of three cuts) ranged between 19.81 and 20.40% in sainfoin, 13.55-15.90 in grasses; 64-68 digestibility coefficient in sainfoin, respectively 61 - 65 C.D. in grasses; obtained un forage with 0.86-0.92 nutritive units at sainfoin and 0.79-0.87 at grasses. The increasing of percentage of drought resistant species has a positive economic impact for forage producers, enabled to produce with low inputs.

Table 5. The quality indicators of sainfoin and grasses mixtures. Fundulea, first year of vegetation

Indicators of quality	Variants/seedlings rate					
	b1		b2		b3	
	Sainfoin/ 70 kg ha^{-1}	Smooth brome/ 9 kg ha^{-1} + hybrid ray grass/6 kg ha^{-1}	Sainfoin/ 70 kg ha^{-1}	Perennial rye grass/ 9 kg ha^{-1} *	Sainfoin/ 70 kg ha^{-1}	Smooth brome/ 9 kg ha^{-1} + perennial rye grass/ 9 kg ha^{-1}
Crude protein (%)	20.40	13.55	20.04	14.81	19.81	15.90
Neutral detergent fiber (%)	34.55	45.39	34.09	42.52	37.85	41.30
Acid detergent fiber (%)	25.52	27,81	28.30	29.06	29.23	27.75
Digestibility coefficient	67	61	68	65	64	65
Net energy (kcal)	1306	1117	1339	1233	1215	1234
Nutritive units (oat)	0.92	0.79	0.95	0.87	0.86	0.87

Beside the high forage yields obtained with these mixtures, one must point out that the sainfoin, as perennial leguminous, had a positive impact on soil, and did not require nitrogen fertilization. In addition, the sainfoin releases nitrogen in soil for next crop and it is able to uptake the nitrogen leached, from the deep soil layers. This behaviour makes sainfoin perfectly compatible with the modern concept of environmental conservation and ecological agriculture.

Conclusion

- Forage mixtures consisting in sainfoin, smooth brome, perennial rye grass and hybrid rye grass provide a high dry matter yield (11-13 t ha^{-1}), with good nutritive value (0, 85-0, 95 nutritive units, 1117-1339 kcal, net energy), and protein - energy ratio optimal for animal nutrition.
- The best combination was: sainfoin (70 kg ha^{-1}) + smooth brome (9 kg ha^{-1}) + hybrid rye grass (6 kg ha^{-1}) and sainfoin (70 kg ha^{-1}) + smooth brome (9 kg ha^{-1}) + perennial rye grass (9 kg ha^{-1});
- This experimental results support the increasing of the surface cultivated with sainfoin and smooth brome due to their superior behaviour under drought conditions
- Cropping of these forage mixtures (compatible with ecological agriculture concept) is performed with low fertilizers inputs, without herbicides, having a positive economic impact for farmers.

Istraživanje smeša nekih krmnih useva otpornih na sušu

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Rezime

U periodu 2006-2008. godine na NARDI Fundulea su ispitivane smeše krmnih biljaka otpornih na sušu (esparzeta, bezosni vlasen). Istraživači su istakli ekonomski aspekt dobijanja krme sa visokim nutritivnim vrednostima kao i primenu adekvatne tehnologije u slučaju produžene suše. Proučavan je uticaj vremena setve (4 varijante setve u jesen, u odnosu na klasičnu prolećnu) i žetve na prinos krme tri smeše. U godini vegetacije, ostvaren je prinos suve materije između 12.6 i 13.5 t ha^{-1} za varijante sejane u jesen, naspram samo 9.3 do 9.9 t ha^{-1} za sejanje u proleće. Prosečan prinos suve mase za period prva-treća godina je bio između 10.5 i 12.7 t ha^{-1}. Istaknuta su vremena setve 1. i 10. septembra, a smeše su se sastojale od: 70 kg ha^{-1} esparzete + 9 kg ha^{-1} bezosnog vlasena + 6 kg ha^{-1} hibridnog ljulja; 70 kg ha^{-1} esparzete + 9kg ha^{-1} bezosnog vlasena + 9 kg ha^{-1} engleskog ljulja, koji su dali prinos između 12.0 i 12.7 t ha^{-1}, sa vrlo značajnim povećanjem prinosa semena (8-18%) naspram prolečnih varijanti. Žetva je obavljena u fazi početka cvetanja esparzete. Vreme žetve je uticalo na nivo prinosa, pri čemu je postignuto 12.2 i 12.7 t ha^{-1} za period 7-14 dana žetve.

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PRODUCTIVITY TRAITS OF LOCAL ALFALFA CULTIVARS

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Abstract: An experimental study was conducted in 2004-2005 i.e. during the fourth and fifth years of alfalfa stand life to evaluate the productivity traits of alfalfa. The study involved seven local alfalfa cultivars: NS-Banat ZMS II, NS-Slavija, NS-Novosađanka H-11 and NS-Tisa developed at the Institute of Field and Vegetable Crops, Novi Sad, K–23 and K–28 bred at the Forage Crops Research Centre, Kruševac, and Zaječarska 83 released by the Institute of Agriculture, Zaječar. The average annual hay yield obtained in this study was 11.35 t ha^{-1}. Hay yield was highest in cvs. Zaječarska 83 and NS Slavija (12.85 t ha^{-1} and 12.10 t ha^{-1}, respectively) and lowest in K-28 and NS Tisa (11.35 t ha^{-1} and 11.05 t ha^{-1}, respectively). No significant differences in dry matter quality were observed among the cultivars tested. The cultivars NS Slavija and NS Novosađanka H-11 gave the highest crude protein content (19.86% and 19.53%) and the lowest crude fibre content (26.02% and 26.05%, respectively).

Key words: alfalfa, yield, hay, quality

Introduction

Alfalfa is one of the most important perennial forages grown both worldwide and in Serbia. In this country, it is cultivated on an area of 192,800 ha, giving average annual yields of about 5.0 t ha^{-1} dry matter *(Statistical Yearbook of Republic of Serbia, 2007).* Apart from the fact that alfalfa typically remains productive for five to seven years under average agro-environmental conditions and proper utilization practices, it is also valued as a high-quality feed source for all types and categories of farm animals; moreover, alfalfa biomass is processed to provide components required for the feed concentrates fed to monogastric animals *(Đukić et al.2008).* Notwithstanding the rather low average yields of dry matter of alfalfa produced in Serbia, the genetic potential of local cultivars indicates much higher productive potential of alfalfa. *Đukić et al. (2007)* reported high dry matter yield potential, ranging from 11-18.9 t ha^{-1}, exhibited under varied agro-environmental conditions by 24 alfalfa cultivars developed at research institutes in Novi Sad (13 cultivars), Kruševac (6 cultivars), Zaječar (4 cultivars) and Aleksinac (1 cultivar).

Dry matter quality of alfalfa is affected by growing conditions, genotype, and particularly developmental stages at the time of harvest. Crude proteins stand out among the chemical substances found in alfalfa in that they serve as major structural components of organic matter which, however, often become deficient in livestock diets. Another important indicator of feed quality is the quantitative relationship between protein and fibre. In other words, plant ageing induces a decrease in crude protein content, but an increase in crude fibre content, and slight variations in the proportion of NFE in all cuts of alfalfa *(Ocokoljić et al., 1977; Negovanović et al., 1992; Lukić, 2000)*.

The objective of this study was to evaluate the forage productive potential and quality of local alfalfa cultivars under the agro-environmental conditions in southern Serbia, in an attempt to significantly contribute to the cultivation and knowledge of this plant species.

Materials and Methods

An experiment was conducted at the "Ledena Stena" site in the suburbs of Niš during 2004-2005 in fourth- and fifth-year alfalfa stands i.e. during the third (A_3) and fourth (A_4) years of alfalfa stand utilization. The study involved seven local alfalfa cultivars: NS-Banat ZMS II, NS-Slavija, NS-Novosađanka H-11 and NS-Tisa developed at the Institute of Field and Vegetable Crops, Novi Sad, cv. K–23 and K–28 bred at the Institute for Forage Crops, Kruševac, and cv. Zaječarska 83 released by the Institute of Agriculture, Zaječar. The soil used in the trial was alluvial. The trial was set up as a randomized block design in four replications, with each elementary plot measuring 5 m^2 (5x1 m) and with maize being used as the preceding crop. The quality of dry matter was determined from the samples taken during the third year of alfalfa stand and first cutting of alfalfa.

The region of Niš was characterized by long dry summer months. The alfalfa stand was cut four times in 2004 i.e. during the third year of alfalfa stand utilization (A_3) which was marked by a dry period lasting from mid-May to the end of June, as well as by abundant precipitation in August (Table 1). The weather conditions in 2005, being the fourth year of alfalfa stand utilization (A_2), were more unfavourable for alfalfa development, resulting in substantially lower yields as compared to the previous year.

Table 1. Mean monthly temperatures (^{o}C) and total precipitation (mm) - Niš, 2004-2005

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII
Temperatures (^{o}C)													
2004	2.1	7.6	10.2	12.6	19.3	20.5	25.0	22.8	17.5	12.1	7.9	1.8	13.3
2005	0.8	4.4	9.9	12.0	20.1	24.2	26.3	25.2	18.7	12.6	5.6	2.8	13.5
Total precipitation -mm													
2004	26.3	19.4	52.7	76.4	46.3	64.3	69.4	114.8	64.9	35.9	26.7	62.8	659.9
2005	65.3	22.8	17.5	56.3	68.3	45.2	45.6	32.5	54.5	115.6	17.3	14.9	555.8

The obtained data were subjected to analysis of variance. An LSD- test was used to evaluate differences between means. The coefficient of variation (CV%) was calculated for the dry matter quality parameters of alfalfa.

Results and Discussion

The average hay yield of alfalfa during the period under study (2004-2005) was 11.35 t ha^{-1} (Table 2). The yield was highest in cvs. Zaječarska 83 and NS Slavija and lowest in cvs. NS Tisa and K-28. Averaged across years, a significantly higher hay yield was produced in 2004 (A_3), being 13.81 t ha^{-1}, whereas the average hay yield in 2005 (A_4) was 8.88 t ha^{-1}. The differences in hay yield between the two years were due to unfavourable agro-environmental conditions in 2005 including low rates and unfavourable distribution of precipitation. The yield decline in this year may have been associated with the alfalfa stand age as well.

Table 2. Alfalfa hay yield in 2004-2005 (t ha^{-1})

Cultivar	Year		Average
	2004	2005	
NS Banat ZMS II	13.95	9.15	11.55
NS Slavija	14.45	9.75	12.10
NS Novosađanka H-11	13.85	7.35	10.60
NS Tisa	12.48	7.62	10.05
K – 23	14.22	9.68	11.95
K – 28	12.80	7.90	10.35
Zaječarska- 83	14.95	10.75	12.85
Average	13.81	8.88	11.35
LSD 0.05	0.95	0.75	1.15
0.01	1.35	1.05	1.75

The highest alfalfa hay yield in 2004 was produced by cv. Zaječarska 83 (14.95 t ha^{-1}), being significantly above the average yield produced during the year. Conversely, compared to the average, statistically significantly lower yields were obtained with cv. K-28 and NS Tisa which gave the lowest hay yields (12.80 and

12.48 t ha^{-1}, respectively). The highest hay yield in 2005 as the fourth full production year (A_4) was also recorded for cv. Zaječarska 83 (10.75 t ha^{-1}), which was highly significantly above the average. The cultivars NS Slavija and K-23 also gave significantly higher yields (9.75 and 9.68 t ha^{-1}, respectively), as compared to the average. Highly significantly lower yields, as compared to the average, were produced by cv. NS Novosađanka and NS Tisa, whereas the yield of cv. K-28 (7.90 t ha^{-1}) was significantly below the average hay yield produced in 2005 (Table 2).

The results obtained suggest that the yields were satisfactory and more than 100% above the average yields produced in Serbia. The yields obtained were significantly affected by growing conditions. The hay yields of alfalfa cultivars during the first three years of stand life, as reported by *Beković et al. (2009)*, averaged 10.68 t ha^{-1}, the yield falling within the 10.23 t ha^{-1} (NS Tisa) to 11,13 t ha^{-1} range (NS Slavija). Similar results were reported by *Kallenbach et al. (2002)* who produced an average five-year yield of 9.14 t ha^{-1}, 9.58 t ha^{-1} and 10.14 t ha^{-1} with cv. Cody, Alfagraze and Pioneer 5373, respectively. In a study by *Stanisavljević et al. (2003)***,** the average two-year yield of alfalfa dry matter was 11.1 t ha^{-1}, with the yield ranging from 7.7 t ha^{-1} to 13.9 t ha^{-1}, depending on nitrogen fertilization. Higher yields were also reported by *Delgado et al. (2003),* the dry matter yield ranging from 12.93 t ha^{-1} to 13.84 t ha^{-1}. *Stevović et al. (2004)* produced substantially higher alfalfa dry matter yields, averaging 8.4 t ha^{-1} and 18.32 t ha^{-1} in the establishment year and in the second year of the stand life (A_1), respectively. By contrast, the yields of K-42, a new alfalfa cultivar, as reported by *Radović et al. (2007)*, ranged from 6.84 t ha^{-1} in the year of sowing to 16.24 t ha^{-1} in the first year of utilization.

As for the dry matter quality of the alfalfa cultivars tested in this study, the crude protein content as the most important quality parameter ranged from 18.99% (NS Banat ZMS II) to 19.86% (NS Slavija), giving an average of 19.29% (Table 3). *Ivanov (1980)* classified alfalfa cultivars according to crude protein content into three groups, being as follows: cultivars having a high CP content (20% and above), a medium content of CP 18-20% and those having a low CP content (below 18%). According to the classification, the tested cultivars fall into the medium CP content group. *Petkova et al. (2005)* reported that the four-year average CP yield of seven genotypes and five cultivars of alfalfa of diverse origin ranged from 18.85% (NS Banat ZMS II) to 21.79% (Višelisna – Ax 93/3).

Table 3. Chemical composition of alfalfa hay (%) (*Beković et al., 2009*)

Cultivar	Quality parameters				
	CP	CFib	CAsh	CFat	NFE
NS Banat ZMS II	18.99	27.50	8.62	2.31	42.58
NS Slavija	19.86	26.02	8.82	2.22	43.08
NSNovosađ. H-11	19.53	26.05	9.01	2.34	43.07
NS- Tisa	18.94	27.74	8.80	2.27	42.25
K – 23	19.45	26.81	8.71	2.18	42.85
K – 28	19.20	26.96	8.77	2.33	42.74
Zaječarska- 83	19.10	26.80	8.61	2.44	43.05
Average	19.29	26.84	8.74	2.30	42.80
CV %	4.40	8.64	2.29	1.13	4.08

CP- crude protein; CFib - Crude fibre; CAsh - Crude Ash; CFat- Crude fat; NFE – Nitrogen free extract

The average content of crude fibre was 26.84%, crude ash 8.74%, crude fat 2.30%, and nitrogen-free extracts 42.80% (Table 3). Similar results on alfalfa dry matter quality were reported by *Stevović et al. (2004)* who obtained the following average values for the tested quality parameters: crude protein 19.67%, crude ash 8.90%, crude fats 1.64%, crude fibre - much higher (31.25%), and NFE somewhat below the values in this study (38.14%). In a study by *Stanisavljević (2006*), four cultivars tested under the agro-environmental conditions in the Timočka Krajina region gave an average of CP 197.5 g kg^{-1}DM, CFib 264.1 g kg^{-1}DM, CFat 42.2 g kg^{-1}DM, CAsh 86.5 g kg^{-1}DM, and NFE 411.8 g kg^{-1}DM, which was comparable to the values obtained in this study.

Conclusion

The above results of the two-year study on the performance of local alfalfa cultivars suggest that high yields of alfalfa forage substantially exceeding the average yields produced in the country can be obtained under the agro-environmental conditions in southern Serbia. The average alfalfa hay yield produced was 13.81 t ha^{-1} in the first year of study i. e. in the third year of stand utilization (A_3) and 8.88 t ha^{-1} in the fourth year of stand utilization (A_4). The average hay yields were highest in cvs. Zaječarska 83 and NS Slavija (12.85 t ha^{-1} and 12.10 t ha^{-1}, respectively) and lowest in cvs. NS Tisa and K-28 (10.05 t ha^{-1} and 10.35 t ha^{-1}, respectively). The average content of crude proteins as the most important quality component was 19.10%, that of crude fibre 26.84%, crude ash 8.74%, crude fats 2.30% and nitrogen-free extracts 42.80%. The cultivars NS Slavija and NS Novosađanka H-11 gave the highest crude protein content (19.86% and 19.53%) and the lowest crude fibre content (26.02% and 26.05%, respectively).

Proizvodna svojstva domaćih sorti lucerke

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Rezime

U cilju utvrđivanja proizvodnih svojstava lucerke izvršena su eksperimentalna istraživanja u periodu od 2004.-2005. godine, odnosno u toku četvrte i pete godine života lucerke. Istraživanjima je obuhvaćeno sedam domaćih sorti lucerke i to: NS-Banat ZMS II, NS-Slavija, NS-Novosađanka H-11 i NS-Tisa stvorene u Institutu za ratarstvo i povrtarstvo u Novom Sadu, K–23 i K–28 stvorene u Centru za krmno bilje u Kruševcu i Zaječarska 83 stvorena u Zavodu za poljoprivredu u Zaječaru. Ostvaren je prosečan godišnji prinos sena od 11,35 t ha^{-1} sena. Po visini prinosa sena izdvajaju se sorte Zaječarska 83 i NS Slavija sa najvišim prinosima (12,85 t ha^{-1}, odnosno 12,10 t ha^{-1}), odnosno K-28 i NS Tisa sa najnižim (11,35 t ha^{-1}, odnosno 11,05 t ha^{-1}). U pogledu kvaliteta suve materije između ispitivanih sorti nije bilo značajnijih razlika. Sorte NS Slavija i NS Novosađanka H-11 imale su najviši sadržaj sirovih proteina (19,86% odnosno 19,53%) i najniži sadržaj sirove celuloze (26,02% odnosno 26,05%).

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DYNAMICS OF ALFALFA GROWTH AND DEVELOPMENT IN THE SEEDING YEAR

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Communication

Abstract: A study was carried out at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in 2009. The goal was to monitor the dynamics of growth and development of the alfalfa cultivar Banat VS in the seeding year by determining the dates and duration of each pheno-phase. The paper describes the stages of alfalfa growth and development from emergence to start of flowering. It was determined that the crop produces a robust root system and develops lateral shoots from axillary buds and a crown in the year of establishment. Weather conditions had a significant effect on the length of the growth period. High temperatures and rainfall deficits speeded up the rate of alfalfa development and caused the plants to make faster transitions from one pheno-phase to the next. This resulted in a shorter growth period up to start of flowering than usual.

Key words: alfalfa, emergence, flowering, pheno-phase

Introduction

Alfalfa is a perennial forage crop that repeats its vegetative growth cycle several times a year and for several years in succession *(Đukić and Erić, 1995)*. In the year of establishment, alfalfa develops a deep root system, lateral shoots, and a crown, which is why the period of vegetative growth is the longest in the first year (120-130 days). In that first year, after the sowing, alfalfa goes through several developmental stages: start of vegetative growth (germination, emergence), the rosette stage (7-8 true leaves), elongation of internodes, appearance of flower buds, and start of flowering, full flowering, and pod maturation.

Of the climatic factors, the progress of the above stages of development is affected the most by soil temperature and moisture and by the presence of light. The minimal soil temperature for alfalfa germination and emergence is 1ºC, with 20-25 ºC being the optimum *(Fick et al., 1988)*. At emergence, young alfalfa plants need larger amounts of water in the uppermost part of the soil because of their underdeveloped root system and poorer resistance to drought. Alfalfa germinates less rapidly in field conditions (10-15 days), as they require more energy for germination and emergence. At 20 ºC in a laboratory, the species germinates rapidly, in 3-7 days *(Lukić, 2000)*. Alfalfa seeds lack the nutritive tissue of the endosperm, because the germ absorbs it completely during its development, so the seeds obtain their nutrition during germination from substances stored in the cotyledons *(Spedding and Diekmahns, 1972)*.

The objective of this study was to investigate the stages of alfalfa growth and development in the seeding year (from emergence to start of flowering) and determine the dates and duration of each pheno-phase. In-depth knowledge of how alfalfa develops can make it possible to implement cultural practices more effectively and thus obtain higher yields and greater persistence in this crop.

Materials and Methods

The trial was carried out at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad in 2009. Planting was done on May 21, 2009 using a seeding rate of 16 kg/ha. The alfalfa variety Banat VS was used. Size of plots was 6 m^2, in three replications.

From emergence to start of flowering (June-August), the pheno-phases were monitored regularly (emergence, early seedling growth, vegetative plant development, all the way through to the stage regarded as the start of flowering). In this study 12 pheno-phases were monitored. The phase of emergence was determined when 50% of plants emerged. The phase from the first to the ninth trifoliate leaf and the phase of beginning of flowering began when 10% of plants were at the given stage. Full flowering was rated when 50% of plants were in full bloom. The phase of start of pod maturing began when 50% of pods had green colour. In the course of the trial, meteorological factors were also monitored, and the usual cultural practices were implemented to manage the crop and protect it from weeds and pests.

Weather conditions: According to data provided by the Rimski Šančevi Weather Station, the mean monthly air temperature (MMT) during the 2009 growing season (April-October) was 19.5ºC, or 1.7 ºC more than the long-term average (1964-2004, 17.8 ºC).

As for rainfall, the growing season in 2009 had a total of 271.5 l/m^2 of rain, which is 93.8 l/m^2 less than the long-term average (1964-2004). The distribution of rainfall from April through to the end of September was rather uneven. The most rainfall was recorded in June (127.2 l/m^2) and the least in April (3.6 l/m^2). Table 1 shows mean daily temperatures and total rainfall by pheno-phase in our study.

Table 1. Mean daily temperature (ºC), total sum of rainfall (mm) and cumulative rainfall (mm) by pheno-phase during the 2009 growing season

Indicator	Pheno-phase									
	Planting	Germination	Emergence	Unifoliate leaf	1 trifoliate leaf	2 – 4 trifoliate leaves	5 trifoliate leaves	6 - 9 trifoliate leaves	Start of flowering	Full flowering
t ºC	22.0	18.9	19.1	20.6	21.4	20.6	20.3	22.1	24.1	22.2
mm	0.3	30.0	15.4	26.0	0	75.1	52.4	13.2	7.9	14.7
CR	0.3	30.3	45.7	71.7	71.7	146.8	199.2	212.4	220.3	235.0

In the present study, just before alfalfa plants were sown and for several days afterwards, there was enough moisture in the soil and the temperature was favourable, which provided highly favourable conditions for the start of germination and emergence. The daily temperature at germination was 18.9 C°, and there was 30 l of rain per m^2 (Table 1).

Results and Discussion

By monitoring the dynamics of alfalfa growth and development from germination to start of flowering in the seeding year, the more important pheno-phases were observed in the plants. Table 2 shows an overview of the pheno-phases observed and their duration and characteristics. The seedling root is the first structure to emerge from the seed during germination. Once the seedling root is anchored firmly in the soil, the seedling axis below the cotyledons elongates in an arch pulling the cotyledons upward to the soil surface. Seed germination and seedling emergence occur in about three to seven days *(Meyer, 1999)*.

Emergence started 10 days after planting, as the cotyledons of 50% of the plants emerged from the soil surface. This pheno-phase lasted 10 days, during which the mean daily air temperature was 19.1°C, and there was 15.4 l of rain per m^2. In the early stages of development, young alfalfa plants develop their root systems faster than their above-ground parts, as the root penetrates into the deeper soil layers in search of water and nutrients. The first, unifoliate leaf developed from the axil of the cotyledon at the first node. In a certain smaller number of plants, the unifoliate leaf appeared nine days after emergence, and by the 12th day these simple leaves had been formed in all of the plants. According to *Meyer (1999)*, under good growing conditions, the seedling is developed fully 10 to 15 days after planting. This pheno-phase lasted three days. The weather conditions were favourable, as the average air temperature was 20.6°C and the amount of rainfall 26 mm.

The first trifoliate leaf appeared on the 12th day after emergence in 10% of the plants and had been fully formed in all the plants by the 14th. At this stage, nodules were observed on the roots of some plants. The nodules contain symbiotic bacteria of the species *Rhizobium meliloti*, which have the ability to fix elemental nitrogen in large quantities. During this phase, there was no rainfall, but there were sufficient soil moisture reserves and the temperature was favourable for the formation of the first true leaf (21.4°C). The development of the first trifoliate leaf was followed by the successive formation of subsequent true leaves on the opposite sides of the primary stem, accompanied by the elongation of internodes. The second trifoliate leaf appeared on the 15th day after emergence, the third on the 18th, and the fourth on the 21st. Leaf formation continued and 42 days after the cotyledons emerged from the ground the plants had eight true leaves (Table 2).

Table 2. Pheno-phases identified in the alfalfa variety Banat VS in the seeding year and their duration and characteristics

Pheno-phase	Date	Days from emergence	Phenoph duration (days)	Phenological observations	Cultural practices
Sowing	21.05.				Planting by hand seeder.
Emergence-appearance of cotyledons	01.06.	10	10	50% of plants emerged	
One unofoliate leaf	10.06.	9	3	Emergence of unifoliate leaf in 10% of plants	
One trifoliate leaf	13.06.	12	3	Start of formation of 1st trifoliate leaf in 10% of plants.	Weeding
2-4 trifoliate leaves	16.06.	15	13	Appearance of 2nd. 3rd and 4th trifoliate leaves in 10% of plants	Treatment with Pivot, 0,5 l/ha.Weeding
5 trifoliate leaves, formation of axillary buds	29.06.	29	5	Start of formation of 5th trifoliate leaf in 10% of plants.	Weeding
6 trifoliate leaves	03.07.	35	3	Appearance of 6th trifoliate leaf in 10% of plants	Weeding
7 trifoliate leaves, development of lateral shoot	06.07.	38	4	Appearance of 7th trifoliate leaf in 10% of plants.	Weeding
8 trifoliate leaves	10.07.	42	4	Appearance of 8th trifoliate leaf in 10% of plants	Treatment with Pivot, 0,5 l/ha, and insecticide.
9 trifoliate leaves formation of buds (crown)	14.07.	46	11	Appearance of 9th trifoliate leaf in 10% of plants.	Insect and pest control
Start of flowering	25.07.	57	24	Start of flowering in 10% of plants	Early cutting
Full flowering	18.08.	81	23		Medium early cutting
Start of mature pod stage	10.09.	103	-	50% of pods are green, 30% brown, and 10% yellow in color.	Late cutting

At the stage of five trifoliate leaves (29^{th} day after emergence), the first buds appeared in the cotyledonary axils and axils of the first two true leaves. Later on, at the stages of seven and eight true leaves (38-42 days after emergence), these buds would start producing lateral shoots (secondary stems, branches). The growth and development of such shoots gives young alfalfa plants their branched appearance. How many of these branches develop depends on the conditions under which the plant develops, most notably the amount of daylight. During the leaf formation stage, weather conditions were favourable until the fifth trifoliate leaf app-eared, after which a period of drought set in. Between the formation of the sixth true leaf and the start of flowering, there was only 13.2 l of rain per m^2, which, along with fairly high daily temperatures, caused the plants to develop more rapidly and make a faster transition from the vegetative to the reproductive stage.

The lower internodes or nodes of the primary or secondary stems may produce adventitious or crown buds. Crown buds produce the vegetative re-growth following harvest the seedling year, or these buds may give rise to branch rhizomes in the fall *(Meyer, 1999)*.The first buds on the crown were observed on the 46th day after emergence. Bud formation lasted 11 days in total. The young alfalfa plant at about 8 to 10 weeks of age, undergoes a growth phase known as contractile growth. This process in plant involves a change in the shape of cells in the hypocotyls or seedling axis below the cotyledons and upper portion of the primary root from long and narrow to short and wide as a result of carbohydrate or food storage *(Meyer, 1999)*. Generative buds appeared at the tip of the central stem at the same. This stage is termed budding.

The crown consists of the perennial portions of the stem *(Grove and Carlson, 1972)*. The crown buds from which shoots spring up after cutting, give the plant a shrubby appearance. Mokeyeva designates the root collar as a part of the crown *(Grove and Carlson, 1972)*. The number of shoots depends on plant age. Young alfalfa plants produce two to three stems in the first year of life and may develop over 30 shoots in the subsequent years.

Conclusion

We studied the dynamics of alfalfa growth and development in the seeding year and determined that the plant produces a robust root system and develops lateral shoots from axillary buds and a crown during the period between sowing and start of flowering. It was also determined that alfalfa goes through the following pheno-phases in the year establishment: germination and emergence; first leaf (unifoliate); first true leaf (trifoliate); formation of 7-8 trifoliate leaves simultaneously with internode elongation and formation of axillary buds; appearance of lateral shoots; and budding, when buds appear on the crown and generative buds develop at the tip of the central stem.

Weather conditions had a significant effect on the rate of development. High temperatures and inadequate soil moisture caused the plants to make faster transitions from one pheno-phase to the next, thus shortening the growth period compared to what its duration is when conditions are optimal.

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Dinamika rasta i razvića lucerke u godini zasnivanja

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Rezime

Istraživanje je obavljeno na oglednom polju Instituta za ratarstvo i povrtarstvo u Novom Sadu, na lokalitetu Rimski Šančevi, tokom 2009. godine. Tokom vegetacije praćena je dinamika rasta i razvića lucerke (sorta Banat VS) u godini zasnivanja određivanjem datuma početka i dužine trajanja pojedinih fenoloških faza. U radu su opisane faze rasta i razvića lucerke u prvoj godini života, od faze nicanja do faze početka cvetanja biljaka. Ustanovljeno je da u godini zasnivanja lucerka obrazuje jak korenov sistem, bočne izdanke iz pazušnih pupoljaka i krunicu. Klimatski uslovi su značajno uticali na dužinu trajanja vegetacionog perioda. Visoke temperature i nedostatak padavina ubrzali su razviće biljaka lucerke i prelazak iz jedne u drugu fenološku fazu pa je vegetacioni period do faze cvetanja trajao kraće nego uobičajeno.

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GENOTYPIC RESPONSE OF TWO MAIZE HYBRIDS TO APPLICATION OF DIFFERENT NITROGEN LEVEL

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Abstract: The aim of this investigation was to estimate the effects of different amounts of nitrogen on the plant height, stalk mass and grain yield in two maize hybrids (ZP 544 and ZP 684). Studied hybrids belong to different maturity groups (ZP 544 – FAO 500 and ZP 684 – FAO 600). Four treatments of fertilization were tested: 0 kg N ha^{-1}, 60 kg N ha^{-1}, 120 kg N ha^{-1} and 180 kg N ha^{-1}. Field trials were carried out in dry land farming, at location Putinci, during the years 2008 and 2009. In both research years, hybrid ZP 684 had higher plant height, stalk mass and grain yield then hybrid ZP 544. The results of the study showed that application of 180 kg N ha^{-1} increased the plant height, stalk mass and grain yield of maize more than application of other treatments (0, 60 and 120 kg N ha^{-1}). Application of the lowest nitrogen level (60 kg N ha^{-1}) in trails brings to significant increase of grain yield compared to the control. This result could be useful for farmers in areas where supply of nitrogen fertilizer is low and in cases when farmers cannot afford the cost of high fertilizer input.

Key words: grain yield, hybrid, maize, nitrogen level, plant height, stalk mass

Introduction

Maize (*Zea mays* L.) is an important cereal crop which ranks the third after wheat and rice in the world. Besides its industrial uses and food value for human being, it is equally useful as a feed for animal domestics. Nitrogen is a vital plant nutrient and a major yield determining factor for maize production *(Shanti et. al,. 1997)*. Nitrogen is the motor of plant growth and it makes up 1 to 4% of dry matter of the plant *(Anonymous, 2000)*. Nitrogen is a component of protein and nucleic acids *(Haque et. al., 2001)*. Maize grain yield increased with increase in N applied up to 100 kg N ha^{-1} *(Mkhabela et al., 2001)*. *Wajid et al. (2007)* reported that nitrogen application increased plant height and grain yield of maize. According to their results, the higher grain yield (8.09 t ha^{-1}) was produced in treatment 250 kg N ha^{-1} then in 200 kg N ha^{-1} (7.57 t ha^{-1}) and 150 kg N ha$^{- 1}$ (6.59 t ha^{-1}) treatments. *Gungula et al. (2005)* obtained the highest grain yield of maize at the level of 120 kg N ha^{-1}. *Olness et al. (2008)* found that increasing of N level (from 10 to 190 kg ha$^{−1}$) is more important for higher maize grain yield then different tillage practices. *Sharifi and Taghizadeh (2009)* concluded that nitrogen levels (0, 80, 160 and 240 kg ha$^{−1}$) had significant effects on yield and yield components in three maize hybrids (SC-301, DC-370 and SC-404). *Tsai et al. (2006)* studied N use efficiency and grain protein quality in trails with eight maize hybrids grown under several N levels. Their results indicated that maize hybrids can be grouped into three categories based on grain yield: (1) low N-responsive types which reach their maximum yield with 134 kg ha^{-1} of applied N; (2) intermediate types that respond to moderate N levels (134 to 201 kg N ha^{-1}); and (3) high N-responsive types that respond to higher levels of N (201 kg N ha^{-1}). The aim of this investigation was to estimate the effects of different amounts of nitrogen on the plant height, stalk mass and grain yield in two hybrids maize (ZP 544 and ZP 684).

Materials and Methods

Effects of mineral nitrogen nutrition on plant height, stalk mass and grain yield of maize were analyzed in two-year researches, conducted on the experimental fields in the village Putinci (region of Srem), during years 2008 and 2009. The tests were carried out on calcareous chernozem soil type. The main characteristics of the soil (depth: 0-50 cm) were: pH in KCl – 6.8 (neutral reaction); pH u H_2O – 7.6 (weakly alkaline reaction); $CaCO_3$ – 7.8% (carbonate); humus – 2.54 (weakly humus), total N – 0.16% (good equipped). The soil contained 16.2 and 24.6 mg/100g soil phosphorus and potassium, respectively.

Two maize hybrids, ZP 544 (FAO 500) and ZP 684 (FAO 600), were used as material. Plots were organized as completely randomized block system design in four replications. Four treatments of fertilization were tested: 0 kg N ha^{-1} (control), 60 kg N ha^{-1}, 120 kg N ha^{-1} and 180 kg N ha^{-1}. Mineral nutrition *KAN* (27% N) was applied. One half of the nitrogen was applied at the time of sowing while the remaining half at the stage of 3 leaves. Plant density was 60000 plants per hectare (70 x 24 cm). Preceding crop was winter wheat. A standard cultivation practice was applied.

Maize harvesting was performed manually. Before maize harvest, plant height (cm) was measured, and than ten plants from each plot were taken for measuring of stalk mass (g) and grain yield (t ha^{-1}). Grain yield was calculated on a 14% moisture basis. Data were processed by ANOVA. Test of difference significance between treatments were estimated by LSD.

Results and Discussion

Plant height, in average for two years, two hybrids and four nitrogen levels, was 274.6 cm (Table 1). In 2008, average plant height was lower for 11.9 cm (4.4%) then in 2009 (280.5 cm).

All nitrogen fertilization levels significantly increased the plant height, within interval from 4.8% (60 kg N ha^{-1}) to 9.7% (180 kg N ha^{-1}). In both research years, maximal plant height (281.1 cm and 290.2 cm) was recorded for level of 180 kg N ha^{-1} in average for both hybrids. Minimal plant height was recorded in control (253.3 cm in 2008 and 267.7 cm in 2009).

Table 1. Effect of nitrogen levels (kg ha^{-1}) on the plant height, stalk mass and grain yield in studied maize hybrids

Year	N levels (B)	Traits								
		Plant height (cm)			Stalk mass (g)			Grain yield (t ha^{-1})		
		Hybrids (A)								
		ZP 544	ZP 684	M	ZP 544	ZP 684	M	ZP 544	ZP 684	M
2008	0	248.0	258.6	253.3	499.0	517.0	508.0	9.24	9.91	9.58
	60	264.7	267.5	266.1	529.0	527.0	528.0	11.18	11.43	11.31
	120	268.6	278.8	273.7	531.0	554.0	542.5	11.59	12.51	12.05
	180	275.1	287.1	281.1	550.0	574.2	562.1	11.91	12.89	12.40
	M	264.1	273.0	268.6	527.2	543.0	535.1	10.98	11.69	11.34
2009	0	263.6	271.7	267.7	535.0	546.8	540.9	10.57	11.32	10.94
	60	273.2	286.4	279.8	549.0	564.0	556.5	12.61	13.08	12.84
	120	279.1	289.5	284.3	559.0	578.0	568.5	13.20	13.83	13.52
	180	284.5	296.0	290.2	576.0	586.0	581.0	13.45	14.16	13.80
	M	275.1	285.9	280.5	554.8	568.7	561.7	12.46	13.10	12.78
M	0	255.8	265.2	260.5	517.0	531.9	524.4	9.90	10.62	10.26
	60	269.0	277.0	273.0	539.0	545.5	542.2	11.90	12.26	12.08
	120	273.9	284.2	279.0	545.0	566.0	555.5	12.40	13.17	12.79
	180	279.8	291.6	285.7	563.0	580.1	571.6	12.68	13.53	13.10
	M	269.6	279.5	274.6	541.0	555.9	548.4	11.72	12.40	12.06

Year	LSD	Plant height (cm)			Stalk mass (g)			Grain yield (t ha^{-1})		
		A	B	A * B	A	B	A * B	A	B	A * B
08	5%	6.44	9.10	12.77	11.54	16.32	22.90	0.16	0.22	0.31
	1%	8.76	12.39	17.31	15.71	22.22	31.04	0.21	0.30	0.41
09	5%	2.44	3.46	4.85	4.30	6.09	8.54	0.15	0.21	0.30
	1%	3.93	4.71	6.58	5.86	8.29	11.58	0.20	0.29	0.40

In average for both studied years, hybrid ZP 684 had higher plant height (279.5 cm) then hybrid ZP 544 (269.6 cm). Hybrid ZP 684 had higher plants for 8.9 cm (3.4%) then hybrid ZP 544 (264.1 cm) in 2008, i.e. for 10.8 cm (4.0%) then hybrid ZP 544 (275.1 cm) in 2009. These values differed statistically significant.

In both researches year interaction between genotype and nitrogen level was not significant. These results are in agreements with research of *Wajid et al. (2007)*.

Average stalk mass for years, hybrids and nitrogen levels, was 548.4 g. In 2008, average stalk mass lower for 26.6 g (5.0 %) then in 2009.

All nitrogen fertilization levels significantly increased the stalk mass, within interval from 17.8 g (60 kg N ha^{-1}) to 47.2 g (180 kg N ha^{-1}). In both research years, maximal stalk mass (562.1 g and 581.0 g) was recorded for level of 180 kg N ha^{-1} in average for both hybrids. Minimal stalk mass was recorded in control (508.0 g in 2008 and 540.9 g in 2009).

In average for both studied years, hybrid ZP 684 produced higher stalk mass (555.9 g) then hybrid ZP 544 (541.0 g). In 2008, hybrid ZP 684 had higher stalk mass for 15.8 g (3.0 %) then hybrid ZP 544 (527.2 g), while in 2009 it had 13.9 g (2.5 %) higher stalk mass then hybrid ZP 544 (554.8 g). These values differed statistically significant.

In both research years, interaction between genotype and nitrogen levels was not significant.

Average grain yield, for two years, two hybrids and four nitrogen levels was 12.06 tha^{-1}. In 2008, average grain yield was lower 1.44 tha^{-1} (12.7%) then in 2009.

Average grain yield for all years and hybrids was the highest (13.10 t ha^{-1}) when nitrogen was applied at the level of 180 kg ha^{-1}. Application of 120 kg N ha^{-1} (12.79 tha^{-1}) gave higher grain yield then application of 60 kg N ha^{-1} (12.08 tha^{-1}). Statistically minimal grain yield was recorded in the control (10.26 tha^{-1}). Increase in grain yield with an increase in nitrogen levels was also observed by *Younas et al., (2002); Glamočlija et al., (2007)*.

Results have shown that maize hybrids ZP 684 (12.40 tha^{-1}) produce higher yield than hybrid ZP 544 (11.72 tha^{-1}). Hybrid maturity group 600 had higher grain yield for 0.71 tha^{-1} (6.5 %) then hybrid maturity group 500 in 2008 (10.98 tha^{-1}), i.e. for 0.64 tha^{-1} (5.1 %) in 2009. These differences were statistically significant.

In 2008, interaction between genotype and nitrogen level was significant, while this interaction was not significant in 2009.

Results suggested that the hybrid ZP 684 and nitrogen level 180 $kgha^{-1}$ could be used successfully for improving maize yield in the region of Srem.

Conclusion

The plant height, stalk mass and grain yield of maize was significantly affected by genotype and nitrogen level. Hybrid ZP 684 (maturity group 600) had higher plant height, stalk mass and grain yield than hybrid ZP 544 (maturity group 500). All levels of nitrogen fertilizer significantly increased the plant height, stalk mass and grain yield of the hybrids comparing to the control. The highest values of these parameters were recorded in treatment 180 kg N ha^{-1}. High yield in the region of Srem could be obtained by using hybrid ZP 684 and nitrogen level 180 $kgha^{-1}$.

Genotipski odgovor dva hibrida kukuruza na primenu različitih količina azota

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Rezime

Cilj istraživanja bio je da se odredi uticaj različite količine azota na visinu biljaka, masu kukuruzovine i prinos zrna dva hibrida kukuruza (ZP 544 i ZP 684). Ispitivani hibridi pripadaju različitim grupama zrenja (ZP 544 – FAO 500 i ZP 684 – FAO 600). Upoređivane su četiri varijante prihrane biljaka N: 0 kg N ha^{-1}, 60 kg N ha^{-1}, 120 kg N ha^{-1} i 180 kg N ha^{-1}. Ogledi su izvedeni u suvom ratarenju, na lokaciji Putinci, tokom 2008. i 2009. godine. U obe godine istraživanja, hibrid ZP 684 imao je veću visinu biljke, masu kukuruzovine i prinos zrna u odnosu na hibrid ZP 544. Rezultati istraživanja su pokazali da je primena 180 kg N ha^{-1} povećavala visinu biljaka, masu kukuruzovine i prinos zrna više nego primena drugih tretmana (0, 60 i 120 kg N ha^{-1}). Primena i najmanje količine azota u ogledu (60 kg N ha^{-1}) dovela je do značajnog povećanja prinosa zrna u odnosu na kontrolu. Taj rezultat u velikoj meri može koristiti poljoprivrednicima u oblastima gde je loše snabdevanje mineralnim hranivima i u slučajevima kada ga ne mogu priuštiti zbog visoke cene.

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THE YIELDING OF MAIZE SOWN ON DIFFERENT DATES

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Abstract: Field experiment with maize was carried out in 2004–2006 at the Experimental Station in Zawady, belonging to the University of Podlasie. The following factors were taken into account: I – three terms of maize sowing: early (18^{th} April), recommended (28^{th} April), late (8^{th} May); II – maize cultivars (Polish and French): two early factors FAO 220 and two middle early factors FAO 240. During the harvest the yield of fresh mass of all plants and the yield of fresh mass of cobs were determined, and after the threshing and drying for 15% humidity the grain yield was determined. The contents of total protein and crude fat were determined in grain. The course of weather conditions in years of research and the factors of experience had a significant impact on the analyzed yields and the content of true protein in grain. Season in 2004 was the most favourable for the growth and development of maize plants. Maize which was sown in early term gave the highest yield of fresh mass of plants and the yield of fresh mass of cobs, and the maize which was sown in optimal term – the highest yield and it collected the highest amount of protein and fat in grain. The size of analyzed features was significantly diversified depending on weather conditions, but the examined factors did not always have a significant impact on the researched features.

Key words: maize, yield of grain, yield of total protein, cobs, sowing of terms, cultivars

Introduction

The dynamic development of hybrid maize breeding, expressing by obtaining early and yielding cultivars caused, that in Poland there is higher possibility of growing this species on grain and reduces the negative correlation between earliness and yielding *(Pabin et al., 2005)*. According to *Adamczyk (2001)* about the amount and quality of the harvested yield of maize in 40% decides the agronomic factor in 30% climatic conditions and also in 30% decides the proper selection of cultivars. It means that prober selection of cultivars is nearly one-third success in the cultivation. Lots of authors claim that for maize, as for every other species, the selection of proper cultivar is the factor which decides about the success of cultivation *(Michalski, 2001; Wróbel et al., 2006).* The pace of maize development depends on the course of thermal conditions in a given year, the earliness of cultivar, but largely from the appropriate date of sowing *(Borowiecki, 1992).* Also other authors *(Bruździak, 1988; Gesch and Arche, 2005)* agree that the factor which has significant influence on the maize yield is its term of sowing. *Otegui et al. (1995)* and *Sulewska (2004)* think that early sowing influences good yielding. Therefore, the aim of researches was the assessment of the impact of three terms of sowing on yielding and the content of selected components in the grain of four maize cultivars cultivated in central-eastern region of Poland.

Materials and Methods

Field experiments were carried out in 2004–2006 at the Experimental Station in Zawady, belonging to the University of Podlasie. Experimental Station is located on the φ - 52º 06 north latitude, λ - 22º 56 east longitude. The experiment was intended in method of random blocks in three variants, on soil belonging to the quality class IVa on a very good cereal complex with a slightly neutral pH.

The soil characterized with low to average abundance of phosphorus, average abundance of potassium. Two factors were taken into account: I factor – three dates of maize sowing varied every 10 days: I early term of sowing - 18th April, II recommended term of sowing - 28th April, III late term of sowing - 8th May); II factor– maize cultivars: Prosna FAO 220 (polish), Wiarus FAO 220 (Polish), Veritis FAO 240 (French), Bahia FAO 240-250 (French). In every year of the study the fore-crop to maize was winter cereal. Agro-technical treatment was conducted in accordance with accepted principles. Number of sowing seeds corresponded to the manning of 10-12 plants /m². The maize harvest took place in full maturity of grain. During the harvest from every combination the field of fresh mass of All plants and the field of fresh mass of cobs without cover leaves were determined counted on the surface of 1 ha. After the threshing the grain was drying for 15% humidity and then the grain yield was determined. In the laboratory the content of total nitrogen was determined by Kjeldahl method, and the content of total protein as the product of the nitrogen content and coefficient equal 6,25. Crude fat was determined by Soxlet method. The results were analyzed statistically, performing for each of the characteristics the analysis of variance test.

Results and Discussion

Weather conditions in the years of researches were varied. The growing season of 2004, in comparison to years 2005 and 2006 was significantly more favourable for the growth and development of maize plants. The lack of drought is Confirmed by hydrotermic factor K = 1.2. In the growing season of 2005 the average air temperature was similar to the long-term average, but with large water deficits. It was drought year, hydrotermic factor was K = 0.9. Distribution of rainfalls was very uneven. Season of 2006 was very warm, but uneven distribution of rainfalls in season has caused that the weather conditions were very unfavourable for the cultivation of grain maize.

About the yield of fresh weight of plants decided the weather conditions in years of researches and genetic determinants of cultivars (Table 1). The highest yield of fresh mass of plants was achieved in 2004 (50.33 t ha^{-1}). Received yield was higher by more than 57% of the crop harvested in 2005 and almost 53% higher compared to the yield in 2006. Such a large variation in the yield of fresh mass of plants shows how important is for obtaining a high yield an adequate system of weather conditions during the growing season of maize. The influence of weather factor is confirmed by many authors *(Jha et al., 1998).* According to *Sulewska (2001)* higher yields of fresh mass are achieved by planting late cultivars, which was not confirmed in own studies. Significantly higher yield of fresh mass was produced by corn of Veritis cultivars in relation to Bahia cultivar. The yield between Veritis cultivar and Prosna and Wiarus cultivars did not diversify significantly. Sowing dates had no significant effect on the yield of fresh weight of plants, which is not confirmed by the results obtained by *Bruździak (1988).* Yielding of each cultivars was modified by weather conditions during the growing season. In 2004 the highest significant yield created Wiarus cultivar, but in years 2005 and 2006 there were no significant differences in the yield of tested cultivars.

Table 1. Yield of fresh matter of plants and yield of fresh matter of cobs in t ha^{-1}

Yield of fresh matter of plants in t ha^{-1}					
Cultivars/years	2004	2005	2006	mean	$LSD_{0.05}$ for:
Prosna	45.6	21.3	23.9	30.3	years =5.9;
Wiarus	60.8	18.7	22.9	34.1	cultivars = 5.4;
Veritis	54.2	22.8	26.0	34.3	years × cultivars = 8.5
Bahia	40.8	23.6	22.3	28.9	
mean	50.3	21.6	23.8	31.9	
Yield of fresh matter of cobs in t ha^{-1}					
Sowing terms/years	2004	2005	2006	mean	$LSD_{0.05}$ for: years = 2.77;
I - 18 april	28.12	13.79	11.67	17.86	sowing terms = 2.77;
II – 28 april	23.95	15.45	9.50	16.30	years × sowing terms = 4.80
III – 08 may	19.17	8.46	13.83	13.82	
mean	23.75	12.56	11.66	16.00	

The yield of fresh mass of the cobs depended significantly on the course of weather conditions in the years of researches and sowing dates (Table 1). The highest yield of fresh mass of cobs was achieved in 2004 (23.75 t ha^{-1}). Significantly lower yields were in years 2005 and 2006. The highest yield was collected from objects on which the sowing was made in 18^{th} April (17.86 t ha^{-1}). These results are consistent with the results which were received by *Jeśmanowicz and Ruszkowski (1981).*

The results of analysis of variance showed that only the weather conditions during the years of researches significantly affected on the grain yield of maize (Table 2). The influence of weather conditions was confirmed by *Szmigiel and Oleksy (2006)* studies. The highest significant grain yield was achieved in favourable year 2004 (5.40 t ha^{-1}). In very warm year 2006 a strong drought appeared during the flowering, which resulted in obtaining very low yields. The results which were obtained are confirmed by the view of many authors *(Lipski, 2003; Michalski et al., 1996; Żarski, 2004).* In own studies sowing dates did not significantly affect grain yields which were obtained. Different results were obtained by *Berzsenyi et al. (2006).* The Grain yield did not significantly depend on the cultivar, in contrast to the results obtained by *Szmigiel and Oleksy (2006)*. Usually early cultivars give lower yield in comparison with later cultivars *(Michalski et al., 1996).*

Table 2. Yield of grain in t ha^{-1}

Cultivars/years	2004	2005	2006	mean
Prosna	4.42	5.72	1.50	3.88
Wiarus	4.31	5.08	2.61	4.00
Veritis	3.58	4.95	4.83	4.45
Bahia	3.78	5.84	2.36	3.99
mean	4.02	5.40	2.82	4.08
$LSD_{0.05}$ for: years = 1.47; interaction – years × cultivars = 1.56				
I - 18 april	4.49	5.38	3.22	4.36
II – 28 april	3.99	7.68	1.98	4.55
III – 08 may	3.58	3.13	3.28	3.33
mean	4.02	5.40	2.82	4.08
$LSD_{0.05}$ for: years = 1.47; interaction – years × sowing terms = 2.54				

In own researches about the total protein content in grain maize decided the weather conditions during the years of research (Table 3). The highest average content of total protein was obtained in 2005 (14.81% DM), and lowest in 2004 (10.36% DM). In the literature, the protein content in grain also varies widely *Dubas (2003).* In *Szmigiel and Kiełbasa (2004)* studies the protein content in grain was the highest in a year characterized with high air temperatures during the growing season and moderate rainfalls. Own obtained results did not confirm that dependence, as well as results achieved by *Szmigiel and Oleksy (2006).* The results of researches carried out by *Korniewicz et al. (2000)* showed that about total protein content decided the cultivar, which was not confirmed by own studies. The protein content, unlike the *Bruździak (1988) and Sulewski (2004)* studies did not depend also on the time of sowing.

Table3. The content of total protein and raw fat in grain in %

years	2004	2005	2006	mean
Total protein in %				
	10.36	14.81	11.58	12.25
$LSD_{0.05}$ for: years = 0.79				
Raw fat in %				
Prosna	4.38	3.55	3.70	3.88
Wiarus	5.25	3.64	2.68	3.86
Veritis	5.78	3.61	2.76	4.05
Bahia	5.90	3.54	4.12	4.52
mean	5.33	3.58	3.32	4.08
$LSD_{0.05}$ for: years = 0.20; cultivars = 0.18; interaction – years × cultivars = 0.29				
I - 18 april	5.14	3.57	3.94	4.22
II – 28 april	5.37	3.78	2.80	3.98
III – 08 may	5.46	3.39	3.21	4.02
mean	5.33	3.58	3.32	4.07
$LSD_{0.05}$ for: years = 0.20; sowing terms = 0.20; interaction – years × sowing terms =0.34				

Statistical analysis of results showed a significant influence of weather conditions in the years of research and experience factors on the content of crude fat in grain maize (Table 3). The largest crude fat had grain maize harvested in 2004 (5.33%) and the lowest in 2006 (3.32%). Similar values of this component showed *Górny (2005) and Dubas (2003)* in their own studies. The highest amount of this component accumulated the grain, when the maize was sown in the first term (4.22%). The highest amount of crude fat was in grain of Bahia cultivar (4.52%), and significantly less in other cultivars. Significant genetic variability of this characteristic also highlighted *Górny (2005)* and *Sulewska (2004). Korniewicz et al. (2000)* did not show the correlation of fat content from the class of cultivar maturity.

Conclusion

On the growth and yielding of maize the decisive influence had weather conditions in the years of research. The course of weather in the years of researches significantly diversified the yield of fresh mass of plants, the yield of fresh mass of cobs and grain yield. While the factors of experience: sowing dates and cultivars did not always have an influence on its size. However, proven interaction between years of researches and sowing dates and years of researches with cultivars indicate the importance of sowing dates and cultivar selection in maize cultivation. The content of total protein and crude fat in maize grain depended on the weather conditions in the years of researches. The significant influence on fat content also had the sowing date and cultivars. In agricultural practice, about the choice of the date of sowing grain maize mainly decided the weather conditions during the spring, but the aim should be to the earlier sowing of maize.

Prinos kukuruza posejanog u različito vreme

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Rezime

Poljski eksperimenti na kukuruzu su izvedeni 2004.-2006. godine u Eksperimentalnoj stanici u Zawady. Poljska. Ispitivani su: I-tri termina gajenja kukuruza: rani (18. april), preporučeni (28. april) i kasni (8. maj); II-sorte kukuruza (poljske i francuske): dva rana faktora FAO 220 i dva srednje rana faktora FAO 240. Za vreme žetve su određeni prinos sveže mase svih biljaka i prinos sveže mase klipa, a nakon vršidbe i sušenja do 15% vlažnosti, određen je i prinos semena. Vremenski uslovi u godinama istraživanja i faktor iskustva imali su značajan uticaj na analizirani prinos i na sadržaj proteina u semenu. Vremenski uslovi u 2004. godini su bili najpovoljniji za rast i razvoj kukuruza. Kukuruz posejan u ranom terminu je dao najviši prinos sveže mase biljaka i sveže mase klipova, a kukuruz posejan u optimalnom periodu najbolji prinos semena koje je sadržalo i najveću količinu proteina i masti u zrnu. Vrednost određivanih parametara se značajno razlikovala zavisno od vremenskih uslova, a ispitivani faktori nisu uvek imali značajan uticaj na određivane osobine.

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EFFECT OF FOLIAR ORGANIC FERTILIZING ON CEREAL-LEGUME MIXTURE

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Abstract: During the 2007-2009 period in the Research Institute of Mountain Stockbreeding and Agriculture, Troyan the effect of foliar fertilizing with the organic fertilizer BioLIFE on the yield of dry matter and weed infestation of annual late spring cereal-legume mixture was tested. The following doses of foliar fertilizing were tested as variants in a mixed stand of maize and white lupine: 1. Untreated (Standard); 2. Treatment with 2 l ha^{-1}; 3. Treatment with 3 l ha^{-1}; 4. Treatment with 4 l ha^{-1}. It was found that the application of 4 l ha^{-1} BioLIFE increased the obtained dry matter by 12.81%, as compared to the control. The exceeding in the productivity of the same variant, as against the obtained ones at the lower doses of foliar fertilizer was by 4.16% and 7.29%. These variants were superior to the standard in yielding capacity by 5.52 and 8.65%, respectively. The weed infestation of the crop was lower in case of the treatment with foliar fertilizer and the tested doses did not influence it significantly.

Key words: late spring mixture, foliar fertilizer, yields, weed infestation.

Introduction

Recently the suspension fertilizers for foliar or soil application find increasingly great application for the needs of organic farming. Up to now the studies in Bulgaria *(Pavlova et al., 1986; Pavlova and Bachvarov, 1992; Atanasova et al., 2000a; Atanasova et al., 2000b; Zhelyazkova et al., 2004; Sabev and Kertikov, 2008)* and abroad *(Hersley and Paul, 1981; Eibner, 1986*) were mainly directed to cereal and grain legume crops, vegetables, vineyards and ornamental plants. Some of the researchers *(Pavlova and Bachvarov, 1992)* found that the use of the foliar fertilizer "Lactofol" increased the grain yield of wheat and its protein content. However other authors *(Andreinska and Andreinski, 1996)* indicated that after foliar treatment with the same fertilizer, the forage of field pea was lower as compared to that obtained in the untreated control variant.

The organic fertilizer BioLIFE is a bacterial-fermentation derivative for use in different annual agricultural crops (cereals and legumes, vegetables, flowers, etc.), as well as in different kinds of perennial plantations. Different modifications are manufactured depending on its use. Alfalfa Blend 5-5-5 is used for plants fixing atmospheric nitrogen. The fertilizer has balanced content of nitrogen, phosphorus and potassium and high levels of micronutrients, iron, copper, boron, molybdenum and cobalt and is suitable for foliar application. It increases plant growth and development during the growing season.

The objective of this study was to determine the effect of foliar fertilizing with organic fertilizer BioLIFE (modification Alfalfa Blend 5-5-5) on the yields of dry mass and weed infestation of annual late spring mixture of maize and white lupine cultivated in the agro-ecological conditions of the Central Balkan Mountains (Troyan region).

Materials and Methods

Trial was carried out during 3 years in spring (at the end of April-beginning of May) at the experimental field of RIMSA, Troyan on light grey (pseudopodzolic) soil by the block randomized method with 4 replications and size of harvest plot of 5 m^2. The following doses of foliar fertilizing with the organic fertilizer BioLIFE (modification Alfalfa Blend 5-5-5-5) were tested as variants in a mixed stand of maize and white lupine: 1. Untreated (Standard); 2. Treatment with 2 l ha^{-1}; 3. Treatment with 3 l ha^{-1}; 4. Treatment with 4 l ha^{-1}. Before sowing the necessary kinds of pre-sowing tillage (shallow ploughing, disking, rotary cultivation) were conducted up to obtaining of a garden status of soil. The sowing rates for the different components of the mixture were as follows: for maize (Hybrid 509) – 15 germinable seeds/m^2 and for white lupine – 100 germinable seeds/m^2. The weight ratio of cereal to legume component in the mixture was 1:3. The row to row distance was 50 cm for maize and 15 cm for white lupine and the sowing depth 5-7 cm. The trial plot was rolled before and after sowing.

Every year the foliar fertilizing with BioLIFE was conducted twice – at formation of 4-5 leaves and at branching of lupine and the stand harvesting at its full flowering. One cut was conducted per each year. The characteristics of “productivity of dry mass” (t ha^{-1}) and “weed infestation of stand” (%) were recorded. The yielding capacity was determined by the cut method with subsequent drying at 105^0C of average samples of fresh mass to constant weight and on the basis of% dry matter it was recalculated per 1 ha. The weed infestation of the stand was determined by weight from fresh average samples for each variant, recording separately the% participation of the sown crops and weeds (as a total).

The statistic processing of data on dry mass productivity was performed by the method of variance analysis.

Results and Discussion

The data of dry matter yield by years and in average for the 3-year period is given in Table 1. It is evident that in 2007 the obtained plant production had similar values for the variants treated with foliar fertilizer, varying from 4.60 t ha^{-1} (var.2) to 5.00 t ha^{-1} (var.4). The exceeding over the standard varied from 5.26% (var.2) to 14.42% (var.4). The highest-yielding var.4 was superior to the other two lower doses of foliar fertilizing (var. 3 and 2) by 6.87 and 9.16%. During the harvesting in 2008 the obtained plant production from the variants treated with foliar fertilizer had relatively similar values and varied from 3.65 t ha^{-1} (var.2) to 3.93 t ha^{-1} (var.4), the exceeding as against the untreated standard variant being 7.67 to 15.93%, respectively. The treatment with 4 lha^{-1} BioLIFE was the most productive and was superior by 2.07 and 8.26% to the other two lower doses of foliar fertilizing.

Table 1. DM yields (in t ha^{-1} and %) by years and average for the period

Variant	2007		2008		2009		Average	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
1.Untreated (St.)	4.37	100.00	3.39	100.00	3.94	100.00	3.90	100.00
2.BioLIFE–2 l/ha	4.60	105.26	3.65	107.67	4.10	104.06	4.12	105.64
3.BioLIFE–3 l/ha	4.70	107.55	3.86	113.86	4.16	105.58	4.24	108.72
4.BioLIFE–4 l/ha	5.00	114.42	3.93	115.93	4.28	108.63	4.40	112.82
$GD_{5\%}$							12.14%	
$GD_{1\%}$							16.13%	

It is evident from the same table that in the last year of the study (2009) the obtained dry matter from the variants treated with foliar fertilizer also varied within insignificant limits – from 4.10 t ha^{-1} to 4.28 t ha^{-1}, the exceeding against the untreated standard variant being from 4.06 to 8.63%. The highest-yielding var.4 was superior in dry mass to the other two lower doses of foliar fertilizing (var.3 and 2) by 3.05 and 4.57%. On average for the 3-year period of study the maximum productivity of dry mass (4.40 t ha^{-1}) was obtained for foliar fertilizing at the dose of 4 l ha^{-1}, exceeding the untreated standard by 12.82%. The exceeding of the productivity of the same variant compared to those obtained at the lower doses of foliar fertilizer was by 4.10% and 7.29%. They were also superior to the control in yielding capacity by 5.64 and 7.18%, respectively. The performed analysis of weed infestation of the stand presented in Figure 1 shows that for the variants with application of foliar fertilizer in 2007 it varied from 10.80% (var.4) to 13.70%

(var.2) as compared to 15.50% for the standard. The percentage participation of maize in them varied from 32.90% (var.4) to 36.40% (var.2) and that of white lupine from 49.90% (var.2) to 56.30% (var.4). In the standard variant their percentage participation reached to 33.60 and 50.90%, respectively. In all studied variants treated with foliar fertilizer the weed infestation in 2008 varied from 12.87% (var.4) to 15.16% (var.2), whereas in the standard variant it reached to 19.00%. The percentage participation of maize in the treated stands varied from 35.15% (var.3) to 40.35% (var.4) and that of white lupine from 45.51% (var.2) to 51.46% (var.3). In the unfertilized standard variant their participation was 32.20 and 48.80%, respectively.

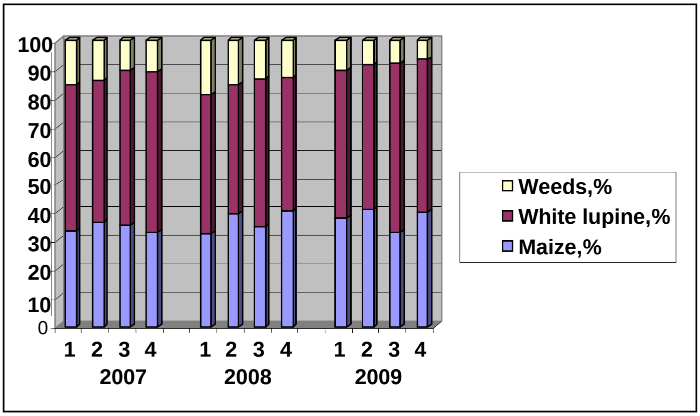

Figure 1. Crops weed infestation in% by years

In 2009 in the variants treated with foliar fertilizer the weed infestation varied from 6.58% (var.4) to 8.28% (var.2), as compared to 10.56% for the standard variant. The percentage participation of maize in the treated stands varied from 33.03% (var.3) to 40.98% (var.2) and that of white lupine from 50.74% (var.2) to 59.31% (var.3). In the unfertilized standard variant their percentage participation was 37.89 and 51.55%, respectively.

Conclusion

The foliar fertilizing with the organic fertilizer BioLIFE of annual late spring mixture of maize and white lupine influenced the yield formation. The use of the dose of 4 l ha^{-1} increased the obtained dry matter by 12.81% as against the untreated standard. The exceeding of the productivity of the same variant as against those obtained at the lower doses of the foliar fertilizer was by 4.16% and 7.29%.

These variants were superior to the control in yielding capacity by 5.52 and 8.65%, respectively. When treating with the foliar fertilizer BioLIFE the weed infestation of the stand was lower, but the different doses having no significant effect on it.

Efekti folijarnog organskog đubrenja na smešu žitarice i leguminoze

V. Lingorski, B. Churkova

Rezime

U periodu 2007-2009. godine na Institutu za planinsko stočarstvo i poljoprivredu u Trojanu testiran je efekat folijarnih đubriva sa organskim đubrivom BioLIFE na prinos suve materije i zakorovljenost jednogodišnje kasne jare smeše žitarica i leguminoza. Testirane su sledeće varijante doza folijarnih đubriva u združenom usevu kukuruza i bele lupine: 1. Bez tretiranja (Kontrola); 2. Tretman sa 2 l ha^{-1}; 3. Tretman sa 3 l ha^{-1}; 4. Tretman sa 4 l ha^{-1}. Utvrđeno je da primena 4 l ha^{-1} BioLIFE dovodi do porasta prinosa suve mase za 12,81%, kada se uporedi sa standardnim rezultatima. Povećanje prinosa ove varijante u poređenju sa onim koje su dobijene primenom nižih doza folijarnih đubriva je 4,16 i 7,29%. Ove varijante su, pak, bile bolje u odnosu na prinos standarda za 5,52, odnosno za 8,65%. Zakorovljenost za tretmane sa folijarnim đubrivima je bila niža, a testirane doze nisu bile od značajnog uticaja.

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PROSPECTS FOR NON-TRADITIONAL PLANT SPECIES CULTIVATED FOR FORAGE IN LITHUANIA

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Original scientific paper

Abstract: The changing climate and increasing number of animal species raised, especially in the organic farming systems, require a new approach to the expansion of the range of plant species grown for forage. Collections of some non-traditional plant species and varieties, during 2006-2007, have been tested at the Lithuanian Institute of Agriculture, for their forage yield and chemical composition. Many tested species and varieties produced rather good yield and had good chemical quality. Amaranth, broomcorn millet, foxtail bristle grass and some other plant species can be successfully grown for food and fodder in Lithuania's agro-climatic conditions on organic and conventional farms. In southern countries these species are widely grown for fodder and are used for green mass, hay or silage. Green mass, dry matter yield and chemical composition showed that many of the tested varieties and accessions of the non-traditional plants for our agro-climatic region in both dry 2006 and wet 2007 year produced rather high yield and can be used as forage for many animal species as green mass, hay or silage.

Key words: amaranth, broomcorn millet, foxtail bristle grass, yield, chemical composition.

Introduction

About 1800 plant species grow on Lithuania's territory, of which 400 are cultivated, however only a few dozens are used on wider range. Intensive anthropogenic effect results in consistent reduction in the diversity of habitats suitable for flora and fauna *(Vilkonis, 2001)*. On the other hand, changing climate, increasing number of animal species reared, especially in ecological farming systems, require a new approach to the expansion of the range of plant species grown for food and fodder.

Common millet, foxtail bristle grass, sorghum and amaranthus are grown in many countries for food and forage *(Anderson, 1999; Nalborczyk etc. 1999)*. Only common millet is sown on 65 million hectares in the world *(Skorniakov,* *1985)*. For many years we have been testing and breeding miliary crops, amaranth and other species for seed production *(Svirskis, 2005, Svirskis, 2007)*. In many southern countries those crops are grown for green grass, hay and silage and are good forage for livestock.

Various collections of plant species have been tested at the Lithuanian Institute of Agriculture for several decades. As a rule they used to be obtained from the former All Union Plant Production Institute in St. Petersburg, Forage Institute in Moscow and later from other institutions. During the last 15-20 years we have tested varieties and breeding lines of the following plant species: amaranth – 20, common millet -3, foxtail bristle grass – 27, soya – 5, buck wheat – 7, paiza- 4 and some other plant species. Most of them were tested for seed yield. The best performing varieties and breeding lines are repeatedly tested, multiplied on a larger area and selected. This paper presents the data of forage mass yield of some non-traditional plant species.

Materials and Methods

Collections of some non-traditional plant species and varieties, during 2006-2007, have been tested at the Lithuanian Institute of Agriculture, for their forage yield and chemical composition. All the tested varieties, except for canary grass (*Phalaris canariensis* L.) 'Judita', fodder mallow (*Malva verticillata* L.) 'Dolina' from Czech Republic and Persian clover (*T.resupinatum* L.) are bred in Lithuania. The check variety was the earliest ripening amaranth variety 'Geltonukai'.

The soil of the experimental site was light and medium heavy loam. Its arable layer at the 25-30 cm depth contained pH_{KCl} 6.7-7.5, humus 1.7-3.6 %, total nitrogen 0.15-0.26 %, mobile phosphorus and potassium 201-270 and 101-175 mg kg^{-1} of soil, respectively. The Lithuanian climate varies between maritime and continental. The warm period in Lithuania (mean daily temperature above 0^0C) lasts from 230 to 270 days, and the period with mean temperature of $+5^0$C lasts for 187-198 days. The weather conditions during the 2006-2007 periods were diverse and had a different impact on non-traditional plant species growth and yield. The year 2006 was very dry, while 2007 was normal.

The soil in spring was harrowed and prepared by "Germinator". The soil was rolled before and after sowing. Experiments were sown by a hand operated sowing machine "Senjor" into seed beds made by marker, with 50 x 50 cm inter-rows. Each plot was a two-row band, 7 m in length, with a 1 m distance between the bands. The trials involved 3 replications. After the rows of emerged plants became visible, the soil was 1-2 times loosened by a four-row rototiller. Weeds were pulled out by hand in rows. In the trials we did not use any pesticides, only 30-40 kg ha^{-1} N. Analyses were done at LIA chemistry laboratory, by standard analyses. The results were processed by the method of analysis of variance using the LSD test.

Results and Discussion

Herbage and dry matter yield data presented in Table 1 suggest that both in the droughty and normal year most of the tested species and varieties produced rather satisfactory green material and dry matter yield. Although in the normal year 2007 the yield was higher, but in the droughty year when conventional barley was severely affected by the drought the plants survived the drought better. A good trait of those crops is good recovery after lodging. All the plant species were cut twice. The highest dry matter yield was obtained by the fodder mallow ‘Dolina’, amaranth ‘Geltonukai’, common millet ‘Juosvės’, ‘Gelsvės’ and ‘Rudes’, foxtail bristlegrass ‘Rudukės’ and ‘Auksės’ and traditional oats ‘Migla’ (in 2007). Rather good yield was persian clover and seradella ‘Neris’. Unexpectedly low yield was obtained by canary grass, which is a valuable plant and in many countries is grown as annual timothy.

Table 1. Herbage and DM yield of the varieties of non-traditional plant species Dotnuva, 2006-2007

Species and variety name	Plant height (cm		GMY t ha^{-1}		DM t ha^{-1}		annual GMY		annual DM	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	t ha^{-1}	%	t ha^{-1}	%
2006	**02 08**		**10 10**							
Amaranth,Geltonukai	100	80	21.0	13.3	1.85	2.56	34.3	100.0	4.41	100.0
Fodder mallow Dolina	60	190	7.3	51.3	0.67	9.24	58.7	170.9	9.91	224.9
Canary grass	60	20	3.3	4.7	0.57	0.79	8.0	23.3	1.37	31.0
Seradella, Neris	20	40	3.7	13.3	0.59	2.24	17.0	49.5	2.83	64.0
Persian clover	20	36	4.0	8.0	0.57	1.47	12.0	34.9	2.04	42.3
C. millet, Rudės	40	50	5.3	8.3	0.75	1.57	13.7	39.8	2.31	52.5
C. millet, Gelsvės	60	60	7.0	8.0	0.76	2.13	15.0	43.7	2.88	65.4
C. millet, Juosvės	60	66	5.3	8.0	0.53	1.50	13.3	38.8	2.04	46.2
F. millet,Rudukės	50	45	7.0	4.3	0.92	0.81	11.3	33.0	1.74	39.4
T. millet Auksės	60	60	4.3	9.3	0.49	1.57	13.7	39.8	2.05	46.6
LSD_{05}			1.8	0.34	0.21	0.55	3.3		0.58	
2007	**19 07**		**01 10**							
Amaranth,Geltonukai	80	60	16.3	10.70	5.22	2.25	27.0	100.0	7.47	100.0
Fodder mallow Dolina	120	120	24.2	32.0	7.73	5.96	56.2	208.2	13.70	183.4
Canary grass	42	36	4.9	1.1	2.34	0.21	6.0	22.2	2.55	34.1
Seradella, Neris	45	50	6.5	22.1	2.20	3.62	28.6	105.9	5.82	77.9
Persian clover	64	46	9.3	14.2	2.24	2.72	23.5	87.0	4.96	66.4
C. millet, Rudės	90	60	13.0	3.5	4.16	0.74	16.5	61.1	4.90	65.6
C. millet, Gelsvės	90	58	15.0	4.2	5.11	0.90	19.2	71.1	6.01	80.5
C. millet, Juosvės	118	64	16.6	5.5	5.65	1.15	22.1	81.8	6.80	91.0
F. millet,Rudukės	98	50	12.3	3.3	6.89	0.72	15.6	57.8	7.61	101.9
F. millet Auksės	100	54	12.5	5.1	5.73	1.21	17.6	65.2	6.94	92.9
Oats Migla	64	40	13.9	3.1	6.66	0.73	17.0	45.9	7.39	98.9
LSD_{05}			2.4	8.0	0.93	1.7	9.0		2.12	

The data on the chemical composition of dry matter presented in Table 2 shows a great variability of the chemical composition and good quality of the tested varieties. Those crops are expected to spread in Lithuania both in conventional and organic farming systems and will supplement forage base both for conventional and unconventional animals. It is noteworthy that threshed straw of seed millet according to chemical composition were nearly identical with conventional hay, since their stems with leaves remain green until seed harvesting. The varieties of amaranth and miliary crops developed in Lithuania are characterised by early maturity, disease resistance, are adapted to long day and produce satisfactory dry matter yield. These varieties are already being tested in Sweden and some Baltic countries.

Table 2. Chemical composition of DM (%) of the varieties of non-traditional plant species Dotnuva, 2006-2007

Species and variety name	Crude protein	Fibre	Digestibility in vitro	NDF*	MADE**	VTA***
1st cut 01 08 2006						
Amaranth ,Geltonukai	26.8	13.4	70.0	22.2	14.4	4.76
Fodder mallow, Dolina	26.4	17.1	77.8	22.7	16.2	6.15
Canary grass	15.0	32.6	56.8	63.2	33.0	6.89
Seradella, Neris	20.3	25.4	67.8	32.8	25.1	9.21
Persian clover	20.9	23.2	74.4	28.1	20.2	8.89
C. millet, Rudes	14.3	35.6	52.2	65.3	30.8	7.26
C. millet, Gelsves	15.3	28.7	50.5	60.0	25.3	9.29
C. millet, Juosves	12.6	28.0	49.4	61.9	26.1	11.39
F. millet, Rudukes	11.4	32.6	57.8	64.2	31.6	11.09
F. millet, Aukses	9.5	33.3	47.8	68.8	31.5	9.26
2nd cut, 10 02 2006						
Amaranth ,Geltonukai	26.0	23.8	62.2	25.5	19.8	8.23
Fodder mallow, Dolina	21.7	29.8	70.0	40.3	30.0	8.42
Canary grass	25.5	25.0	63.9	44.8	24.2	8.03
Seradalla, Neris	12.4	33.2	60.9	40.1	32.7	10.6
Persian clover	17.4	26.5	73.2	29.7	26.0	15.69
C. millet, Rudės	14.7	28.8	76.9	53.5	28.0	9.18
C. millet, Gelsvės	20.2	29.1	49.4	55.2	27.6	5.84
C. millet, Juosves	16.8	30.9	44.7	55.2	28.5	7.08
F. millet, Rudukes	17.3	33.1	41.5	48.0	29.7	4.76
F. millet, Aukses	15.8	28.4	46.2	52.3	33.1	4.99
Threshed straw, Aukses	9.3	34.3	27.1	71.4	37.0	4.93
Threshed straw, Gelsves	9.4	37.6	33.7	70.3	38.7	4.19
Threshed straw, Juosves	11.3	33.2	32.0	68.6	34.8	4.80
1st cut 16 07 2007						
Amaranth, Geltonukai	19.7	23.5	83.7	26.7	22.3	1.71
Fodder mallow, Dolina	20.5	25.4	67.2	33.2	25.2	8.64
Canary grass	19.0	30.6	61.6	55.4	27.4	6.97

Persian clover	24.3	29.5	85.8	30.0	19.6	8.08
Seradella, Neris	11.8	36.2	66.2	39.9	28.3	11.1
Oat, Migla	16.8	32.7	59.9	59.0	30.5	8.33
C. millet, Rudes	17.7	30.7	55.5	58.6	28.3	6.70
C. millet, Gelsves	19.6	29.5	62.2	56.6	26.9	8.06
C. millet, Juosves	15.7	29.8	48.4	65.2	33.1	4.55
F. millet, Rudukes	18.2	34.3	59.5	62.0	31.2	4.92
F. millet, Aukses	15.5	35.6	55.9	58.4	31.3	6.63
2nd cut 27 09 2007						
Amaranth, Geltonukai	17.0	23.5	64.8	Not done		
Fodder mallow, Dolina	19.6	25.4	82.0			
Canary grass	15.8	30.6	49.1			
Persian clover	14.0	29.5	70.8			
Seradella, Neris	14.4	36.2	60.6			
Oat, Migla	13.9	32.7	35.6			
C. millet, Rudes	15.1	30.7	41.9			
C. millet, Gelsves	13.9	29.5	42.4			
C. millet, Juosves	13.4	29.8	42.8			
F. millet, Rudukes	11.8	34.3	38.7			
F. millet, Aukses	11.2	35.6	38.4			

* NDF –Neutral deterget fibre
**MADF – Modified acid deterget fibre
***VTA – water soluble carbohydrates

Conclusion

Many tested species and varieties produced rather good yield and had good chemical quality. Amaranth, miliary crops, fodder mallow and some other plant species can be successfully grown for food and fodder in Lithuania’s agro-climatic conditions on organic and conventional farms.

Mogućnost gajenja nekih netradicionalnih biljaka za krmu u Litvaniji

A. Svirskis

Rezime

Promena klime i porast broja životinjskih vrsta su u porastu, posebno u sistemima organske poljoprivrede, što zahteva nov pristup u povećanju opsega biljnih vrsta koje se gaje radi krme. U toku 2006-2007 godine na Litvanijskom Institutu za poljoprivredu, ispitivan je prinos i kvalitet krme biljnih vrsta koje se inače u Litvaniji ne gaje za krmu. Zelena masa, prinos suve mase i hemijski sastav

pokazuju da mnoge od testiranih sorti netradicionalnih biljaka u klimatskom regionu Litvanije su u u sušnoj 2006. i vlažnoj 2007. godini ostvarili veće prinose i mogu se gajiti kao krmne vrste za mnoge životinjske vrste, kao zelena masa, seno ili hrana.

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YIELD PERFORMANCE OF WINTER TRITICALE CULTIVATED FOLLOWING DIFFERENT PREVIOUS CROPS

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Original scientific paper

Abstract: A study was conducted in the period 2004-2007 to examine the effect of six previous crops (spring triticale, oats, field pea, yellow lupine, binary mixtures of 50 + 50% spring triticale/field pea and 50 + 50% oats/yellow lupine) on yield performance of winter triticale. The field experiment was conducted at the Zawady Experimental Station owned by the University of Podlasie in Siedlce (Poland). The experiment was designed as randomized complete blocks with three replicates on soil suitable rye cultivation. It was demonstrated that grain and straw yields of winter triticale planted following pulses and cereal/pulse mixtures were significantly higher than the yields of winter triticale after cereal components of mixtures grown in pure stand. Additionally, pulses and pulse/cereal mixtures grown as previous crops significantly increased total nitrogen content in and nitrogen uptake with winter triticale grain and straw. The highest winter triticale yield as well as total nitrogen uptake were after yellow lupine and field pea. The impact of oats/yellow lupine mixture on winter triticale characteristics examined in the experiment was similar to the influence of field pea.

Key words: grain and straw yields, previous crop, total nitrogen content and uptake, winter triticale

Introduction

In the world and in Poland winter triticale is predominantly grown for forage purposes. Due to its high yield potential and good nutritive value, triticale has become a successful competitor with other cereal species. The soil and weather conditions in Poland favour winter triticale cultivation *(Rozbicki, 1998)*. Levels of winter triticale yield and yield quality depend not only on soil and weather conditions but also an appropriate cropping sequence *(Chrzanowska-Drożdż, 1997; Kuś and Siuta, 1995; Mazurek and Kuś, 1992; Szempliński, 1997; Szurpnicka-Połtarzewska and Koc, 1997; Zając et al., 2006)*. Triticale is an artificial cereal combining the characteristics of demanding wheat and tolerant rye and, as such, has demonstrated different levels of susceptibility to unfavourable previous crop *(Kuś and Siuta, 1995; Mazurek and Kuś, 1992; Smagacz, 1999)*. Agricultural researchers have long been looking for ways of reducing negative effects of too frequent inclusion of cereals in rotations. The factors that prevent soil depletion include: increased rates of mineral fertilization, agrochemical protection of crops, farmyard manure application, introduction of plants stimulating soil regeneration, introduction of break crops and cultivation of plant mixtures *(Maidl et al., 1996; Nemecek et al., 2008; Rudnicki and Wasilewski, 2000; Wanic and Nowicki, 2000)*. The aim of the study was to determine the yield performance of winter triticale following selected cereals, pulse crops and their mixtures under the soil and weather conditions of the Siedlecka Upland.

Materials and Methods

A field experiment was conducted at the Zawady Experimental Station (52º06' N, 22º50' E), owned by the University of Podlasie in Siedlce (Poland). The soil of the experimental site was on soil suitable rye cultivation; it had neutral pH and was moderately rich in available phosphorus, potassium and magnesium. The experiment was designed as one-factor randomized complete blocks with three replicates. The harvested plot area was 20 m^2. The factor of the experiment was a winter triticale previous crop of: spring triticale cv 'Kargo', field pea cv 'Sokolik' (fodder cultivar), yellow lupine cv 'Legat' (in the 1st and 2nd year) and 'Taper' (in the 3rd year), a 50 + 50% spring triticale/field pea mixture and a 50 + 50% oats/yellow lupine mixture. The proportion of species in the mixtures was determined relative to the number of seeds sown per m^2 in pure stand that is 400 seeds of spring triticale and oats, 110 seeds of field pea and 120 seeds of yellow lupine.

Winter triticale cv Witon was treated with either Univeral 19.5 WS (triadimenol + imazalil + fuberidazol) or Vitavax 200 WS (carboxin + tiuram) and sown at 400 seeds per m^2 in the second decade of September. Winter triticale was cultivated following spring barley. Preplant nitrogen fertilizer was applied at split rates of 55 kg N ha^{-1} in spring at the start of vegetation, and 40 kg N ha^{-1} at the stem elongation stage. Monocotyledonous and dicotyledonous weeds were controlled in spring at the start of the stem elongation stage by means of Apyros 75 WG (sulfosulfuron) + Atpolan 80 EC (paraffin oil 11-13) whereas pests were controlled using Decis 2.5 EC (deltamethrin) and Fastac 100 EC (alpha-cypermethrin). The agrochemicals were applied at rates and dates recommended by Institute of Plant Protection in Poznań, Poland. Winter triticale was harvested at full maturity in the third decade of July or the first decade of August. The following characteristics were examined: winter triticale grain and straw yields at

15% moisture content, total nitrogen content in grain and straw as well as total nitrogen uptake with grain and straw. Total nitrogen content in winter triticale grain was determined using the Kjeldahl procedure.

The data from the study were statistically analysed by variance analysis. Significance of differences between treatment means was checked by Tukey test at $\alpha = 0.05$.

Weather conditions over the study period were rather changeable. Precipitation sums from September to August of 2004/2005, 2005/2006 and 2006/2007 were 368.1 mm (71.5% of long-term mean), 415.1 mm (80.6% of long-term mean) and 458.2 mm (89.0% of long-term mean), respectively. The respective average air temperatures were: 8.2, 7.6 and 9.9°C, the long-term mean being 7.5°C. The most and least favourable weather conditions, in terms of precipitation and temperature, for winter triticale growth were in 2004/2005 and 2005/2006, respectively. Precipitation in 2005/2006 was low in the period from September to July (apart from April) whereas air temperature in July was high (by 4.7°C higher than the long-term mean).

Results and Discussion

Grain and straw yields of winter triticale (Table 1) were influenced by weather conditions and the previous crop. The significantly highest winter triticale yield was recorded in 2005 when precipitation and thermal conditions were quite beneficial in the spring and summer. Also studies by other workers indicated substantial differences in winter triticale grain and straw yields over the study years *(Chrzanowska-Drożdż, 1997; Zając et al., 2006)*. In this experiment, higher winter triticale grain and straw yields (by 7.0 and 9.1%, respectively) were harvested following oats compared with spring triticale. Oats is believed to be a relatively good previous crop in the cultivation of cereals. It reduces negative effects of cultivation of cereals in monoculture *(Kuś and Siuta, 1995; Mazurek and Kuś, 1992)*. In the present experiment, winter triticale planted after pulses (field pea, yellow lupine) and cereal/pulse mixtures significantly increased grain and straw yields compared with winter triticale following spring cereal components of the mixtures (triticale, oats). The increase in the grain and straw yields of winter triticale following pulses amounted to 18.5 and 33.1% (an average for the whole study period), compared with the respective yields of winter triticale after cereals. Many authors have reported positive effects of previous pulse crops on winter triticale grain and straw yields *(Chrzanowska-Drożdż, 1997; Kuś and Siuta, 1995; Mazurek and Kuś, 1992; Sadowski and Krześlak, 1997; Siuta et al., 1998; Szempliński, 1997; Zając et al., 2006)*. Increased cereal yields following pulses result from a greater amount of nitrogen contained in their residues as well as better disease control and weed suppression, improved soil structure and availability of the remaining macroelements *(Maidl et al., 1996; Sadowski and Krześlak, 1997; Szempliński, 1997)*. The universally recognized characteristics of pulses condition their marked value as previous crops. They are also revealed, though to a lesser extent compared with cultivation in pure stand, when pulses are grown in mixture with cereals *(Wanic and Nowicki, 2000*). In the present experiment, the grain yields of winter triticale following spring triticale/field pea and oats/yellow lupine were by 13.9 and 11.9% higher compared with the respective cereals cultivated alone. The straw yields of both mixtures amounted to 22.3 and 21.5%, respectively. Some cereal/pulse mixtures may be the elements of rotation which reduce negative effects of cereals frequently cultivated in monoculture *(Rudnicki and Wasilewski, 2000; Wanic and Nowicki, 2000)*. In the present study, the grain yield of winter triticale following spring triticale/field pea did not differ significantly from the yield harvested following oats/narrow leaf lupine. By contrast, a significantly higher winter triticale straw yield (by 8.3%) was obtained after oats/yellow lupine compared with spring triticale/field pea. The value of cereal/pulse mixtures for subsequent crops in rotation depends on the species composition of the components, their proportion in a mixture, selection of cultivars, levels of yields and soil conditions *(Rudnicki and Wasilewski, 2000; Siuta et al., 1998; Wanic and Nowicki, 2000)*.

Table 1. Grain and straw yield of winter triticale (t ha^{-1})

Previous crop	Grain				Straw			
	2005	2006	2007	mean	2005	2006	2007	mean
Spring triticale	6.13	3.51	5.44	5.03	6.80	4.77	6.96	6.18
Oats	6.70	3.66	5.79	5.38	7.76	4.94	7.52	6.74
Field pea	7.56	4.13	6.64	6.11	9.30	6.28	9.76	8.45
Yellow lupine	7.85	4.09	6.70	6.21	10.06	6.43	9.79	8.76
Spring triticale 50% + field pea 50%	7.10	3.86	6.23	5.73	8.39	5.71	8.59	7.56
Oats 50% + yellow lupine 50%	7.61	3.96	6.49	6.02	9.51	6.05	9.02	8.19
Mean	7.16	3.87	6.22	–	8.64	5.70	8.61	–
$LSD_{0.05}$								
years				0.23				0.29
previous crop				0.31				0.42
intraction				0.38				0.51

In the present experiment, similarly to the study by *Szurpnicka-Połtarzewska and Koc (1997),* it was found that weather conditions and the previous crop significantly influenced the total nitrogen content in winter triticale grain and straw (Table 2). The highest total nitrogen content was determined in the grain and straw of winter triticale harvested in 2006 which was characterised by low precipitation in the period May – July and higher than the long-term mean air temperature *(López-Bellido et al., 2000)*. In this experiment total grain and straw nitrogen contents of winter triticale following pulses and cereal/pulse mixtures were significantly higher compared with spring cereals. It is probably due to the fact that winter triticale was better supplied with soil nitrogen *(Chrzanowska-Drożdż, 1997; Maidl et al., 1996; Szempliński, 1997; Szurpnicka-Połtarzewska and Koc, 1997; van Kessel and Hartley, 2000; Zając et al., 2006)*. The greatest total nitrogen content was determined in the grain and straw of winter triticale after pulses. An impact of oats/yellow lupine on total nitrogen content in winter triticale grain and straw did not differ significantly from an influence of field pea and the spring triticale/field pea mixture.

Total nitrogen uptake with winter triticale grain and straw was influenced by the study years and previous crop (Table 3). In the experiment discussed, pulses and cereal/pulse mixtures significantly influenced total nitrogen uptake with winter triticale grain and straw, compared with the cereal components of the mixtures in pure stand. Most total nitrogen was taken up with the grain and straw of winter triticale preceded in rotation by pulses. *Szurpnicka-Połtarzewska and Koc (1997)* reported similar findings.

Table 2. Total nitrogen content in winter triticale grain and straw (g kg^{-1}DM)

Previous crop	Grain				Straw			
	2005	2006	2007	mean	2005	2006	2007	mean
Spring triticale	16.5	21.2	17.7	18.5	5.31	6.41	5.62	5.78
Oats	16.8	21.2	17.8	18.6	5.36	6.44	5.66	5.82
Field pea	18.3	22.5	19.2	20.0	5.79	6.68	6.04	6.17
Yellow lupine	18.6	22.3	19.7	20.2	5.87	6.70	6.10	6.22
Spring triticale 50% + field pea 50%	17.8	22.1	18.6	19.5	5.67	6.56	5.86	6.03
Oats 50% + yellow lupine 50%	18.3	22.1	19.1	19.8	5.72	6.62	5.93	6.09
Mean	17.7	21.9	18.7	–	5.62	6.57	5.87	–
$LSD_{0.05}$								
years				0.1				0.07
previous crop				0.3				0.12
interaction				0.5				0.16

Table 3. Total nitrogen uptake by winter triticale grain and straw (kg ha^{-1})

Previous crop	Grain				Straw			
	2005	2006	2007	mean	2005	2006	2007	mean
Spring triticale	98.8	71.7	93.6	88.0	34.8	29.2	37.6	33.9
Oats	110.0	74.8	100.2	95.0	40.1	30.3	40.9	37.1
Field pea	135.2	89.8	124.2	116.4	52.1	40.0	56.8	49.6
Yellow lupine	142.8	88.1	128.6	119.8	57.1	41.1	57.5	51.9
Spring triticale 50% + field pea 50%	123.5	82.2	112.9	106.2	45.9	35.7	48.5	43.4
Oats 50% + yellow lupine 50%	136.2	84.4	120.7	113.8	52.6	38.2	51.5	47.4
Mean	124.4	81.8	113.4	–	47.1	35.8	48.8	–
$LSD_{0.05}$								
years				2.9				1.4
previous crop				5.6				2.7
intraction				7.5				3.6

Conclusions

Cultivation of winter triticale after field pea, yellow lupine, spring triticale/field pea and oats/yellow lupine mixtures significantly increased grain and straw yields, total nitrogen contents and total nitrogen uptake with yield, compared with winter triticale following the cereal components of the mixtures in pure stand.

Under the experimental conditions, yellow lupine and field pea most beneficially influenced winter triticale grain and straw yields as well as total nitrogen content and uptake, compared with the remaining previous crops. Oats/yellow lupine did not significantly influence the aforementioned winter triticale characteristics, compared with field pea.

Prinos i kvalitet ozime tritikale gajene nakon različitih preduseva

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Rezime

Ispitivanje je sprovedeno u periodu 2004-2007 da bi se ispitao efekat šest različitih preduseva (prolećna tritikala, ovas, stočni grašak, žuti zvezdan, smeša 50+50% prolećna tritikala/stočni grašak i 50+50% smeša ovas/žuti zvezdan) na prinos i kvalitet tritikale. Eksperimenti su izvedeni u eksperimentalnoj stanici u Zawady, na oglednom polju Univerziteta Podlasie u Siedlice (Poljska). Eksperiment je dizajniran kao slučajni blok sa tri ponavljanja na zemljištu koje je pogodno za gajenje raži. Rezultati istraživanja su pokazali da je prinos zrna semena i slame ozime tritikalee gajene na zemljištu nakon mahunarki ili smeše žitarice/mahunarke bio značajno viši u odnosu na ozimu tritikalu gajenu nakon žitarica kao čistih kultura. Gajenje mahunarki ili smeše mahunarki sa žitaricama kao predusevom dovodi do značajnog porasta ukupnog sadržaja azota u senu i semenu ozime tritikale. Najviši prinos ozime tritikale kao i ukupno usvajanje azota je na zemljištu nakon gajenja žutog zvezdana i stočnog graška. Uticaj smeše ovsa/žutog zvezdana kao preduseva je sličan uticaju stočnog graška.

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PRODUCTIVITY OF BULGARIAN SEMI-SUGAR BEET HYBRIDS IN DROUGHT CONDITIONS

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Original scientific paper

Abstract: In 2007-2009, on the experimental fields of Agricultural Institute – Shumen, Bulgaria, six fodder beet origins (diploid and tetraploid) and their semi-sugar beet hy6brids with five monogerm MS sugar beet lines have been tested. The period of study includes extreme water deficiency years and the performance of the semi-sugar beet hybrids is indicative of their drought resistance, and for their high potential as a forage crop. The increased dry matter content in the tetraploid fodder beet origins brings to balanced higher productivity and forage quality of the triploid semi-sugar beet hybrids, and especially these of the fodder beet origin SKR (4x), which exceed significantly the standard Bulgarian commercial varieties. The maintained fodder beet origins and sugar beet male sterile lines are good precondition for creation of new generation of semi-sugar beet hybrids to meet the increased requirements of farmers for high productivity forage crops.

Key words: semi-sugar beet hybrids, drought, dry matter content

Introduction

The inclusion of fodder beet and semi-sugar beet in the rations of ruminants during the autumn-winter period is determined by the high yields and concentrations of nutritive substances and vitamins, the good taste qualities *(De Vliegher et al., 1994)*. These crops are valuable also for the maintenance of the normal exchange of matters in the animal's organism, improving the digestibility and the assimilation of the rough and concentrated forages *(Hoden et al., 1988)*. The increased interest in growing sugar x fodder beet hybrids (semi-sugar beet) for rich forage *(Orlov and Yatsenko, 1999)* is due to the favourable combination of the positive economical qualities of the sugar beet with the high productivity of the fodder beet in the crosses between them *(Dalke, 1996; Kikindonov, 2009)*. The modern semi-sugar beet varieties are hybrids between monogerm male sterile sugar beet lines and the traditionally high productivity fodder beets on different ploidy levels *(Van Bockstaele, 1990)*. The monogermity, the resistance to diseases, the increased dry matter content, the uniformity of roots by form and position in the ground *(Kajiyama et al., 1992; Fomichev, 1997; Badawi et al., 2000)* are preconditions for the application of intensive technologies for growing, conservation and nourishing feeding of livestock.

The aim of our study is to test some fodder beet origins and their semi-sugar beet hybrids in conditions of severe drought. The tests allow the estimation of their economic qualities and ecological plasticity.

Material and Methods

The test was carried out during 2007-2009, under severe water deficiency conditions during the vegetation. The soil type of the experimental fields of the Agricultural Institute-Shumen was a carbonate black-earth with a good mechanical structure and weak alkaline reaction of the soil solution. Six fodder beet origins of the breeding program of the Institute (three diploid and three tetraploid) and their diploid and triploid semi-sugar beet hybrids with five diploid monogerm male sterile lines of sugar beet have been included in the tests. The multigerm fodder beet populations differ significantly in regard to the form and colour of the roots and also in regard to dry matter content. The monogerm sugar beet lines have good combining ability. The quantity of the fully sterile plants of these lines is over 95%, and their monogermity - over 98%. The sugar x fodder beet crosses were made in 2005, with a simultaneous flowering of the maternal and the paternal components. The monogermity of the hybrid seeds is over 98%, and the seeds germination of all the tested sugar x fodder beet hybrid combinations is over 90%. The randomization of the field tests was according the two-seated lattice method *(Shanin, 1977)*, the area of the experimental plot is 10.8 m^2 – in 4 repetitions, and a Group Standard is used, formed by the commercial semi-sugar beet varieties Vessi (2x) and Pliska (3x).

The assessed parameters are root yield (t ha^{-1}), dry matter content (%), measured refractometrically and dry matter yield (t ha^{-1}). The results are treated statistically by a dispersion analysis *(Lidanski, 1988)*.

Results and Discussion

In Table 1 is given information about the precipitation amount during the last ten years. It is seen that for the entire period (2000-2009) the quantities of rainfall in the pre-sowing period (October-March), vegetation rainfall (April-September) and the sum of annual rainfall, do not differ significantly from the 50 years norm, although the distribution of rainfall during the separate years is quite uneven – in 2005 the sum of annual rainfall was 929.2 mm, and the vegetation rainfall was 668.2 mm, while in 2000 the sum of annual rainfall was 413.3 mm, and the vegetation rainfall quantity in 2009 was only 167.9 mm. The period of test is characterized with a significant water deficiency – the annual sum of rainfall for the period 2007-2009 (455.9 mm) is substantially less than the normal one (559.0 mm). The rainfall in the pre-sowing period (October-March), forming the soil's moisture reserves, are with 50 mm less than the normal quantity. The vegetation rainfall has the biggest effect on the productivity of beet. In this regard the period of our study is extremely unfavourable for the culture development – the measured vegetation rainfall was by 89.3 mm less than the normal. And all this accompanied by higher than normal daily temperatures and lower air humidity. So the test results to a great extent give an idea about the drought-resistance of the tested semi-sugar beet hybrids. In the conditions of global warming up this quality will attain greater significance.

Table 1. Rainfall quantity (mm) for 2000-2009

Year	Pre-sowing rainfall (October-March)	Vegetation rainfall (April-September)	Years rainfall sum (January-December)
2000	275.8	239.6	413.3
2001	167.1	290.7	456.6
2002	208.4	374.6	674.8
2003	252.4	287.8	538.4
2004	287.7	384.4	607.9
2005	252.9	668.2	929.2
2006	256.0	330.8	550.3
2007	112.7	206.8	378.1
2008	295.1	288.2	538.4
2009	188.3	167.9	451.2
2000-2009	229.6	323.8	553.8
2007-2009	198.7	220.7	455.9
Norm for 50 years	249.0	310.0	559.0

The basic economic indices of the fodder beet are the yield of roots, the dry matter content and the yield of dry matter from a unit of area. Crossing MS sugar beet lines with fodder beet pollinators makes it possible to combine the high root yield of the fodder beet with the high dry matter content of the sugar beet – the result is a higher dry matter yield.

The root yield in our study varies for the different ploidy levels of tested materials. It is obvious (Table 2), that the tetraploid fodder beet pollinators realize higher root yields than the diploid fodder beet pollinators, and that the tested triploid sugar x fodder beet hybrids show higher productivity than the diploid semi-sugar beet hybrids. There is a strong correlation between the root yield of the fodder beet parents and the yield of the relevant semi-sugar beet hybrids. Data of our tests, carried out in years with normal vegetation rainfall, show exactly the same tendency. Even not statistically proven, the higher than standard's mean values of the root yield from the tested tetraploid fodder beets and their triploid semi-sugar beet hybrids, show that we have already a new generation of materials with better productivity, and what is more, successfully realizing it in conditions of severe drought. A good confirmation of that could be the proved higher productivity values of the semi-sugar beet hybrids of the tetraploid fodder beet pollinator SKR. Of course, we should continue our work with the diploid fodder beet and sugar x fodder beet hybrids – their seed production is cheaper and the seed qualities- better, and as with the sugar beet – they have a higher resistance to fungal diseases.

Table 2. Productivity of sugar x fodder beet hybrids in drought conditions (2007 – 2009)

Variant	Root yield t ha^{-1}	% of St.	Dry matter %	% of St.	Dry matter yield t ha^{-1}	% of St.
Gr.Standard	37.99	100.0	16.68	100.0	6.34	100.0
Vessi (2x)	36.37	95.7	17.12	102.6	6.23	98.3
Pliska (3x)	39.60	104.3	16.24	97.4	6.43	101.7
Diploid fodder beet pollinators	34.79	91.6	15.74	94.3	5.48	86.4
Tetraploid fodder beet pollinators	38.86	102.3	15.0	89.9	5.83	92.0
Diploid semi-sugar beet hybrids	35.80	94.2	17.85	107.0	6.39	100.8
Diploid hybrids of BR(2x)	35.90	94.5	18.45	110.6	6.63	104.6
Triploid semi-sugar beet hybrids	39.61	104.3	16.81	100.8	6.66	105.1
Triploid hybrids of SKR(4x)	42.13	110.9	17.05	102.2	7.18	113.2
GD 5%	3.30	8.7	1.53	9.2	0.89	14.0
P%	2.96		3.40		5.06	

In water deficiency conditions the dry matter content of the roots is increased. It is seen, that the diploid fodder beet pollinators have higher dry matter content than that of the tetraploid fodder beet pollinators. The fact that the dry matter content is higher in the diploid semi-sugar beet hybrids compared to that of the triploid hybrids could be easily explained with the bigger genomic dose of the fodder beet pollinator (lower dry matter content) in the triploid crosses with sugar beet. At the other hand – all the tested semi-sugar beet hybrids have proven higher dry matter content than the relevant fodder beet parents. And that is the reason why all the breeding companies in Europe work in this direction. Comparing the dry matter content of the tested hybrids with that of the Standard commercial varieties we should note that applying some traditional breeding methods we have managed to increase the dry matter content of the fodder beet origins, and as a result we have received a higher dry matter content in the tested sugar x fodder beet hybrid combinations. For the diploid hybrids of the fodder beet pollinator BR this exceeding over the Standard's dry matter content is statistically proved.

The yield of dry matter from a unit of area is the most important economical index, determining the value of these forage crops. In water deficiency years the diploid fodder beet origins (included in our tests) give the lowest dry matter yield, significantly below the yield of the Standard varieties. This is due to their proven lower root yield combined with low dry matter content. The tested tetraploid fodder beet origins also fall back the standard varieties, due to the fact that they have the lowest dry matter content. Having in mind that here are discussed the mean values of 15 of each ploidy level semi-sugar beet hybrids we could be satisfied by the high dry matter yields of the tested diploid and the triploid semi-sugar beet hybrids. The balanced high values of root yield and dry matter concentration of the triploid sugar x fodder beet hybrids bring to the highest values of dry matter yield – the mean of the tested 15 triploid semi-sugar beet hybrids is 105.1% of the Group Standard. The majority of these hybrids could be given for tests in the system of the State Variety Trials Agency for approval and certification as new Bulgarian varieties of semi-sugar beet. We have no doubt for the good performance and results of the tested triploid semi-sugar beet hybrids of the fodder beet tetraploid SKR. With proved higher productivity and stable high dry matter content they realized significantly higher dry matter yields in our study.

Conclusion

The semi-sugar beet hybrids show higher productivity potential than the tested fodder beet origins in drought conditions. In years with water deficiency during the vegetation the tested sugar x fodder beet hybrid combinations form significantly higher dry matter content than the fodder beet origins and realize much higher yields of dry matter yield.

Produktivnost bugarskih polu-šećernih hibrida repe u uslovima suše

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Rezime

U periodu 2007-2009. godine na eksperimentalnim poljima Poljoprivrednog instituta – Šumen, u Bugarskoj su testirana porekla 6 krmnih repa

(diploidnih i tetraploidnih) i njihovi polu-šećerni hibridi sa 5 monogermnih MS linija šećerne repe. Period istraživanja uključuje godine sa ekstremnim nedostatkom vode. Kvalitet hibrida polu-šećerne repe ukazuje na njihovu otpornost prema suši kao i na njihov visok potencijal kao krmne biljke. Povećani sadržaj suve mase u tetraploidnoj krmoj repi *origines* dovodi do umereno veće produktivnosti i kvaliteta krme tetraploidnih polu-šećernih hibrida, a posebno SKR (4x) krmnih repi *origin*, koje su premašile standardne bugarske komercijalne varijetete. Održavanje krmne repe *origin* i linije šećerne repe koje nose muški sterilitet je dobar preduslov za formiranje nove generacije polušećernih hibrida repe, koji treba da zadovolje povećane potrebe farmera za visoko produktivno krmno bilje.

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ANNUAL PASTURES ON ARABLE LANDS – PROSPECT OF THEIR FAST INVOLVING IN FORAGE PRODUCTION

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Original scientific paper

Abstract: There is too much high degree of ploughed up agricultural lands (78%) in Ukraine. It results in absence of balance in rural landscapes and to decline of their ecological stability. Fast transformation of arable lands, it is especial slopes and the areas near to stock-rearing farms in a perennial agro phytocenosis it is complicated with series of social and economic problems and global climate fluctuations. It constrains omnipresent realization of measures on a grassing, creation of pastures of long-term use and dictates necessity of search of new approaches to rational system of cattle feeding. Creation of annual pastures allows involving accelerated field grounds in a forage production and is a transient stage to grazing perennial grasses. Researches have shown, that use on a grazing of mixes of annual cultures as more phytocenotic active and ecologically plastic components of an agro phytocenosis, today is not alternative to perennial cultivated grasslands. But in extreme situations, it is specific to the areas near farms as one of factors of reception of low-cost forages for animals they are completely justified.

Key words: annual pastures, arable lands, forage production.

Introduction

In Ukraine there is too high degree of ploughed agricultural lands - 78%. It results in absence of balance in rural landscapes with the dominance of destructive subsystems (tilled lands, ore dumps, infrastructure and others) over stabilizing ones (meadows, forests, swamps and others), decrease of ecological stability of landscapes and development of heavy erosions *(Petrychenko and Kovtun, 2006)*. In 2000 a program was started on the exclusion of 10 million ha of arable slopes from the intensive cultivation and further grassing of 8 million ha, foresting of 2 million ha providing equal correlation of pastures and arable lands. First of all, it is necessary to stop tillage of lands in flood control river zones with the increased level of erosion as well as all slopes with 7^{o}, partly saline soils and lands of the melioration fund that perform important ecological function *(Petrychenko et al., 2007)*. Then the grassing area should be increased by the plots with complicated configuration and less convenient for regular cultivation as well as arable lands near farms and complexes engaged in livestock production. Today there are no doubts as for expediency of pasturing such lands, especially in the zone of adequate moisture supply of the soil *(Veklenko, 2007)*. But difficult relations of the industrial and agrarian sectors, high cost of machinery, fuel and oil materials, mineral fertilizers, lack of seed of perennial grasses and legumes produced in Ukraine restrain the measures on land grassing, creating pastures of long-term use and cause the necessity of searching new approaches to the system of rational livestock feeding *(Vorobel and Veklenko, 2008)*. Insufficiency of financial resources for creation of high productive cultivated perennial pastures and unreasonableness of use of faraway natural forage lands attract attention to possible creation on the base of close-by farm crop rotation of annual pastures from forage crops and their mixtures as one of the factors of getting low-cost forages for livestock. In this case it is reasonable to have species with low sowing rates of the seed and its low cost. It is mainly winter and spring cereal, legume and crucial crops which are the base of pasture mixtures. The use of different annual mixtures with different sowing terms is not an alternative to perennial cultivated pastures, but in conditions of growing deficiency of atmosphere water supply of the pastures, especially of lands near farms, it can be quite reasonable.

Materials and Methods

Researches were carried out in 2007-2009 on the mid-washed off gray podzol soil of 6-8^0 slopes of the central Forest-Steppe of Ukraine (49^{o}10'N, 28^{o}23'E, average precipitation 586 mm, air temperature +6.7^0). Humus content in the arable layer 1.6-1.9%, pH 5.1-6.0, 100 g of the soil contain 10-12 mg of easily hydrolyzed nitrogen, 10-14 mg of the exchange potassium, 10-15 mg of labile forms of phosphorus. During the period of research, in 2007 and 2009 it was dry, in 2008 - normal. The systems of fertilization were not used in annual pastures.

Trial 1. Productivity of annual pastures of winter cereal and crucial mixtures was investigated. They were sown on September 20-25. Triple pasturing (grazing) was used starting in the middle of April till the end of August.

Trial 2. Yield of the mixtures of spring early forage crops under pasturing was studied. Sowing term – the third decade of April. Three cycles of pasturing were used – from the beginning of June till the end of August.

Trial 3. Efficacy of the use of late spring annual forage crops for creating of annual pastures was determined. Sowing term – the second decade of May. The regime of grass stand use was triple pasturing. Pasturing period was the beginning of July – the middle of October. Determination of the yield of top biomass (YM), dry matter (DM), output of crude protein (CP) and digestible protein (DP), concentration of gross energy (GE) and metabolically energy (ME) in dry matter of forage and its nutritious value in fodder units (FU) was carried out according to the generally accepted procedures. Mathematical yield data processing was carried out by the methods of dispersion, correlation and regression analysis using computer software.

Results and Discussion

The main purpose of creation annual pastures is to provide livestock with high quality fodder in critical periods of the pasturing season (early spring, digression of perennial grasses, late autumn). It is advisable to sow crops that are able to re-grow after grazing. That is why we have studied pasture use of different variants of cereals, cabbage-cereal and legume-cereal mixtures of annual forage crops of different ripeness groups: winter, spring early and spring late.

Table 1. Productivity of winter cereal and cabbage-cereal annual pasture grass stands, (in average for 2007-2009)

Swards	YM, $t\ ha^{-1}$	DM, $t\ ha^{-1}$	FU, $t\ ha^{-1}$	CP, $t\ ha^{-1}$	GE, $GJ\ ha^{-1}$	ME, $GJ\ ha^{-1}$	DP, $g\ kg\ FU^{-1}$
Secale cereale L./ *Lolium multiflorum var. Westerwoldicum* Mansh.	24.23	5.91	5.53	0.87	114.8	62.8	112.5
S. cereale L./*Brassica napus* L./ *L. multiflorum* Lam.	26.65	5.75	5.08	0.83	110.2	58.7	127.7
xTriticosecale Wittmack/ *L. multiflorum* Lam.	23.36	5.48	5.50	0.94	104.7	60.6	129.3
xT. Wittmack/ B. napus L./ *L. multiflorum* Lam.	22.91	5.01	4.67	0.85	94,3	52.3	140.3
Triticum durum Desf./ *L. multiflorum* Lam.	20.11	4.77	3.67	0.76	91.3	44.4	137.6
T. durum Desf./*B. napus* L./ *L. multiflorum* Lam.	20.92	4.93	3.74	0.87	94.0	45.6	154.7
$LSD_{0.05}$	1.86	0.41	0.35	0.02	2.1	1.2	3.8

Trial 1. In the group of forage crops we have studied mixtures of winter rye (*Secale cereale* L.), winter triticale (*xTriticosecale Wittmack*) and winter wheat (*Triticum durum* Desf.) with winter rapeseed (*Brassica napus* L.) and westerwold ryegrass (*Lolium multiflorum var. Westerwoldicum* Mansh.). Cereal pasturing at the phase of tilling – the beginning of heading; crucial and legume – at the phase of branching provides at least three-cycle pasturing per season. Observations have

shown that the quickest growth and development had mixtures with winter rye, and lowest – with winter wheat that can be started to be pastured from the beginning of the second decade of April. Table 1 contains data on the productivity of annual pastures of winter forage mixtures. The yield of green mass of mixtures with winter rye was 24.23-26.65 t ha^{-1}, that is substantially more than in other variants. Green mass output of mixtures with winter triticale is smaller than the previous one, but it is higher than in mixtures with winter wheat – 22.91-23.36 against 20.11-20.92 t ha^{-1}. Such tendency was proved by the output of dry matter and fodder units, but it should be mentioned that there was some dominance of cereal mixtures over cabbage-cereal mixtures, except for the variants with winter wheat where it was within the trial error. Researches variants showed quite high output of CP that was from 0.76 t ha^{-1} in the mixture of winter wheat with westerwold ryegrass up to 0.94 t ha^{-1} in winter triticale with westerwold ryegrass. 44.4-62.8 GJ ha^{-1} ME was obtained with pasture forage. The highest concentration of DP in 1 fodder unit was in the fodder of variants where winter rapeseed, westerwold ryegrass were sown with winter triticale (140.3 g) or with winter wheat (154.7 g).

Table 2. Productivity of annual forage mixtures of early-spring sowing term, (in average for 2007-2009)

Swards	YM, t ha^{-1}	DM, t ha^{-1}	FU, t ha^{-1}	CP, t ha^{-1}	GE, GJ ha^{-1}	ME, GJ ha^{-1}	DP, g kg FU^{-1}
Secale cereale L./*Lolium multiflorum var. Westerwoldicum* Mansh.	16.91	3.86	3.45	0.58	73.7	40.7	123.8
S. cereale L./Raphanus sativus L./ *L. multiflorum* Lam.	23.55	4.91	4.76	0.77	90.5	54.1	130.9
S. cereale L./Pisum arvense L./ L. multiflorum Lam.	21.26	4.43	4.19	0.75	86.0	48.0	132.8
Hordeum vulgare L./ L. multiflorum Lam.	17.55	3.59	3.01	0.61	68.0	36.4	137.7
H. vulgare L./ *R. sativus* L./ *L. multiflorum* Lam.	22.41	4.54	4.14	0.83	86.3	49.6	150.8
H. vulgare L./ P. arvense L./ *L. multiflorum* Lam.	18.64	4.03	3.62	0.78	77.7	50.0	148.5
Avena sativa L./ *L. multiflorum* Lam.	17.77	3.62	3.03	0.57	69.1	35.8	133.1
A. sativa L./*R. sativus* L./ *L. multiflorum* Lam.	20.99	3.96	3.82	0.65	73.6	43.1	138.3
A. sativa L./ *P. arvense* L./ *L. multiflorum* Lam.	21.86	4.38	3.94	0.72	84.7	45.1	135.6
LSD $_{0.05}$	2.16	0.41	0.27	0.01	1.8	1.1	3.4

Trial 2. In the group of early spring sowing term of spring crops pasture mixtures having different intensity of biomass growing and consisting of spring rye (*Secale cereale* L.), spring barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) in

combination with radish (*Raphanus sativus* L.) or pea (*Pisum arvense* L.) were studied. Due to entire sowing under the above-mentioned grass stands of westerwold ryegrass, three cycles of livestock pasturing per season beginning in the second decade of June till August were conducted. Average data on the productivity of these mixtures for the years of research are shown in table 2. Cereal-legume and cabbage-cereal mixtures had the highest yield of YM, DM and FU in comparison to cereals. They also helped to increase CP output from the forage area from 0.57-0.61 to 0.65-0.83 t ha^{-1} and obtaining of ME from 35.8-40.7 to 43.1-54.1 GJ ha^{-1}. Pasture forage of the researched grass stands had high protein content: one fodder unit contained 123.8-150.8 g of DP. Spring rye matured as pasture quicker, oats was the last to mature.

Trial 3. In the group of spring forage crops sown in mid May to create annual pastures mixtures based on Japanese millet (*Echinochloa frumentaceae (*Roxb.) Link.) and Sudan grass (*Sorghum sudanense* (Piper.) Stapf.) with cabbage component white mustard (*Sinapis alba* L. and legume – spring vetch (*Vicia sativa* L.) compressed by westerwold ryegrass were studied.

Table 3. Productivity of the variants of pasture mixtures of late spring forage crops, (in average for 2007-2009)

Swards	YM, t ha^{-1}	DM, t ha^{-1}	FU, t ha^{-1}	CP, t ha^{-1}	GE, GJ ha^{-1}	ME, GJ ha^{-1}	DP, g kg FU^{-1}
*Echinochloa frumentaceae (*Roxb.*)* Link./*Lolium multiflorum var. Westerwoldicum* Mansh.	17.99	3.22	2.42	0.46	59.9	29.2	101.1
*E. frumentaceae (*Roxb.*) Link./Vicia sativa* L./ *L. multiflorum* Lam.	18.46	3.13	2.42	0.48	58.9	29.4	118.9
E. frumentaceae (Roxb.) Link./Sinapis alba L./L. multiflorum Lam.	21.41	3.63	2.75	0.53	68.0	33.9	118.5
Sorghum sudanense (Piper.) Stapf./ *L. multiflorum* Lam.	24.94	4.25	3.99	0.59	78.4	44.2	105.4
S. sudanense (Piper.) Stapf./ *Vicia sativa* L./*L. multiflorum* Lam.	25.19	4.10	3.66	0.69	76.2	42.9	127,4
S. sudanense (Piper.) Stapf./ *S. alba* L./*L. multiflorum* Lam.	28.22	4.63	3.99	0.70	85.4	47.5	124.1
LSD $_{0.05}$	1.72	0.34	0.25	0.01	1.2	0.8	2,5

Due to after-grassing and drought resistance of the above-mentioned species of this group, triple full value pasturing of livestock during extreme period, beginning in the beginning of June till mid October, was conducted. As it is shown in table 3, in average for the years of researches mixtures of late spring forage crops provided output of 17.99-28.22 t ha^{-1} of pasture forage, 3.13-4.63 t ha^{-1} DM and 2.42-3.99 t ha^{-1} FU. Mixtures with Sudan grass substantially prevailed over grass mixtures based on Japanese millet by these characteristics. Comparison of the variants reveals the dominance of cabbage-cereal mixtures over other types of grass stands. Thus, cereal mixtures provided 0.46-0.59 CP output, legume-cereal – 0.48-0.69 and cabbage-cereal 0.53-0.70 t ha^{-1}, correspondingly. Such tendency concerned energy output from pasture forages of the researched variants.

Conclusion

Creation of annual pastures from forage crops of different ripening groups (spring, early spring, late spring) on arable lands that cannot be cultivated intensively enables to involve them in forage production quicker being a transitional stage to grassing of perennial grasses. Researches have shown that pasture use of more phytocenotically active and ecologically plastic mixtures of annual crops (cereal, legume-cereal, crucial-cereal), in conditions of frequent droughts (global warming), extreme periods of vegetation of perennial grasses (pasture digression) partly solves the problems of livestock supply with low-cost full-value pasture forages excluding additional costs on mineral fertilizers.

Jednogodišnji pašnjaci na plodnim zemljištima – mogućnost njihovog brzog uključivanja u proizvodnju krme

V. Petrychenko, Y. Veklenko

Rezime

U Ukrajini se nalazi mnogo preoranog poljoprivrednog zemljišta – 78%. To za rezultat ima jak disbalans u ruralnim područijima i smanjenje njihove ekološke stabilnosti. Brza transformacija plodnog zemljišta, pogotovo padina i zemljišta blizu stočnih farmi, u višegodišnje agrofitocenoze je komplikovan proces koji sa sobom nosi veći broj socijalnih i ekonomskih problema, kao i problem globalnih klimatskih promena. Sužava mogućnost realizacije mera na travnjacima, stvaranje pašnjaka za dugotrajnu upotrebu i dovodi do potrebe nalaženja novih postupaka racionalizacije sistema za proizvodnju stočne ishrane. Stvaranje jednogodišnjih pašnjaka dozvoljava ubrzano uključivanje obradivog zemljišta u proizvodnju krmnog bilja i predstavlja prelaznu fazu za gajenje višegodišnjih trava. Istraživanja su pokazala da korišćenje smeša jednogodišnjih kultura za ispašu, danas nije alternativa višegodišnjim gajenim travnjacima. Ali u ekstremnim

situacijama, kada se gaje blizu farmi, pogodni su kao jeftin izvor krmne hrane za stoku, što je u potpunosti opravdano.

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TALL FESCUE PERFORMANCE UNDER DROUGHT AND CUTTING CONDITIONS

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Original scientific paper

Abstract: Drought and cutting are the two main factors affecting the plant survival and growth under Mediterranean conditions. The above ground dry biomass, leaf and stem dry weight, leaf area, the leaf water potential (Ψ), stomatal conductance and transpiration rate (Tr) were measured in order to evaluate the combined effects of water stress and cutting on tall fescue (*Festuca arundinacea* cv. Festorina) performance. The plants were grown under field conditions, at a semiarid area in Drama (Macedonia, Greece). The plants were subjected to irrigated (Ir) and rain fed (Rf) conditions. Two cutting treatments, light cutting at 7 cm and heavy cutting at 3 cm from soil surface, were applied four times at twenty-day intervals. Leaf area index (LAI), leaf area ratio (LAR), and leaf weight ratio (LWR) were calculated. Moreover, canopy transpiration was estimated. Tall fescue probably manage to overcome drought and survive, through both morphological (leaf area index, leaf area ratio and leaf weight ratio), and physiological (leaf water potential, stomatal conductance, transpiration rate and canopy transpiration) mechanisms. On the other hand, the two cutting levels did not produce any differences on the above mentioned characteristics. In addition, yield reduced during warm season, independently of water regime used.

Key words: drought, leaf area, transpiration, water potential, yield, *Festuca arundinacea*

Introduction

Drought is worldwide problem, seriously constraining global crop production and quality. Recent global climate changes have made this situation more serious. In semi-arid Mediterranean grasslands drought conditions considered to be the most important environmental factor limiting plant survival and yield. In this sense, more information is required on factors that make crops more tolerant to changing conditions. In addition, a change in the management practices of grasslands is needed; it is necessary to drive practices towards sustainable grasslands which permit high production and environmental goals *(Kemp and Michalk, 2007; Moreno et al., 2008)*.

The common practice of cutting is a principal and drastic factor affecting the growth pattern and yield of forage species *(Brummer and Moore, 2000)*. Moreover a positive effect of cutting on water balance of plant tissues has been reported for several species *(Tsiouvaras et al., 1986, Paez and Gonzalez, 1995, Lazaridou et al., 2002, Lazaridou and Noitsakis, 2005)*. However, the specific mechanisms contributing to this mitigation have not been completely investigated.

The effect of drought on growth and yield of a species depends upon the severity, duration and timing of water deficit. The degree of plant response to water stress varies among species. Plants develop morphological and physiological adaptation mechanisms such as reduction of leaf area, stomatal closure, and regulation of water potential, in order to minimize water loss and, therefore, to survive during water stress *(Passioura, 1997; Sarker et al., 2004; Karatassiou et al., 2009)*.

Tall fescue is a perennial C_3 cool-season grass species, with high forage production, well adapted all over the Greece *(Iliadis, 2006)*. Tall fescue has broad adaptability, persistence, and high nutritive value *(Burns and Chamblee, 1979)*. However, few are the reports on morphological and physiological adaptation mechanisms that it develops when it grows under drought and cutting conditions and little is known about drought survival and recovery in perennial grasses generally *(Moreno et al., 2008)*. A more detailed knowledge of these mechanisms in this species may be important for the improvement of management practices, as well as for future selection of species for drought prone areas.

The subject of this research was to investigate both morphological and physiological characteristics, that tall fescue develops as adaptation mechanisms in order to survive and produce biomass under the combined effect of drought and cutting.

Material and Methods

The field experiment was performed in Drama (41°09′ N lat, 24°09′ E long, 130 m alt) of Macedonia Greece. The climate is semi-arid, with mean annual temperature 15.2°C, and mean total precipitation 589.4 mm. The dry period is from middle of the June to September. The years of experimentation the dry period began from May (Figure 1). The soil is medium textured with pH value equal to 7.5. The studied species was tall fescue (*Festuca arundinacea* cv Festorina).

Two water regimes were applied: a) irrigation by sprinkler to maintain field capacity (Ir) and b) no water was applied; the plants received only the rain water (rain fed, Rf) (Figure 1). Within each water regime, two cutting treatments were applied: light cutting at 7 cm from soil surface (C7) and heavy cutting at 3 cm from soil surface (C3). The cutting treatments applied four times at twenty-day intervals, during spring and early summer.

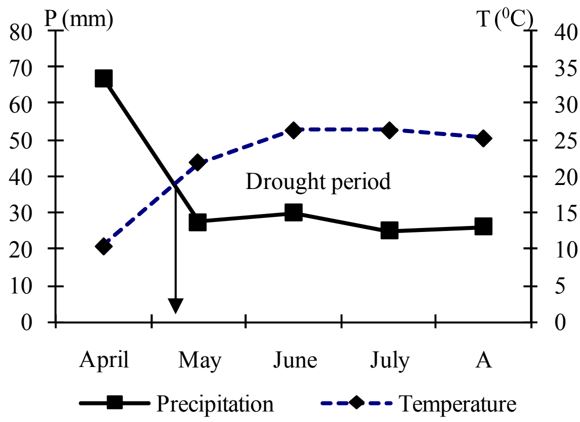

Figure 1. Ombrothermic diagram based on the data of the two years of experimentation.

The yield (above ground dry biomass in g m^{-2}) was determined each time the plants were cut. The cut material was oven dried at 75°C for 48 hours. In order to study the canopy structure the morphological characteristics of leaf area and leaf and stem dry weight determined. The leaf surface measured using Area measurement system, (Delta-T-Devices, Cambridge, UK). Leaf area Index (LAI) calculated as leaf area per ground area. Leaf Area Ratio (LAR) calculated as leaf area per dry weight of above ground plant tissue. Leaf Weight Ratio (LWR) calculated as leaf weight per dry weight of above ground plant tissue *(Beadle, 1993)*. Physiological characteristics of leaf water potential (Ψ), stomatal conductance (G) and transpiration rate (Tr) were measured. Midday G and Tr was measured in mmol m^{-2} s^{-1}, on the abaxial leaf surface, with a steady state porometer (Li-1600, LiCor Nebraska U.S.A.). Ψ (Mpa) was measured using a pressure chamber (ARIMAD–2, Kfar Charuv, Israel).

Plant modulates the water transpired by reducing the transpiration rate, the plant size and/or the leaf area. The last two affect the leaf area index (LAI). It is noticeable that for crops with closed canopies and abundant litter, the soil evaporation could be ignored; water use is then equal to transpiration by canopy. As a consequence of these, the transpirational water losses were estimated as Canopy Transpiration (CT), using the equation *(Lazaridou et al., 2002)*:

CT= transpiration rate x leaf area index (in mmol m^{-2} s^{-1}), The experimental design was completely randomized, with field plot size of 1 m x 1 m, in four replications. The physiological parameters were measured in twelve leaves per treatment. The measurements were performed from April to July over two successive growth seasons.

Results and Discussion

Seasonal changes of yield, namely the above ground dry biomass, are illustrated on Figure 1a. The yield reduced progressively in both water regimes, being higher in April and lower in July. Higher yield all over the season is obvious in irrigated plants compared to rain fed ones. Finally, the yield of plant reduced to about one third of irrigated. The cutting affected the yield in irrigated plants but no significant differences observed between cutting treatments in rain fed plants. Yield reduction of forage species subjected to drought has also been reported by many researchers *(Lazaridou and Noitsakis, 2003; Farooq et al., 2009)*. Canopy structure, photosynthetic processes and internal water status affect biomass production and allocation, but it is difficult to interpret how plants integrate these processes to accumulate biomass *(Farooq et al., 2009)*.

LWR is the main allometric index, showing the changes of photosynthetic tissues. In all treatments LWR was higher in summer and lower in spring (Figure 2b). The increase of photosynthetic tissues means that the plants were in the vegetative stage and there was not any reproductive growth during summer. Between the two water regimes substantial differences were observed only in May and June; less photosynthetic tissues were found under drought. LAI is directly associated with dry matter accumulation and yield through its effect in photosynthesis *(Passioura, 1997; Shao et al., 2008)*. Moreover, it is associated with the water used by the plant as it indicates the transpiring surface per land area *(Blum, 1997)*. In irrigated treatments, LAI was more or less constant during the season, while in rainfed treatments it was significantly reduced after April (Figure 3a). Reduction of leaf area in herbaceous species, as a response to drought, has been reported by many other researchers *(Noitsakis et al. 1991; Tardieu 1997; Moreno et al., 2008)*. Except LAI, a decrease in leaf area per above ground dry biomass (LAR) in plants under drought observed after May, indicating reduction of transpiring surface more than photosynthetic tissues of plant (Figure 2b). The LAI and LAR adjustment suggest that these morphological changes are a strategy to overcome drought *(Pereira, 1995; Moreno et al., 2008)*. It is noticeable that LAR presented higher values during warm season (June and July) compared to cool period (April and May) in both irrigation treatment. The cutting treatment didn’t significantly affect these two allometric indices.

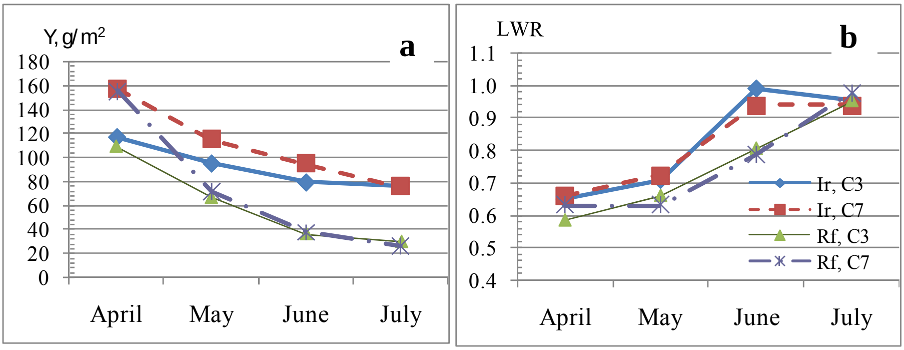

Figure 2. Mean seasonal changes of the two years of experimentation, of a. yield (Y=above ground dry biomass, g/m^2) b. Leaf Weight Ratio (LWR). Ir, irrigation. Rf, rainfed. C3, heavy cutting. C7, light cutting

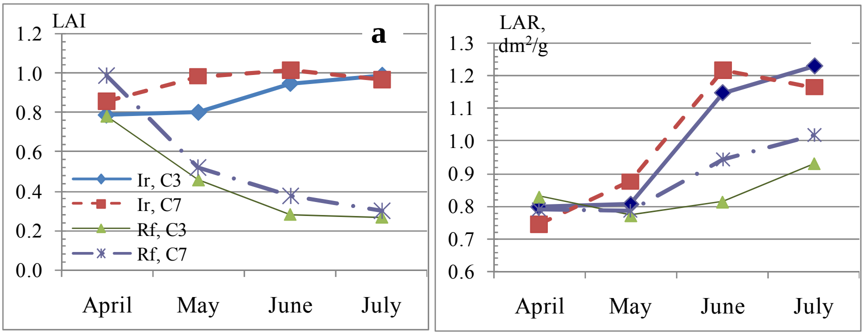

Figure 3. Mean seasonal changes of the two years of experimentation, of a. Leaf Area Index (LAI) and b. Leaf Are Ratio (LAR)

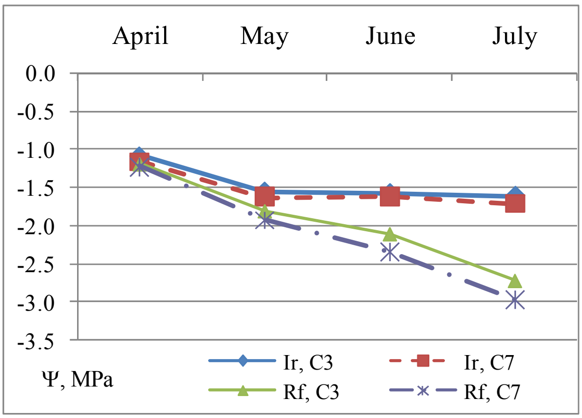

Figure 4. Mean seasonal changes of the two years of experimentation of leaf water potential (Ψ)

Leaf water potential, stomatal resistance and rate of transpiration are important physiological characteristics that influence plant water relations *(Farooq et al., 2009)*. The seasonal changes of midday leaf water potential (Ψ) indicated higher values in irrigated plants compared to rainfed at the same time of growing season (Figure 4). The resulting differences gradually became greater. No differences observed between the cutting treatments under irrigation. Under drought the Ψ values were higher in heavy cut plants only in June and July. It is documented that cutting maintain more favourable internal water status compared to uncut plants in water stress conditions *(Tsiouvaras et al., 1986; Lazaridou and Noitsakis, 2005)*. Generally, it is accepted that in the stressed plants the decrease of Ψ is due to the soil water deficit, while in the irrigated plants it is due to high VPD in the midday *(Lazaridou and Noitsakis, 2005)*.

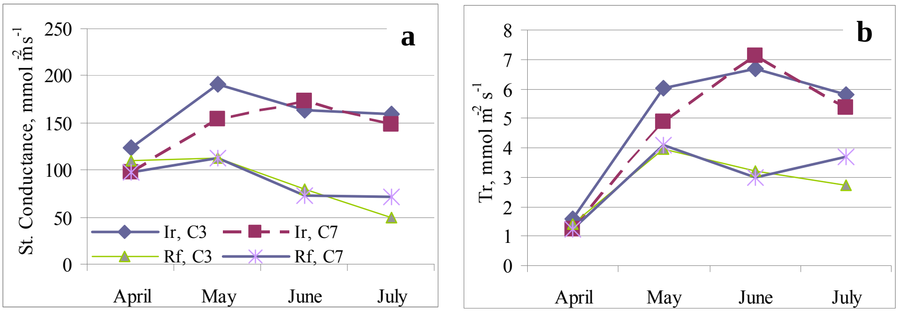

Figure 5. Two years mean seasonal changes of a. Stomatal Conductance and b. transpiration rate (Tr). Ir, irrigation. Rf, rainfed. C3, heavy cutting. C7, light cutting

Leaf water potential (Ψ) controls stomatal aperture under drought affecting stomatal conductance. Thus we could assume that the low values of Ψ in the rain fed treatment would result in lower stomatal conductance and consequently lower transpiration rate *(Lazaridou and Noitsakis, 2003)*. Indeed, as illustrated on the Fig. 5a, b the stomatal conductance and transpiration rate were significantly lower in rain fed than in irrigated treatment, implying less transpiration water losses in both cutting treatments (Figure 5b). No substantial differences between cutting treatments observed concerning G and Tr. The tall fescue seems to control water loss to atmosphere by reducing leaf area and transpiration rate. The combined effect of these two parameters on water used by plant was indicated by canopy transpiration (CT). We consider CT being a better indicator of plant performance than transpiration rate, as it is an expression of total water transpired from crop canopy, although the adaxial leaf surface transpiration is not included. The canopy transpiration (CT) exhibited substantially lower values in Rf treatment after April (Figure 5b). Cutting level didn't affect the total water transpired by plant. Consequently, it seems that tall fescue survives and maintains high productivity during the drought period using morphological and physiological adaptation mechanisms.

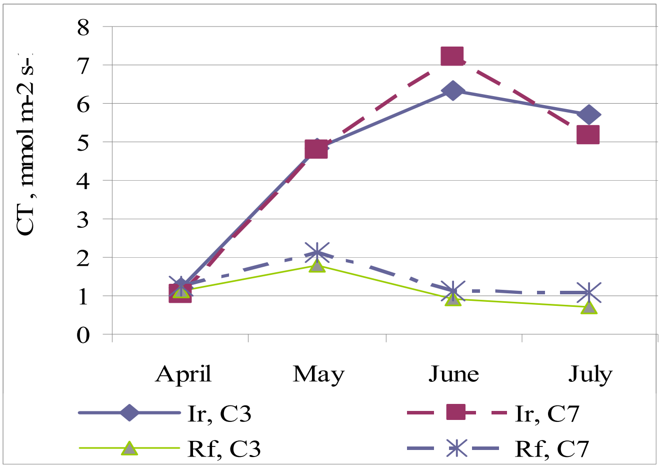

Figure 7. Mean seasonal changes of Canopy Transpiration (CT) for the two years of experimentation

Conclusion

Tall fescue probably manage to overcome drought and survive, through both morphological (leaf area index, leaf area ratio and leaf weight ratio), and physiological (leaf water potential, transpiration rate and canopy transpiration) mechanisms. On the other hand, the two cutting levels did not produce any differences on the above mentioned characteristics. In addition, yield reduced during warm season, independently of water regime used.

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Uticaj suše i visine košenja na visoki vijuk

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Rezime

Suša i košenje su dva glavna faktora koja utiču na preživljavanje i rast biljaka u mediteranskim uslovima. Da bi se utvrdili kombinovani efekti vodnog

stresa i košenja na ponašanje visokog vijuka (sorta Festorina), mereni su suva nadzemna biomasa, suva masa lista i stabla, površina lista, vodni potencijal lista (Ψ), provodljivost stoma i brzina transpiracije (Tr). Biljke su gajene u poljskim uslovima u polu-suvoj oblasti u Drami (Makedonija, Grčka). Biljke su postavljene u uslove navodnjavanja (Ir) i zalivanja kišom (Rf). Primenjena su dva tretmana košenja, visoko košenje na 7 cm i nisko košenje na 3 cm od površine zemlje, četiri puta u intervalima od dvadeset dana. Računati su indeks površine lista (LAI), udeo površine lista (LAR) i udeo težine lista (LWR). Takođe, procenjena je i transpiracija gornjih delova biljke. Visoki vijuk verovatno može da prevaziđe sušu i da preživi koristeći morfološke (indeks površine lista, udeo površine list i udeo težine lista) i fiziološke mehanizme (vodni potencijal lista, provodljivost stoma, brzinu transpiracije i transpiraciju gornjeg dela biljke). Sa druge strane, dva nivoa košenja nisu imala nikakve razlike u navedenim karakteristikama. Takođe, prinos se smanjio u toku tople sezone nezavisno od vodnog režima.

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EFFECT OF MINERAL FERTILIZATION ON YIELD OF *Agrostidetum vulgaris* – TYPE MEADOWS IN MOUNTAINOUS GRASSLANDS IN SERBIA

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Abstract: The trial was carried out during four years (2005 - 2008) on *Agrostidetum vulgaris* -type meadow in the hilly region of western Serbia (near city Valjevo - altitude 750 m). The investigation included six NPK fertilizer rates - $N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ kg ha^{-1}. The increase in NPK fertilization level resulted in corresponding increases in the DM yield. Maximum four-year average dry matter yield of 9,03 t ha^{-1} was achieved with the highest NPK rate NPK ($N_{200}P_{150}K_{150}$ kg ha^{-1}). The increase was 7,1 t ha^{-1} or 464% compared with the control. In particular, the increases in N rate had a favourable effect on DM yield. DM yield was 1.96 and 8.10 t ha^{-1} at the lowest and highest NPK rates, respectively. The maximum yield of hay was obtained in the test with ($N_{200}P_{150}K_{150}$ kg ha^{-1}), since it produced only 12.4 kg of hay per kg of NPK, while in tests with lower dose ($N_{50}P_{50}K_{50}$ kg ha^{-1}) this ratio was higher (16.2 kg).

Key words: *Agrostidetum vulgaris,* NPK fertilization meadow, yield DM

Introduction

Natural meadows cover large areas in the mountainous region of Serbia *(Mijatović and Pavešić-Popović, 1972)*. They are of considerable importance for forage and soil utilization and protection. Because of poor management and careless utilization these grasslands are rather degraded, with low production and poor quality. In past decades the yield response of grasslands to mineral (NPK) fertilization has been frequently studied. The use of NPK fertilizer is an important factor in intensive grass-based dairy farming, as NPK affects dry matter yield and crude protein content of herbage, and thus the amount and protein concentration in supplementary feed, and rate of nitrogen losses through ammonia volatilization, nitrate leaching, nitrous-oxide emission and denitrification *(Jarvis, 1996;*

Whitehead, 2000). The results of other tests performed in Serbia showed that mineral fertilizer had a favourable effect on the yield, protein, ash and fat content while decreasing the cellulose content of herbages *(Vučković et al. 2004, 2005a,b,c, 2007, 2009)*.

Recommendations for application rates of mineral fertilizer have been defined as a function of soil, grassland type and climate (*Vellinga and André, 1999*). The criteria for optimum application rates of mineral fertilizer were merely economic, for example, a marginal N response of 10-5 kg DM of herbage per kg N *(Unwin and Vellinga, 1994; Whitehead, 1995; Reid, 1970; Morrison et al., 1980)*.

Bearing in mind the above mentioned, the objective of this study is to assess the effect of mineral fertilization on the yield of natural meadow type *Agrostidetum vulgaris,* with respect to agro-ecological and economical conditions as well. The study is of great importance both for scientific and practical points of view.

Materials and Methods

On a Serbian acid soil, swards were compared at different levels of mineral (NPK) fertilization. The trial was carried out during four years (2005 - 2008) on natural meadows dominated by *Agrostidetum vulgaris* in the hilly region of Serbia (village Leskovica near thr city of Valjevo, 750 m altitude). Soil analysis was made before the experiment was established. The investigation included six NPK fertilizer rates - $N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ kg ha^{-1}. All fertilizers were broadcast directly after the melting of snow (early March). Dry matter (DM) yield was determined on the basis of total DM amount per plot and calculated as DM yield per unit of area.

Soil properties. The grasslands vegetation of *Agrostidetum vulgaris* on hilly region in the village Leskovica near Valjevo is formed on the acid soil. These are shallow to medium deep soils, skeletal to different degrees, with high humus level (5.37%) and the acidity of 4.9. According to phosphorus and potassium content (2.8 and 18.4 mg 100g^{-1}, respectively) the soil belongs to a medium type.

Table 1. Soil properties

Depth	pH in KCl	Humus %	P_2O_5	K_2O
0-30 cm	4,9	5,37	2,8	18,4

Weather conditions

Sum of precipitation (particularly for growing season) and temperatures affected the number of cuttings and annual DM yield. Observation of important meteorological elements was done only for (2005 - 2008) (Table 2). The mean monthly and annual temperatures were rather low. The mean annual temperature was 10.8°C in 2005, 11.6°C in 2006, 12.8°C in 2007 and 12.9°C in 2008. Late spring frosts occur even in April (or even later), while early frosts start in October.

The amount of precipitation (Table 2) was relatively high. Although the amount of precipitation has a high effect on the productivity of meadows and pastures, especially during growing season. The mean annual precipitation was 843.9 mm in 2005, 821.7 mm in 2006, 843.9 mm in 2007 and 656.1 mm in 2008.

Table 2. Mean monthly temperatures (^{0}C) and monthly precipitation (mm) during 2005-08.

Valjevo	Years	Months												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII
Temperature	2005	0.8	-2.0	4.5	11.4	16.6	19.3	21.6	19.8	17.2	11.3	5.1	2.8	10.8
	2006	-1.8	1.4	5.6	12.6	16.2	19.7	23.2	20.0	17.8	13.2	7.2	3.1	11.6
	2007	6.1	6.4	9.2	13.0	18.0	22.3	24.4	22.9	15.1	10.7	4.0	0.5	12.8
	2008	2.4	4.8	7.7	12.8	18.0	21.8	22.4	22.5	15.7	13.1	8.3	4.6	12.9
Precipitation	2005	34.6	75.3	47.1	56.9	70.9	72.7	122.1	128.9	81.9	27.0	68.5	58.0	843.9
	2006	41.8	51.0	121.6	77.9	46.4	104.5	35.6	183.4	17.7	27.5	30.6	83.7	821.7
	2007	53.4	40.6	77.6	7.2	125.0	72.7	22.5	65.4	100.4	119.7	107.5	51.3	843.3
	2008	36.0	28.2	96.5	46.0	72.9	77.8	72.6	21.9	68.9	22.4	59.7	53.2	656.1

Results and Discussion

Forage yield of the natural meadow dominated by *Agrostidetum vulgaris* was affected by the mineral fertilizer rates (Table 3). DM yield varied depending on the year of study, i.e., on precipitation sum and schedule of individual years. The obtained data indicate that mineral fertilizer had a favourable effect on DM yield (Table 3). Maximum four-year average DM yield of 8.12 t ha^{-1} was achieved with the highest NPK rate ($N_{200}P_{150}K_{150}$ kg ha^{-1}), which was an increase of 7.1 t ha^{-1} or 464% compared with the control. The yields of the ($N_{150}P_{100}K_{100}$) treatment (on average 6.80 t ha^{-1}) illustrate a high production on the site. On the ($N_{100}P_{100}K_{100}$), ($N_{100}P_{50}K_{50}$), ($N_{50}P_{50}K_{50}$) and control ($N_0P_0K_0$) plots high average yields were obtained – 5.80 t ha^{-1}, 5.80 t ha^{-1}, 4.37 t ha^{-1}, and 1.96 t ha^{-1}, respectively. Although the total average DM yield was lower in the year with the lower amount of precipitation (growing season). The obtained results showed also that mineral fertilization not only provided higher yields but also stabilized fodder production, since better nourished grasses used water more economically.

Tabela Biomass and dry matter yield of the meadow type *Agrostidetum vulgaris* as affected by different rates of fertilizer during 2005-2008

	Dry matter (t ha^{-1})					
Treatments	2005	2006	2007	2008	Average	DM/kg
$N_0P_0K_0$	2.32	2.15	1.47	1.89	1.96	-
$N_{50}P_{50}K_{50}$	5.20	4.52	3.20	4.59	4.37	16.2
$N_{100}P_{50}K_{50}$	6.22	5.80	4.34	6.62	5.80	19.0
$N_{100}P_{100}K_{100}$	6.25	5.86	4.70	6.39	5.80	12.8
$N_{150}P_{100}K_{100}$	7.01	7.90	5.73	6.62	6.80	13.9
$N_{200}P_{150}K_{150}$	8.27	8.11	7.11	9.03	8.12	12.4
Average	5.87	5.72	4.45	5.86		
LSD 0,05	0.75	1.37	0.83	1.95		

Optimum application rates of mineral fertilizer were changeable. Mineral fertilizer use efficiency decreased from 16.2 to 12.4 kg DM per kg NPK with increasing NPK application levels of $N_0P_0K_0$ and $N_{200}P_{150}K_{150}$ kg per ha^{-1}.

Conclusion

Increases in mineral fertilizer rate had a favourable effect on DM yield. The maximum four-year average DM yield of 8.12 t ha^{-1} was achieved with the highest mineral fertilizer rate ($N_{200}P_{150}K_{150}$ kg ha^{-1}), which was an increase of 7.1 t ha^{-1} or 464% compared with the control.

The maximum fertilizer rate ($N_{200}P_{150}K_{150}$ kg ha^{-1}) produced only 12.4 kg of hay per kg of NPK, while in tests with lower dose ($N_{50}P_{50}K_{50}$ kg ha^{-1}) this ratio was higher (16.2 kg).

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Uticaj primene mineralnih đubriva na produktivnost livade tipa *Agrostidetum vulgaris* u brdskom području Srbije

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Rezime

Ogled je izveden tokom četiri godine (2005-2008) na livadi tipa *Agrostidetum vulgaris* u zapadnoj Srbiji (okolina Valjeva, nadmorska visina 750 mnv). Istraživanje je uključilo 6 doza NPK đubriva - $N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ i $N_{200}P_{150}K_{150}$ kg ha^{-1} po godini. Povećanje količine N, P i K đubriva je vodilo u odgovarajuće povećanje prinosa suve materije krme, posebno je povećan prinos SM sa većim dozama N. Maksimalni prosečan prinos suve mase od 9,03 t ha^{-1} je ostvaren u četvrtoj godini ispitivanja sa najvećom dozom NPK ($N_{200}P_{150}K_{150}$ kg ha^{-1} po godini). Povećanje je bilo 7,1 t ha^{-1} ili skoro 464% u poređenju sa kontrolom. Maksimalan prinos je postignut sa najviše primenjenom količinom hraniva ($N_{200}P_{150}K_{150}$ kg ha^{-1}), što je samo 12.4 kg SM po kg NPK hraniva, dok su niže doze hraniva ($N_{50}P_{50}K_{50}$ kg ha^{-1}) dale veću količinu SM po jedinici hraniva (16.2 kg).

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CHANGES IN FORAGE QUALITY DEPENDING ON CLIMATE CONDITIONS AND GRASSLAND MANAGEMENT

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Original scientific paper

Abstract: Forage quality is the key element of sustainable animal production. Forage of high quality is usually determined by a high content of protein and low content of fibre. The concentration of these quality elements in grassland forage depend on botanical composition, as well as on soil and climate conditions and grassland management. The content of crude protein and crude fibre were studied on grassland forage (at two stages of plant growth, three levels of N application, with and without under-sowing, within two years). The results of these studies indicate that plant maturity and climatic conditions have the biggest effect on crude protein and crude fibre content. Crude protein content ranged from 82.5 to 177.6 g kg^{-1} at the heading stage of the plants growth, and 54.0 to 81.0 g kg^{-1}. Crude fibre content ranged from 213.2 to 302.7 g kg^{-1} at the heading stage of plant growth and 275.9 to 343.8 g kg^{-1}.

Key words: grassland, forage quality, crude protein, crude fibre

Introduction

Forage quality is the key element of sustainable animal production. Forage of high quality is usually determined by a high content of protein and low content of fibre. The concentration of these quality elements in grassland forage depend on botanical composition, soil and climate conditions and grassland management *(Alibegovic-Grbić et al., 2004)*. In general, legumes contain more protein and grasses more fibre. Leaf is richer in protein than stem and that is the reason why plants in earlier stage of growth contain more protein than in the latter. But plants of the same stage of growth can vary in protein content depending on daily temperature and availability of water and soil nutrients *(Popovic, 1987)*.

Since large grassland areas of Bosnia and Herzegovina deliver more than 80 % of roughage forage, its quality plays a very important role in ruminants feeding. Common grassland management practice in Bosnia mean no or very low amount of fertilization, late cutting or permanent grazing and haymaking on the ground *(Alibegovic-Grbić et al., 2005)*. The consequence of the mentioned management practises is roughage forage of low quality.

The aim of this study was to determine the content of crude protein and crude fibre in the forage depending on botanical composition, stage of plant growth, N fertilization and weather conditions

Materials and Methods

The experiment was carried out during 2002-2003 on a two-year old grass sward, which followed barely for seed. We observed the influence of three levels of nitrogen fertilization, under-sowing and harvesting at two stages of plants growth on forage yield, forage quality, and botanical composition of the sward. The trial was located in Gracanica's hilly region, at Vina (300 m a.s.l), with an average annual precipitation of 870 mm and average annual temperature of 10,6 °C. The soil characteristics were as follows: pH-6,1 (in KCl), P-6,4 and K-13,2 mg and N-260 mg in 100 g of soil.

The field experiment design was with random-blocks of four replications per block. Plot size was 6 m^2. Under-sowing (B) was by *Lotus corniculatus* and *Dactylis glomerata* in the spring 2002. Nitrogen fertilizer was applied early in the spring, as follows: 30 (N30), 60 (N60), 80 (N80) N kg ha^{-1} and zero N (N0, control). The swards were harvested in two stages of plant growth: heading and flowering stage.

Total N was determined by Kjeldahl and crude fibre by Weende method. Protein content was calculated (N x 6,25).

Results and Discussion

Usually between 80 and 90% of protein in forage is present in the cell contents *(Tamminga and Suedekum, 2000)*. As the cell contents prevail in leaves and leaves participate with bigger portion in earlier stages of plant's growth that indicate correlation between plant maturity and content of protein. Stage of plant growth had the biggest effect on the protein content in dry matter (Table 1). Average protein content at the heading stage was 159.6 and 100.3 g kg^{-1} DM, ranging from 149.5 to 176.6 in 2002 and from 82.5 to 130.9 g kg^{-1} DM in 2003 year, regardless of the treatment. With increasing maturity to flowering stage of plant growth, protein content decreased markedly, to 69.0 and 70.0 g kg^{-1} DM, ranged from 61.3 to 79.2 in 2002 and 54.0 to 81.0 g kg^{-1} DM in 2003.

The second thing that influenced protein content was weather conditions. Late winter and early spring of 2003 were characterised by low rainfall and rather low temperature influencing slow growth and low uptake of N form the soil. The result was 36,9% less protein (on an average) in the heading stage, but 3,14 % more protein in the flowering stage in 2003 in comparison to 2002.

Table 1. Content of crude protein in DM (g kg^{-1})

Treatment	Heading		Flowering	
	2002	2003	2002	2003
N0	150.3	82.5	61.3	77.0
N30	176.6	101.0	61.7	66.0
N60	156.1	101.0	63.9	81.0
N80	165.7	104.0	67.0	76.0
N0B	154.3	83.0	76.6	57.0
N30B	153.0	100.3	79.1	54.0
N60B	171.7	100.3	63.3	68.0
N80B	149.5	130.9	79.2	81.0
Average	159.6	100.3	69.0	70.0

N application had positive effect on protein content in general and in average (166.1; 102.0; 64.2 and 74.3 in both stage of plant growth and years compared with 150.3; 82.5; 61.3 and 77.0 g kg^{-1} DM, respectively). No clear differences were found between protein contents and rates of N application. Under-sowing did not show any effect on protein content in the forage. This could be explained by slow development of *Lotus corniculatus* and *Dactylis golomerata*, as under-sowed species.

Crude fibre contrary to protein is connected to the stem and cell walls. With increasing plant maturity, portion of stems increases as well and cell walls become more lignified. Concerning these, it is understandable why there is far less crude fibre in the heading than in the flowering stage, 288.7 and 234.8 compared with 324.0 and 301.2 g kg^{-1} DM, (Table 2). The content of crude fibre ranged from 257.2 to 302.7 in 2002 and from 213.2 to 268.2 g kg^{-1} DM in 2003, at the heading stage but from 307.9 to 343.8 and from 275.9 to 337.2 g kg^{-1} DM, respectively, at the flowering stage of plant growth.

Table 2 Content of crude fibre in DM (g kg^{-1})

Treatment	Heading		Flowering	
	2002	2003	2002	2003
N0	289.3	215.0	317.6	291.6
N30	294.9	224.6	324.7	300.3
N60	302.7	268.2	343.8	297.8
N80	299.0	245.1	307.9	299.3
N0B	282.8	247.5	311.8	337.2
N30B	298.3	236.6	318.5	307.2
N60B	286.0	228.5	329.7	303.0
N80B	257.2	213.2	338.6	275.9
Average	288.7	234.8	324.0	301.5

Rather fast plants development in late spring of 2003, due to higher temperatures, resulted in less fibre content in both stages of plant growth (288,7 in 2002 compared to 234.8 at the heading and 324.0 in 2002 compared to 301.5 g kg^{-1} DM in 2003 at the flowering stage). This indicates that weather conditions during the vegetation season also play an important role in influencing forage quality.

N application increased fibre content in general and in average (298.8; 245.9; 325.4 and 299.1 in both stage of plant's growth and years compare to N0 (289.3; 215.0; 317.6 and 291.6 g kg^{-1} DM, respectively).

Conclusion

Crude protein content was positively affected by N application and especially by cutting in earlier stage of plant growth. Weather condition was also very important factor in forage quality, even more when grassland is not fertilized. Forage quality in Bosnia and Herzegovina could be improved by changing daily practice and cutting grasslands during the heading stage of plant growth instead during the flowering stage (or even later).

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Uticaj vremenskih prilika i vremena kosidbe na kvalitet krme sa prirodnih travnjaka

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Rezime

Kvalitet kabaste krme je ključni element održive stočarske proizvodnje. Krma visokog kvaliteta uobičajeno podrazumeva visok sadržaj proteina i nizak sadržaj vlakana. Sadržaj ova dva najvažnija pokazatelja kvaliteta krme sa prirodnih travnjaka zavisna je od botaničkog sastava, vremenskih prilika i naročito od stadijuma razvoja biljaka pri kosidbi. Sadržaj sirovih proteina i sirovih vlakana je proučavan u suvoj masi krme sa travnjaka (dve faze razvoja biljaka, tri nivoa đubrenja azotom, sa i bez podsejavanja) tokom dve godine. Rezultati predstavljenih istaraživanja ukazuju da starost biljaka i vremenske prilike imaju najveći efekat na sadržaj proteina i sadržaj sirovih vlakana. Sadržaj sirovog proteina je bio od 82,5

do 177,6 g kg^{-1} pri kosidbi u fazi klasanja trava, te od 54,0 do 81,0 g kg^{-1} suve mase. Sadržaj sirovih vlakana varirao je od 213,2 do 302,7 g kg^{-1} pri kosidbi u fazi klasanja glavnih trava, te od 275,9 do 343,8 g kg^{-1} suve mase.

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THE EFFECT OF DIFFERENT FERTILIZER TREATMENTS ON NATURAL GRASSLAND YIELD

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Abstract: The study was conducted during two-year period (2007-2008) on natural grassland in Barlovci locality Banjaluka. The influence of mineral fertilizers on green biomass, hay yield and effect of applied fertilizers was studied. The following treatments were applied: 1) Control treatment (no fertilizers), 2) 500 kg ha^{-1} NPK (8:26:26), 3) 500 kg ha^{-1} NPK and 87 kg ha^{-1} UREA, 4) 500 kg ha^{-1} NPK and two side-dressings with 87 kg ha^{-1} UREA, 5) 500 kg ha^{-1} NPK and two side-dressings with 130 kg ha^{-1} UREA, 6) 500 kg ha^{-1} NPK and two side-dressings with 222 kg ha^{-1} KAN. Total amount of complex NPK fertilizers was applied early in the spring, while nitrogen fertilizer was applied after each cutting. The highest biomass yield was achieved in the first experimental year (2007) with application of 500 kg ha^{-1} NPK and two side-dressings with 130 kg ha^{-1} UREA (treatment 5) and reached 21.64 t ha^{-1} green biomass. There was increased yield compared to the control (treatment 5), 31 t ha^{-1} hay or 67.91%. The highest hay yield in both years (2007-2008) was achieved by application of 500 kg ha^{-1} NPK and two side-dressings with 87 kg ha^{-1} UREA (treatment 4) – 7.82 t ha^{-1} hay. There was increase compared to control treatment 17.01 t ha^{-1} or 78.6%. The best effect with N fertilizers was obtained by application of 80 kg ha^{-1} N, reaching the highest hay yield per 1 kg of applied N (32.13 kg).

Key words: mineral fertilizers, yield, natural grassland.

Introduction

The aim of these trials was to determine the impact of applying different types and doses of mineral fertilizers on the productivity of natural grasslands. Grasslands in Bosnia and Herzegovina occupy more than half of total agricultural land (meadows and pastures 1.346 390 ha), and represent an important source of bulky feed. Extensive way grassland utilization was accompanied by low-yield low-quality forage. In order to improve livestock, by increasing the yield and quality of forage, it is necessary to apply the existing grasslands melioration measures. The biggest impact on increasing the yield of bulky feed on grass had cultural measure fertilization. The results of numerous studies show that nitrogen mineral fertilizers (UREA and KAN) and complex NPK mineral fertilizers have a large impact on yield, quality and floristic composition of natural grasslands *Vojin et al. (2003), Ivanovski et al. (2004), Vučković et al. (2004), Dubljević (2007).*

Materials and Methods

Two-factorial trial was set up on natural grassland, in a randomized block system in 4 repetitions. The area of the basic plot was 25 m^2. Spacing between the plots and blocks was 1 m. research encompassed the following treatments of fertilizing : 1) control (non-fertilized), 2) 500 kg/ha NPK 8:26:26, 3) 500 kg/ha NPK 8:26: 26 and 87 kg/ha UREA (46% N), 4) 500 kg/ha NPK 8:26:26, and two side-dressings with 87 kg/ha UREA (46% N), 5) 500 kg/ha NPK 8:26 : 26 and two side-dressings with 130 kg/ha UREA (46% N) and 6) 500 kg/ha NPK 8:26:26, and two side-dressings with the 222 kg/ha of KAN (27% N). The total amount of complex NPK fertilizers was applied in early spring. First side-dressing with nitrogen fertilizers was carried out after the movement of vegetation in I cutting, while the other side-dressing was made after mowing of I cutting. In both years of research two fodder cuttings were achieved. Green fodder yield was determined by measuring the total amount cutting from the main plot mass, and the obtained results are processed and presented by yields height per unit of area. During mowing, samples of 1 kg green mass were taken and dried naturally in order to determine the yield of hay. The effects the amount of applied fertilizer, clean feed, as well as the effect of pure nitrogen in forage yield with natural grasslands were determined by calculation. The results of the research were analyzed using the variances analysis, and significance of differences in yield was determined using LSD test.

Results and Discussion

By testing the effects of applying different treatments of mineral fertilizers on the yield of natural grasslands, the following were analyzed: the yield of green forage and hay, the effect of fertilization on the yield of hay per 1kg of used fertilizer and nutrients and the effect of 1 kg of nitrogen on the hay yield.

Green forage yield. The results of yield by treatments of green forage fertilization, cuttings and years of research are presented in Table 2. The highest yield of green forage in the first year of utilization was achieved by applying treatment 5 (21, 64 t/ha). The attained yields of green forage showed statistically significant difference compared to the control, increased by 17.01 t/ha, or 78.6%.

In the second year of testing (2008) the highest yield of green fodder was achieved using treatments 6, compared to the control treatment achieved a highly statistically significant increase in green fodder yield of 16.29 t/ha or 68.7%. In the first year of testing, the green fodder portion (percentage) of the first cutting in the overall yield was 85.3%, while in the second year was lower by 4.1% (80.4%).

During two years of study the highest green fodder yield was achieved with the treatment 5, where the yield from 22,46 t/ha green fodder used 500 kg/ha NPK in early spring, and two side-dressing of the 130 kg/ha UREA. These achieved yields of green fodder show that proper application of fertilizer in dry years can significantly reduce the negative impact of drought and stabilize the production of forage in grasslands.

Table 1. The influence of different fertilizing treatments on green fodder yield per cut (tha^{-1})

Mark	Fertilizer treatment $N/P_2O_5/K_2O$ (A)	2007./Year(B_1)			2008./Year(B_2)			Average (A)
		Cut (C)			*Cut (C)*			
		C_1	C_2	Σ	C_1	C_2	Σ	
1	Control	3.40	1.23	4.63	6.00	1.40	7.40	6.02
2	40/130/130	9.37	1.50	10.87	14.12	1.84	15.96	13.42
3	80/130/130	13.19	1.50	14.69	18.43	2.05	20.48	17.59
4	120/130/130	14.53	4.31	18.84	17.79	3.44	21.23	20.04
5	160/130/130	16.09	5.55	21.64	19.81	3.46	23.27	22.46
6	160/130/130	16.60	3.79	20.39	19.39	4.30	23.69	22.04
Average(B)		12.20	2.98	15.18	15.92	2.75	18.67	16.92
Cutting percentage (%)		80.4	19.6	100	85.3	14.7	100	-
LSD				A			B	AB
	0,05			2.90			1.67	4.10
	0,01			3.87			2.24	5.84

Hay yield - During the first year of testing an average yield for all tested treatments of fertilization of 5.80 tha^{-1} was achieved (Table 3). The highest yield of hay in this year was achieved by applying treatment 4, and it was 7.89 tha^{-1} of hay. Compared to the control (non-fertilized treatment) showed a highly statistically significant difference in the yield of 5.8 t/ha or 73.52%.

U 2008, an average yield of hay from 6.35 tha^{-1} was achieved, as compared to the first year it was increase of 0.55 tha^{-1} Increase of the average yield of hay in the second year was mostly achieved due to the higher volume and better distribution of precipitation in that year. The Highest yield of hay in 2008 was achieved using treatment 4 (7.74 tha^{-1}).

Table 2. The influence of different fertilizing treatments on hay yield per cut (t ha^{-1})

Mark	Fertilizer treatment $N/P_2O_5/K_2O$ (A)	2007./Year (B_1)			2008./Year (B_2)			*Average (A)*
		Cut (C)			Cut (C)			
		C_1	C_2	∑	C_1	C_2	∑	
1	Control	1.62	0.47	2.09	2.25	0.67	2.92	2.51
2	40/130/130	4.01	0.58	4.59	4.36	1.54	5.90	5.25
3	80/130/130	4.80	0.60	5.40	5.65	0.96	6.61	6.01
4	120/130/130	6.57	1.32	7.89	6.46	1.28	7.74	7.82
5	160/130/130	5.70	1.67	7.37	6.34	1.29	7.63	7.50
6	160/130/130	6.21	1.22	7.43	5.70	1.59	7.29	7.36
Average (B)		4.82	0.98	5.80	5.13	1.22	6.35	6.07
Cutting percentage (%)		83.1	16.9	100	80.8	19.2	100	-
LSD				A			B	AB
	0,05			1.01			0.58	1.43
	0,01			1.35			0.78	1.91

First cutting percentage in the total yield of hay in 2007 amounted to 83.1% and it was by 2.3% higher than the percentage of first cutting in the second year of study (80.8%). The highest average yield of hay, in both studies was achieved by using treatment 4 (7.82 t/ha).

Fertilizing effect on the hay yield with 1kg of used fertilizers and nutrients- Applied fertilizing treatments had different effects on the yield of hay per 1kg given fertilizer, and nutrients (Table 5). The best effect of the amount of fertilizer applied and pure nutrients was achieved with a treatment 4 (7.88 kg 13.97 kg of hay and straw). Analyzing the results by years, it was observed that the fertilizing was higher in the first year of research, and that it decreased in 2008.

Table 3. Increasing of hay yield per 1 kg of used fertilizer and nutrient

Mark	Fertilizer treatment $N/P_2O_5/K_2O$	Year				Average	
		2007		2008			
		1kg fertilizer effect	1kg nutrient effect	1kg fertilizer effect	1kg nutrient effect	1kg Fertilizer effect	1kg nutrient effect
1	0	-	-	-	-	-	-
2	40/130/130	5,00	8,33	5,96	9,93	5,48	9,13
3	80/130/130	5,64	10,34	6,29	11,53	5,97	10,94
4	120/130/130	8,61	15,26	7,15	12,68	7,88	13,97
5	160/130/130	6,95	12,57	6,20	11,21	6,58	11,89
6	160/130/130	5,66	12,71	4,63	10,40	5,15	11,56
Average		6,37	11,85	6,05	11,15	6,21	11,50

The effect of 1 kg of nitrogen on hay yield. Hay yield results generated per kg of nitrogen used by years of research were presented in Table 6. By observing the effect pure nitrogen application by years of research, it was noticed that its influence on the yield of hay was significantly more pronounced in the first year. The average yield for all tested treatments achieved in 2007(27.08 t/ha) was higher compared to 2008 by 7.39 kg per kilogram of used pure nitrogen, or 44.10%.

Table 4. The effect of 1kg of nitrogen on hay yield (kg)

Mark	Fertilizer treatment $N/P_2O_5/K_2O$	Year		Average
		2007.	2008.	
1	0	-	-	-
2	40/130/130	-	-	-
3	80/130/130 (40 kg/ha N)	20.25	17.75	19.00
4	120/130/130 (80 kg/ha N)	41.25	23.00	32.13
5	160/130/130 (120 kg/ha N)	23.17	14.42	18.80
6	160/130/130 (120 kg/ha N)	23.67	11.58	17.63
Average		27.08	16.69	21.89

In both years of research, the best effect of nitrogen fertilization was achieved with the treatment 4 in which 80 $kgha^{-1}$ of pure nitrogen were used. With this of nitrogen fertilizer treatment 41.24 kg of hay yield per kg of nitrogen used was achieved in the first year yield, and 23.0 kg in 2008. By analyzing the effects of nitrogen by years of testing, we can see that in 2007 the effect of 1 kg of nitrogen on hay yield was higher compared to 2008. The highest effect of pure nitrogen application was achieved with the treatment 4, with the average hay yield in both years of 32.13 kg. *Vučković et al. (2004)* reported the maximum yield of hay with application of 160 $kgha^{-1}$ of nitrogen, which is 5.30 t/ha of hay the first year, comparing to the control means an increase by 1.76 t/ha or 50%. In the second year, the yield of hay with a maximum treatment of nitrogen was 4.55 $kgha^{-1}$, as compared to the control there was an increase of 2.75 t/ha or 153%. Lower dose of nitrogen (40 kg/ha) gave significantly lower yields of hay (4.09 t/ha), but provided significantly more hay (35.5 kg) per 1 kg of used nitrogen nutrients.

According to *Dubljević (2007)* maximum average hay yields (5.3 $kgha^{-1}$) were achieved by fertilization with 120 kg/ha, but the best effect of applying nutrients to increase yields was in the treatments with 90 $kgha^{-1}$.

Conclusion

Based on the results of testing the impacts of different fertilizer treatments on grassland productivity, the following can be concluded:

In all fertilizer treatments and trials years, higher average fodder yields were determined (green fodder and hay) in comparison to the control. Green fodder yield increased with increasing of applied fertilizer doses, from 6.02 kgha^{-1} in non-fertilized treatments, to 22.46 kgha^{-1} of green fodder in treatment 5. The highest yield of hay was achieved using treatment 4, from 7.82 kgha^{-1}, as compared to control (non-fertilized) there was increase of 5.31 kgha^{-1} or 67.91%. The best effect of fertilization on the hay yield per kg of used fertilizers and nutrients was achieved by applying treatment 4, while the highest effect of pure nitrogen fertilizers on the hay yield attained with 80 kgha^{-1} of nitrogen, or 1 kg of nitrogen gave 32.13 kg of hay in two-year average.

Efekat primene različitih varijanti mineralnih đubriva na prinos prirodnih travnjaka

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Rezime

Ispitivanje je obavljeno na prirodnom travnjaku, lokalitet Barlovci kod Banja Luke, u dvogodišnjem periodu (2007.-2008. g.). Proučavan je uticaj mineralnih đubriva na prinos zelene mase, sena i efekat primenjenih đubriva. Primenjene su sledeće varijante: 1) kontrola (bez đubrenja), 2) 500 kg ha^{-1} NPK (8:26:26), 3) 500 kg ha^{-1} NPK i 87 kg ha^{-1} UREE, 4) 500 kg ha^{-1} NPK i dve prihrane sa po 87 kg ha^{-1} UREE, 5) 500 kg ha^{-1} NPK i dve prihrane sa po 130 kg ha^{-1} UREE i 6) 500 kg ha^{-1} NPK i dve prihrane sa KAN-om 222 kg ha^{-1}. Ukupna količina kompleksnih NPK đubriva primenjena je rano u proljeće, dok su azotna đubriva po metodici primenjena posle skidanja otkosa. Maksimalan prinos zelene krme u prvoj godini (2007) ostvaren je primenom varijante 5), iznosi 21,64 t ha^{-1} zelene krme, što je u odnosu na kontrolu povećanje od 17,01 t ha^{-1} ili 78,6%. Najveći prinos sena u obe godine (2007-2008) postignut je primenom varijante 4), iznosi 7,82 t ha^{-1} sena, što je u odnosu na kontrolu povećanje od 5,31 t ha^{-1} sena ili 67,91%. Najbolji efekat đubrenja azotom postignut je primjenom 80 kg ha^{-1} azotnog hraniva, gdje je dobijeno prosečno najviše sena po 1kg hraniva (32,13 kg).

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SOLUTIONS OF PROBLEMS CAUSED BY ARID CLIMATE WITH HELP OF THE SOIL CONDITIONERS AND SPECIAL PLANT MIXTURES

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Original scientific paper

Abstract: Within the project „A pilot project of prevention of soil biological degradation under conditions of arid climate“ three types of plant mixtures were proposed and created: regional, landscape and annual. The mixtures were sown on experimental field near Hodonín. At the same time, the auxiliary soil substances were added to improve biological, chemical and physical properties of soil. In the years 2008 and 2009 biomass samples from an area of $0.05m^2$ were harvested. The harvesting was repeated three times per year. The evaluation of botanical groups in samples (clovers, grasses, herbs) was performed. By the analysis of variance a statistically significant differences were found between the tested variants in the yields of green biomass and hay only for the regional mixture in the first harvest year. Yields of the control variant and a hydro absorbent variant were statistically significantly higher than zeolite and lignite variants. Species composition of vegetation was affected by high presence of weeds. In regional and landscape mixture clovers prevailed. On the other hand in the annual mixture grasses predominated. It is necessary to claim that these results are only from the sowing year and the first harvest year. Therefore we cannot consider these results as definitive.

Key words: Mixtures, green matter yield, hay yield, botanical composition, soil conditioners.

Introduction

The pilot project on the area with critical lack of water in Ratíškovice near Hodonín started in 2008. The lack of water is a limiting stress factor for plants. Water in ecosystems has a very fast cycle and its content in soil is sufficient only for a relatively short period in comparison to nutrients *(Procházka, 1998)*. The level of plant resistance to water stress varies during the growing season. Plants suffer from water stress especially in the spring and summer period. The consequence of water stress is a reduced speed of photosynthesis, respiration and uptake of CO_2 *(Kincl and Krpeš, 2000)*. Due to increasing aridity of the landscape (the impact of climate changes) solutions must be sought to help alleviate this situation. The European Economic Association indicates that climate changes have already a substantial impact on the economy, quality of life and ecosystems (http://www.eea.europa.eu/themes/climate). Spontaneous succession could be considered the most natural way of recovery. This process is long-term and depends on surrounding resources of the diaspores. One of the possibilities how to eliminate the lack of water in soil is application of soils conditioners. Raw natural lignite is due to its sorption capacities *(Klučáková and Omelka, 2004)* and high humus content a suitable material for improving soil properties. It supplies the soil with organic matter, regulates release of nutrient elements, immobilizes toxic elements, regulates microbiological climate of soil and improves water retention *(Kučerík et al., 2003)*. Zeolite is a highly porous mineral of volcanic origin with ability to absorb large quantities of water and ions (ionic exchange) and gradually release them into soil. Agrisorb (hydro absorbent) is an organic polymer compound able to bind water into its structure and pass it to the roots. Creation or improvements of crumb structures in soil are the main effects of Agrisorb application *(Hrabě et al., 2003)*.

Materials and Methods

On the experimental area in Ratíškovice near Hodonín (Figure 2) three types of soil conditioners were applied in spring 2008: agrisorb (with the active substance hydro absorbent), lignite and zeolite. Three kinds of legume-grass mixtures were proposed and sowed (Table 1): a) Landscape mixture - with the addition of clovers (sowing rate 200 kg ha^{-1}), b) Regional mixture (sowing rate 100 kg ha^{-1}) c) Annual mixture (sowing rate 70 kg ha^{-1}). The scheme of experimental areas is presented at Figure 1. The composition of sown mixtures is presented Table 1. The first cut was performed in a delayed term to enable spreading of the main species of natural seeds. In the period before the first cut the biomass samples were taken from the area of 0.05 m^2 in three repetitions from each parcel (variants). The samples were divided into four groups (grasses, legumes, herbs and non sown species - weeds) and weighed in the laboratory. The samples were dried and weighed again to determine the hay yields. The results were processed by conventional methods of analysis of variance. The experimental area was mulched, because of its large size. The nomenclature of plant names in the paper was unified according to *Kubát et al. (2002).*

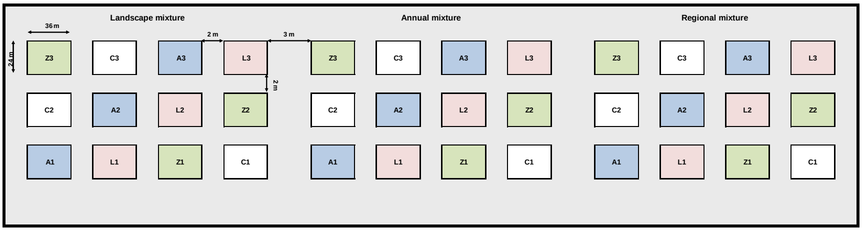

Figure 1. The scheme of experimental plots

Results and Discussion

The production, species composition and forage quality is changing during the years. It is known, that the vegetation succession during the first six years goes through considerable changes of species composition *(Hrabě et al., 2004).* The hairy crabgrass (*Digitaria sanguinalis*) predominated in the first year of the experiment, while in the second year it was the Canadian horseweed (*Conyza canadensis*). Both these species grow on undeveloped soils, anthropically disturbed *(Slavík et al., 2004).* On the experimental area both species grew from the soil seed bank. The representation of grasses, herbs, clovers and weeds (i. e. non sown species) in mixtures in the first two years of the experiment is presented in percentage in Table 2. In the regional and the landscape mixtures in the first year no sown species were found in the term of withdrawing the samples. This fact was caused by the delayed term of the experiment establishment, followed by a considerable drought. In the annual mixture representatives of all the groups (grasses, legumes, herbs and weeds) were found. This diversity was caused by a specific composition of the mixture. The mixture contained grasses: broom millet (*Panicum miliaceum*), Italian ryegrass (*Lolium multiflorum*) and canary grass (*Phalaris canariensis*); legumes: white lupin (*Lupinus albus*), chickpea (*Cicer arietinum*); and herbs: safflower (*Carthamus tinctorius*) and purple tansy (*Phacelia tanacetifolia*). The situation in the second year was completely different. In the annual mixture Italian ryegrass (*Lolium multiflorum*) predominated in all the variants. This species germinated from the seeds that had spread in the year before. The legumes and herbs were not observed in this mixture in the second year, unfortunately they were not able to spread their seeds in the first year. In the regional and landscape mixtures the sown species appeared in the second year. The smallest proportion of grasses in these mixtures was found in the variant with hydro absorbent application and in the control variant. The ratio of legumes was minimal in the landscape mixture with lignite application, but in the regional mixture the proportional share of legumes was balanced in all variants. The legumes were not apparent even in the second year perhaps due to the small number of sown species of herbs in the landscape mixture, while in the regional mixture they covered approximately 24-31%. Biomass production is observed when the stand is evaluated. It is not designated as yield, because this project is not focused on the quantity of biomass production.

Table 1. The composition of mixtures

Annual		Landscape		Regional	
Species	%	Species	%	Species	%
Phalaris canariensis	15	*Festuca rubra – stolonate*	38.3	*Agrostis capilaris*	2
Lolium multiflorum var. westerwoldicum	19	*Festuca rubra – clumpy*	25.5	*Anthoxantum odoratum*	6.5
Panicum miliaceum	6	*Festuca ovina*	12.8	*Arrenatherum elatius*	5
Bromus sp.	10	*Poa pratensis*	7.7	*Cynodon dactylon*	6
		Agrostis tenuis	0.9	*Festuca ovina*	19.9
				Festuca rubra	6
				Festuca rupicola	4.8
				Festuca valesiaca	2.1
				Koeleria macrantha	9.4
				Phleum pholeoides	1.9
				Poa angustifolia	0.2
				Poa pratensis	6
Grasses	50		85.1		69.5
Medicago lupulina	23	*Trifolium repens*	3	*Achilea millefolium*	0.5
Cicer arietum	1	*Lotus corniculatus*	3	*Anthylis vulneraria*	4.7
Trifolium campestre	6	*Securigera varia*	3	*Artemisia vulgaris*	0.5
Melilotus albus	4	*Onobrychis varia*	3	*Astragalus cicer*	0.2
Lupinus albus	7	*Anthylis vulneraria*	3	*Astragalus lasiopetalus*	1.4
Phacelia tanacetifolia	1			*Securigera varia*	6
Carthamus tinctorius	8			*Dianthus carthusianorum*	0.1
				Hypericum perforatum	0.5
				Lathyrus silvestris	1.2
				Lotus corniculatus	0.5
				Lupinus polyphylus	0.4
				Medicago falcata	0
				Onobrychis vicifolia	7.6
				Plantago lanceolata	7.9
				Plantago media	0.1
				Silene vulgaris	0.2
				Trifolium alpestre	0.2
				Trifolium medium	0
				Trifolium repens	3.3
				Trifolium rubens	0.4
				Veronica teucrium	0.1
				Vicia pisiformis	0.7
				Vicia villosa	0.1
Legumes + herbs	50		14.9		30.4
Total	100		100		100

The production of green mass is presented in Table 3 and the production of hay is in Table 4. These values are in both tables converted in to percentage of control variant. With one exception the variants with the lowest and the highest production have the same order concerning the yields of green matter and the yields of hay. In the regional and in the annual mixtures the highest production was in the year 2008 in the variant with applied hydro absorbent. In the landscape mixture in 2008 the highest biomass production was in the variant without soil conditioners. In 2009 the variant with hydro absorbent surpassed the control variant in regional mixture as well as in the landscape mixture. And in the annual mixture no variant with applied conditioners surpassed the control variant.

Table 2. The composition of individual groups in the mixtures

Variant/2008	Grasses (%)	Legumes (%)	Herbs (%)	Weeds (%)	Variant/2009	Grasses (%)	Legume s (%)	Herbs (%)	Weeds (%)
Regional mixture					Regional mixture				
Control	0	0	0	100	Control	92	14.8	23.9	52.1
Hydro absorbent	0	0	0	100	Hydro absorbent	11.5	18.9	30	39.6
Zeolite	0	0	0	100	Zeolite	24.9	14.9	30.9	29.3
Lignite	0	0	0	100	Lignite	15.6	14.7	26.8	42.9
Landscape mixture					Landscape mixture				
Control	0	0	0	100	Control	8.5	17.4	0	74.1
Hydro absorbent	0	0	0	100	Hydro absorbent	0	18	0	82
Zeolite	0	0	0	100	Zeolite	38.5	17	0	44.5
Lignite	0	0	0	100	Lignite	19.9	8.2	0	71.9
Annual mixture					Annual mixture				
Control	30.7	0.9	4.8	63.5	Control	44.1	0	0	55.9
Hydro absorbent	36.0	3.5	0.0	60.5	Hydro absorbent	43.4	0	0	56.6
Zeolite	37.5	1.9	0.0	60.6	Zeolite	56.9	0	0	43.1
Lignite	21.3	0.2	0.3	78.2	Lignite	42.8	0	0	57.2

Differences between individual variants were tested by analysis of variance. Statistically high significant differences were found only in the regional mixture in 2009 in the production of fresh and dry biomass. The differences between other variants were not significant. Statistical contrasts among all the variants were analysed by Tukey´s test. Only in the regional mixture in 2009 there were found statistically significant and high significant differences among the tested variants. These results are shown in Table 5. Statistically high significant differences were found between the variants with lignite and hydro absorbent and zeolite and hydro absorbent in the production of green biomass of the regional mixture. Statistically high significant differences were found between the control and lignite variants and the control and zeolite variants in the production of hay of regional mixture. No statistical differences were found between the variants with lignite and zeolite, and the variants with hydro absorbent and control.

Table 3. Biomass production in years 2008 and 2009

Variant	Regional mix.				Landscape mix.				Annual mix			
	2008		2009		2008		2009		2008		2009	
	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	%of Con.	($t.ha^{-1}$)	% of Con.
Control	5.76	100.0	12.47	100	14.3	100.0	7.2	100.0	5.58	100.0	9.76	100.0
Hydroabsorbent	6.51	113.1	15.27	122.5	12.0	84.0	7.6	105.9	6.47	115.8	6.93	71.1
Zeolite	4.56	79.2	8.11	65.1	9.9	69.2	5.9	82.7	5.64	101.2	9.13	93.6
Lignite	3.00	52.1	8.44	67.7	12.8	89.7	7.0	97.2	4.60	82.5	7.61	72.9
D_T 0,05	4.07		3.92		9.87		4.11		3.75		6.64	
D_T 0,01	6.8		6.55		16.49		8.86		6.27		11.08	

Table 4. Hay production in years 2008 and 2009

Variant	Regional mix.				Landscape mix.				Annual mix			
	2008		2009		2008		2009		2008		2009	
	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.	$t.ha^{-1}$	% of Con.
Control	2.42	100	3.78	100	2.13	100	2.02	100	2.73	100	2.62	100
Hydro absorbent	2.36	97.2	3.47	91.8	3.69	173.3	1.64	81.3	2.73	100	2.16	82.2
Zeolite	1.89	78	1.93	51.2	3.44	161.8	1.16	57.1	2.53	92.7	2.89	110.2
Lignite	1.56	64.2	1.98	52.4	4.49	210.9	1.8	89	2.16	78.9	1.8	68.6
D_T 0,05	1.14		1.03		2.45		1.07		1.32		2.22	
D_T 0,01	1.91		1.73		4.09		1.79		2.2		3.79	

Table 5. The results of statistical analysis (only statistical differences); Tukey´s test

Production of biomass				Production of hay			
Regional 2009	L	Z	H	Regional 2009	L	Z	H
C	4.03*	4.36*	2.8	C	1.8**	1.85**	0.31
H	6.83**	7.16**		H	1.49*	1.54*	
Z	0.33			Z	0.05		

Conclusion

The results show that the soil conditioners had the greatest effect on the production in the group of grasses and weeds. For example, zeolite influenced a greater amount of grasses and decreased the weed occurrence. The annual mixture was not successful, because of a great ratio of weeds in the first year, exceeding always 60%. On the other hand, 40% dominance of annual species *Lolium multiflorum* among cultural grass species was a very positive surprise in the second year. These results are only from two years and it can be supposed that the changes will continue in the next years too.

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Rešenja problema uzrokovanih aridnom klimom uz pomoć dodataka zemljištu i specijalnih biljnih smeša

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Rezime

U okviru projekta „Pilot projekat za prevenciju biološke degradacije zemljišta pod uticajem aridne klime„ stvoreno je tri tipa biljnih smeša: regionalna, pejzažna i godišnja. Smeše su sejane u eksperimentalnim poljima blizu Hodonína. Istovremeno, pomoćne substance su dodavane u zemljište da bi se poboljšale biološke, hemijske i fizičke osobine zemljišta. U 2008. i 2009. godini, požnjeveni su uzorci biomase sa površine od 0.05 m^2. Izvršena je procena botaničkih grupa u uzorcima (deteline, trave, ostale biljke). Analizirajući varijanse, između testiranih varijanti nađene su statistički značajne razlike u prinosu zelene biomase i sena samo za regionalne smeše u prvoj godini žetve. Prinosi kontrolne varijante i vodoupijajuće varijante su bili statistički veći nego kod varijanti sa zeolitom i lignitom. Prisustvo korova je imalo uticaja na sastav vrsta vegetacije. Detelina je preovladavala u regionalnim i pejzažnim smešama. S druge strane, u godišnjim smešama, preovladavale su trave. Neophodno je naglasiti da su ovi rezultati iz godine setve i prve godine punog iskorišćavanja.

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FERTILIZING RESULTS OF HIGH MOUNTAIN GRASSLANDS *Poetum violaceae*

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Original scientific paper

Abstract: Testing of the impact of application of different doses of nitrogen on the production properties of natural grasslands *Poetum violaceae* has been performed on the south-western slopes of Durmitor (Pisce locality) in the period 2004 – 2005. The experiment was placed on the soil type rendzina, and applied the following variations: 0, 30, 60, 90 and 120 kgha^{-1}N. During the study of the influence of fertilization the following was recorded: the yield of hay and crude protein, changes floristic composition and effect (output) received doses of fertilizers. Highest average yield of 4.16 tha^{-1} was achieved in the variant with the highest and the lowest (2.44 tha^{-1}) in variant with the lowest dose of fertilizer, which compared to the control is an increase from 26.01 to 116.64%. Yield of crude protein increased with increasing doses of fertilizers and varied from 218.45 to 422.20 kgha^{-1}. Floristic composition of grasslands is characterized by a dominant share of grass and a small presentation of the legume (< 10%). At higher doses of fertilizers the participation of grass increased, while the share of legume was lower. Effect of fertilizer was higher in variants with medium doses (21 and 22 kg of hay). The results of these studies suggest the conclusion that application of medium doses of nitrogen has positive effect on production traits of high mountain grasslands *Poetum violaceae* community. Higher doses of fertilizers are less rational and more affected by adverse changes in the floristic composition.

Key words: grassland, fertilization, nitrogen, yield, hay, crude proteins, floristic composition

Introduction

Natural grasslands in Montenegro make approx. 88% of the total agricultural areas while in the hilly mountain areas their participation is even greater, about 92%. Forage from natural grasslands is for majority of farms basic, and often the only source of feed, which significantly affect the results in livestock production, which is the most important branch of agriculture in this area. Although they have great importance, grasslands are generally in poor condition, give low yields and poor quality forage. From the aspect of production of animal feed they have unfavourable floristic composition due to the very low participation of legumes, but high share of weeds and leafy weeds, and increasing presence of harmful and poisonous species. On high mountain grasslands the association of *Poetum violaceae* is mainly spread on deeper soils, which creates the oasis of better and more productive grasslands. By regular use which is not followed by appropriate application of fertilizer and other agro technical measures, the production features of grasslands, primarily the yield and nutritive value are in the constant decline. Rational fertilization increases the yield and quality of forage, but significant changes occur in botanical composition *(Mijatovic and Pavesic-Popovic, 1972; Dubljevic, 1988; Stosic and Lazarevic, 2007)*. Grassland fertilization with nitrogen is particularly important because its application increases the forage yield and crude protein, significantly changing floristic composition, highlights the growth and grass clustering, increase the density of grass cover, prolong the vegetation and slow aging of plants *(Mijatovic et al., 1970; Vuckovic et al., 2004; Alibegovic-Grbic et al., 2004; Ivanovski et al., 2004; Ćupina et al., 2007; Dubljević 2007)*. There is a real need to improve production properties of grassland in order to use rational, but more pronounced tendency is to preserve the environment to the maximum by repair measures. The aim of this study was to determine the influence of the implementation of lower and medium doses of nitrogen on production properties of high mountain natural grassland association of *Poetum violaceae*.

Materials and Methods

Research was conducted at the site Lokvice - Pisce, on the south-western slopes of Durmitor (1620m above sea level) in the period 2004 to 2005. The experiment was set up according to random block design in four repetitions, with the main parcel area of 10m^2. The testing included the following variants of fertilization: 0, 30, 60, 90 and 120kg ha^{-1} N. Fertilization in experiment was done by KAN (27% N), early in the spring, immediately after the melting of snow.

Immediately after cutting the yields of fresh forage was measured and samples collected for laboratory analysis (total nitrogen by Kjeldahl) and the calculation of hay yield and crude protein. The effect of fertilizers on the yield increase was obtained as the quotient of the yield difference between fertilized variations and control and the quantity of active ingredients of fertilizer.

Results of the research were analysis of variance and significant differences in yield were determined by LSD test.

Studies were conducted on soil type rendzina (in dolomite), which is sour (pH in H_2O 4.67-5.01), medium supplied with potassium (13.61-15.50 mg 100g^{-1}) and poor in phosphorus (2.65-5.33mg 100g^{-1} soil). Humus content was high (5.68-

6.30%), but of the sour reactions due to poor conditions for mineralization in the cold mountain climate.

Climatic conditions in this area are basically determined by geographical location, distance from the sea and expressed forms of relief. Annual average temperature is about 5.50 C, and in the period from May to September around 14°C. The average annual amount of rainfall is about 1450 mm, of which in average approx. 35% during the vegetation period.

Results and Discussion

The yield of hay and crude proteins. The yields of hay and crude protein were increasing with increase of fertilizer doses (Tables 1 and 2). The highest average yield of hay was from 4.16 t ha^{-1}, and 3.61 in the first and of 4.70 t ha^{-1} in the second year obtained in the variant with the highest dose of fertilizer, which is in comparison to the control average increase of 116.67%. Lowest dose of fertilizer gave the lowest yields, in average of 2.44 t ha^{-1} (2.08 and 2.80 t ha^{-1}), which was 27.08% higher than average yield on unfertilized plot. An average yield increase of fertilized variants compared to the control was 62.81%.

Table 1. The effect of nitrogen fertilization on the yield of hay

Nitrogen kg ha^{-1}	*Yield of hay* (t ha^{-1})				*Average*	*Relat. yield* (%)
	2004		2005			
	t ha^{-1}	%	t ha^{-1}	%		
0	1,66	100,00	2,17	100,00	1,92	100,00
30	2,08	125,30	2,80	129,03	2,44	127,08
60	2,93	176,51	3,49	160,83	3,21	167,19
90	3,38	202,61	4,42	204,69	3,90	203,12
120	3,61	217,47	4,70	216,58	4,16	216,67
Average	2,73	164,58	3,52	162,03	3,13	162,81

LSD $_{0,05}$	0,32	0,37
$_{0,01}$	0,51	0,59

Average yields of crude protein in fertilized variants varied from 208.45 in the lowest to 422.20 kgha^{-1} in the highest dose of fertilizer, which is in relation to unfertilized variant increase of 37.41% to 178.31. Content of crude protein in dry matter increased slightly with the increase of the dose of fertilizer, so their yield was influenced much greater by hay yields.

Table 2. Crude protein yield

Nitrogen kg ha^{-1}	*Crude protein yield* (kgha^{-1})				*Average*	Relat. yield (%)
	2004		2005			
	kg ha^{-1}	%	kg ha^{-1}	%		
0	132,24	100,00	171,16	100,00	151,70	100,00
30	187,12	141,50	229,78	134,24	208,45	137,41
60	234,30	177,18	298,74	174,53	266,52	175,69
90	316,44	239,29	387,86	226,61	352,15	232,14
120	388,52	291,80	455,88	265,35	422,20	278,31
Average	251,72	189,95	308,68	180,20	280,19	184,71

Floristic composition of grasslands

Influence of nitrogen fertilization on botanical composition changes of grassland of *Poetum violaceae*, expressed through the participation of grass, legumes and other leafy species in a total yield of green forage is shown in Table 3.

Table 3. Floristic composition of the grasslands type *Poetum violaceae*

Nitrogen kg ha^{-1}	% of green forrage yield								
	2004			2005			2004 / 2005		
	Poac.	*Fab.*	*Oth.*	*Poac.*	*Fab.*	*Oth.*	*Poac.*	*Fab.*	*Oth .*
0	62	6	32	65	5	30	63,5	5,5	31,0
30	66	6	28	67	4	29	66,5	5,0	28,5
60	65	4	31	62	3	35	63,5	3,5	33,0
90	68	4	28	70	4	26	69,0	4,0	27,0
120	71	4	25	71	4	25	71,0	4,0	25,0
Average	66.4	4.8	28.8	67.0	4.0	29.0	66.7	4.4	28.9

Average total participation of grass in the green forage yield on unfertilized plot was 63.5%, legumes 5.5% and leafy species of 31.0%. On fertilized variants participation of grass grew with increase of nitrogen doses, from 66.5 to 71.0%, participation of legume was from 3.5 to 5.0%, and leafy species 25.0 to 33.0%. Fertilization did not significantly affect the participation of legume, while the share of other species (leafy ones) in the yield decreased with increase of doses. Nitrogen fertilization did not affect significantly changes the composition of floristic composition of grassland because it was applied only one nutritive element and of a short period of its implementation (2 years).

The effect of fertilizers on the yield increase. The largest average effect of fertilizer on hay yield increase was of 22.06 kg in the variant with 90 kgha^{-1}N, while the lowest of 17.50 kg in variants with the lowest dose of fertilizer (Table 4). In the first year of study the effect of fertilizer was 17.65 kg, which is significantly less than in the second year (22.26 kg), which was significantly influenced by favourable climatic conditions, primarily the amount and distribution of rainfall during the vegetation period.

Table 4. The Effect of 1 kg of nitrogen on kg of hay yield

Nitrogen kg ha^{-1}	The Effect of 1kg of nitrogen on kg of hay yield		Average
	2004	2005	
0	-	-	-
30	14,05	20,94	17,50
60	21,17	22,00	21,59
90	19,11	25,05	22,06
120	16,25	21,08	18,67
Average	17.65	22.26	19.96

Influence of nitrogen fertilization on the natural grassland is very complex. Improving productivity of *Poetum violaceae* grassland by nitrogen fertilization is the result of increasing habitat of existing plants (especially grass), and much less the consequence of desirable changes of floristic composition. Results of these studies are similar to those obtained by *Dubljević (2003), Alibegovic-Grbic et al. (2004)* and *Vuckovic et al. (2004)*, applying similar doses of nitrogen on grasslands type *Nardetum strictae* and *Cynosuretum cristati* in the mountain area of Bosnia and Herzegovina and Serbia.

Conclusion

Fertilization with medium and low doses of nitrogen positively influenced the productive characteristics of mountain grasslands association of *Poetum violaceae*. Largest average yield of hay of 4.16 t ha^{-1} was achieved with the highest dose of fertilizer, which is compared to the control increase of 116.67%. Content of crude protein slightly increased with increasing doses of nitrogen, so their yield was much greater influenced by the yield of hay. Nitrogen fertilization led to the expected but less pronounced changes of botanical composition of grasslands. The greatest effect of 1 kg of nitrogen on increase hay yield was in variant with 90 kg ha^{-1} N, 22.06 kg, and lowest in the fertilization with 30 kg ha^{-1} N, 17.50 kg of hay.

Rezultati đubrenja visokoplaninskog travnjaka *Poetum violaceae*

R. Dubljević, D. Mitrović

Rezime

Ispitivanje uticaja primene različitih doza azota na proizvodne osobine prirodnog travnjaka *Poetum violaceae* obavljena su na jugozapadnim padinama Durmitora (lokalitet Pišče - Lokvice, 1620 m.n.v), u periodu 2004.-2005. godine. Ogled je postavljen na zemljištu tipa rendzina, a primenjene su sledeće varijante: 0, 30, 60, 90 i 120 kg ha^{-1} N. Tokom istraživanja praćen je uticaj đubrenja na: prinos sena i sirovih proteina, promene florističkog sastava i efekat (učinak) primenjenih

doza đubriva. Najveći prosečan prinos od 4,16 t ha^{-1} ostvaren je u varijanti sa najvećom, a najniži (2,44 t ha^{-1}) u varijanti sa najmanjom dozom đubriva, što je u odnosu na kontrolu povećanje od 26,91 do 116,64%. Prinos sirovih proteina je rastao sa povećanjem doze đubriva i varirao je od 208,45 do 422,20 kg ha^{-1}. Floristički sastav travnjaka karakteriše dominantno učešće trava i mala zastupljenost leguminoza (<10 %). Pri većim dozama đubriva učešće trava se povećavalo, dok je udeo leguminoza bio manji. Efekat đubriva bio je veći u varijantama sa srednjim dozama (21 i 22 kg sena). Rezultati ovih istraživanja ukazuju na zaključak da se primenom srednjih doza azota povoljno utiče na proizvodne osobine visokoplaninskog travnjaka zajednice *Poetum violaceae*. Veće doze đubriva su manje racionalne i više utiču na nepovoljne promene florističkog sastava.

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COMPARATIVE VALUE OF DRY MATTER YIELD OF RED CLOVER (*Trifolium pratense* L.), ITALIAN RYEGRASS (*Lolium multiflorum* Lam.) AND THEIR MIXTURES

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Orginal Scientific paper

Abstract: Perennial legumes and perennial grasses are, in most cases, the main sources of roughage forage from arable land in Bosnia and Herzegovina. Mixtures of legumes and grasses are more productive compared to pure grass and even legumes. Reasons can be found in utilization of fixed nitrogen in red clover. As there is not much data about the subject in domestic literature, the aim of this study was to investigate the comparative value of the dry matter yield of pure red clover and Italian ryegrass as well as their mixtures with different ratios of these two species. Results of two year studies of yield dry matter indicate that Italian ryegrass is significantly less productive than red clover. Mixtures with high participation of Italian ryegrass are significantly less productive and can not be a competitive dry matter yield (DM) of red clover or mixtures with high participation of red clover. On the other hand, mixtures with higher participation of red clover (above 50 %) are more productive than pure crop and can be considered more productive and more valuable than pure red clover. Overall, for the two years of research, the highest yield of DM was achieved with a mixture of 75 % of red clover (30,78 t ha $^{-1}$).

Key words: red clover, yield, dry mass, Italian ryegrass.

Introduction

Perennial legumes and perennial grasses are, in most cases, the main sources of roughage forage from arable land in Bosnia and Herzegovina. Legumes are often grown in pure stand because of its high forage value, while grasses are usually grown in mixture with legumes, which is why such crops achieve good yields of high quality. As a benefit of growing a mixture instead of pure crops noted *Jose et al. (2001)*, that mineralization of organic mass (and thus the fact and the number of soil organisms) in soil is higher under grass-legumes mixtures than under pure crops.

Red clover is most important among all true clovers but also the most important of all perennial legumes in Bosnia and Herzegovina, due to its high production (up to 25 t ha^{-1} of DM) of high quality. Some varieties *(Wolfhardt and Luftensteiner, 1984)* achieved yields of 23,24 t ha^{-1} DM in the second year of the year of exploitation. According to the research done by *Gail (2008),* red clover growing in mixture with grasses can produce high yields of DM in the first year of use (12.24 to 18.23 t ha^{-1}).

Amount of fixed nitrogen by the red clover and the contribution that makes to the content of nitrogen in plants vary depending on numerous factors, such as climatic and soil conditions, presence and efficiency of *Rhizobium legumionsarum trifolii,* but also associated components and the phase of plant development. *Taylor and Quesenberry (1996),* indicate that the amount of fixed nitrogen by red clover, depending on the region range of 76 to 389 t ha^{-1} per year.
Red clover gave 12,37 t ha^{-1} DM, with 18 % of proteins in DM (2016 kg ha^{-1} proteins), and fixing in that way from the air more than 224 kg ha^{-1} of nitrogen. Part of the fixed nitrogen was used by other components in the mixture *(Caddel and Redmon, 1995).*

Italian ryegrass is one of the most valuable short perennial grass species of high productivity and high forage value. According to the soil and other climatic requirement, it is similar to red clover and that is why is often grown with this clover in mixture.

The aim of the research was the examination of comparative value of DM yield of red clover and Italian ryegrass in pure stand as well as their mixtures at different proportion of tested components. Taking into account that red clover fix nitrogen, which can be used by Italian ryegrass for its nutrition and increased yield of DM.

Materials and Methods

A two-year experiment was carried out during 2004 and 2005 at Butmir near Sarajevo (550 m above sea level). Experiment was set up by randomized block method in four repetitions. The size of the basic plot was 5 m^2. Sowing was manual and the row distance was 12,5 cm.

Characteristics of soil was as follows: pH-5.2 (in KCl), pH 5.6 (in H_2O), humus content 3.7 %, total nitrogen 0.16 %, soil is slightly to moderately provided with phosphorus (11.0 mg/100 g soil), content of potassium is medium (14.6 mg/100 g soils). Weather conditions were favourable, with a good disposition of temperature and precipitation, except August of 2004, when there was only 31 mm of precipitation.

In the experiment there were:
1. Red clover 100 %
2. Italian ryegrass 100 %
3. Red clover 75 % + Italian ryegrass 25 % (S1)
4. Red clover 50 % + Italian ryegrass 50 % (S2)
5. Red clover 25 % + Italian ryegrass 75 % (S3)

Experiment was not fertilized. In the year of sowing were obtained three and in next year four cuts.

DM yield was determined based on the yield of green mass and the factors of dryness. Results were subjected to ANOVA and compared by LSD test.

Results and Discussion

Results of two year investigations of productivity of red clover, Italian ryegrass and their mixtures showed significantly lower productivity of Italian ryegrass compared to red clover. The highest annual DM yield (Table 1.) in the sowing year gave red clover mixture S1 (11.43 t ha^{-1}), while the lowest yield achieved pure Italian ryegrass (6.03 t ha^{-1}). In the next year, the highest total DM yield showed red clover (19.51 t ha^{-1}), then mixture S1 (19.35 t ha^{-1}), while the Italian ryegrass had the lowest yield (8.07 t ha^{-1}).

Delorit et al. (1974). indicate that red clover reached best results if it is grown in soils of pH 6.6-7.6, while Italian ryegrass best results achieved in deep, fertile soils, but it can be grown successfully in a broader areal of different soil types (*Hannaway et al., 1999).* Taking into consideration the characteristics of the soil in Butmir, where research was done, it could be said that this was relatively favourable for both species, having positive effect on obtained results.

Average total DM yield of all the tested varieties was 25.41 t ha^{-1}. The mixture of red clover 75 % plus Italian ryegrass 25 % achieved the highest total yield (30.78 t ha^{-1}) of DM. The least productive was the Italian ryegrass (14.10 t ha^{-1} DM), whose yields are twice lower than the red clover and its mixtures, in which the Italian ryegrass participated up to 50 %.

Highest yield of DM in the sowing year was obtained in the first cut, due to favourable weather conditions, because the amount of precipitation in the June and July was higher than average. Sufficient amounts of precipitation and adequate temperature favoured rapid growth and development of plants of red clover, Italian ryegrass, as well as their mixtures.

Table 1. DM yield of red clover, Italian ryegrass and their mixtures (t ha^{-1})

Variants	Year		Total
	2004	2005	
Red clover 100 %	9,36*	19,51***	28,87***
Red clover 75 % + Italian ryegrass 25 %	11,43**	19,35***	30,78***
Red clover 50 % + Italian ryegrass 50 %	10,39*	18,76***	29,15***
Red clover 25 % + Italian ryegrass 75 %	8,08	16,09***	24,17***
Italian ryegrass 100 %	6,03	8,07	14.10
Average	9,05	16,35	25,41
LSD 0,05	3,31	2,05	2,53
0,01	4,32	2,83	3,41
0,001	5,98	3,91	4,53

Total DM yields achieved during the two years investigations showed that the red clover in pure stand as well as its mixture with Italian ryegrass were highly significantly more productive compared to pure stand of Italian ryegrass. It was obvious that red clover with small portion in mixture can not fix enough nitrogen to support vigorous development of Italian ryegrass in it. For such a mixture would need additional fertilization with diet of mineral nitrogen. This conclusion was indicated by result of *Carlsson et al. (2008).*

Conclusion

Based on the two year research and analysis of the obtained result it can be concluded:

- In Butmir condition, red clover is more productive than Italian ryegrass.
- Mixtures with 75 and 50 % of red clover (30.78 and 29.15 t ha^{-1} DM) are more productive than pure red clover (28.87 t ha^{-1} DM).
- DM yield of Italian ryegrass (14.10 t ha^{-1}) was significantly lower compare to the yield of red clover, but also all variants of mixtures.
- Mixtures with significant participation of red clover (above 50 %) are more productive than pure crop of red clover and could be considered as more valuable than red clover.

Uporedna vrednost prinosa nadzemne mase crvene deteline (*Trifolium pratense* L.), italijanskog ljulja (*Lolium multiflorum* Lam.) i njihovih smeša

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Rezime

Višegodišnje leguminoze i višegodišnje trave su u većini slučajeva glavni izvori proizvodnje krme na oranicama. Gajenjem leguminoza i trava u smeši se dobija veća produkcija biljne mase u odnosu na čiste useve trava, a često i samih leguminoza. Razloge treba tražiti u boljem iskorišćavanju azota fiksiranog od strane crvene deteline. S obzirom da nema previše podataka o tome u domaćoj literaturi, cilj ovoga rada je bio da se ispita uporedna vrednost prinosa čistih useva crvene deteline i italijanskog ljulja, kao i njihovih smeša, sa različitim odnosom ove dve vrste. Rezultati dvogodišnjih istraživanja prinosa suve mase ukazuju na to da je italijanski ljulj značajno slabije produktivan u odnosu na crvenu detelinu. Smeše sa visokim učešćem italijanskog ljulja su značajno manje produktivne i ne mogu biti konkurent u produkciji suve mase crvenoj detelini, ali ni smešama sa visokim učešćem crvene deteline. S druge strane, smeše sa visokim učešćem crvene deteline (iznad 50 %) su produktivnije u odnosu na čist usev, te se mogu smatrati produktivnijim i vrednijim u odnosu na čistu crvenu detelinu. Sveukupno, za dve godine istraživanja najveći prinos suve mase je ostvarila smeša sa 75 % crvene deteline (30,78 t ha^{-1}).

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STUDY OF INTRODUCED MEADOW GRASSES IN MIXTURES WITH BIRD'S FOOT TREFOIL UNDER THE AGRO-ECOLOGICAL CONDITIONS OF TROYAN

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Abstract: During the 2006-2009 period a field trial was carried out with the purpose of determining the productivity and botanical composition of a local population of bird's foot trefoil in binary mixtures with hybrid ryegrass, smooth brome grass, meadow timothy, Kentucky bluegrass and cocks foot. The mixtures were laid out by the block method with 4 replications and size of harvest plot of 5 m^2. We used the pure stand of bird's foot trefoil as a control. It was found that the highest yield of dry mass, on average for the period of study, was obtained from the mixture of bird's foot trefoil with Kentucky bluegrass and cocksfoot, the exceeding being by 7,9 and 7,3%, as compared to the control. The mixture of bird's foot trefoil and timothy ranked second in productivity, being inferior to the control by 6.6%. The botanical composition of the sward showed different percentage participation of the tested meadow grasses and bird's foot trefoil by years and cuts. In the second year bird's foot trefoil predominated in the sward with smooth brome grass (87.96%), in the third one – in the mixture with timothy (52.1%) and in the fourth one – with Kentucky bluegrass (49.1%).

Key words: bird's foot trefoil, herbaceous mixtures, productivity, botanical composition

Introduction

Bird's foot trefoil *(Lotus corniculatus L.)* is a crop with great potential capacities with regard to productivity, as well as in connection with quality of obtained forage. It is a suitable legume component for establishment of high-productive forage herbaceous mixtures on acid soils *(Lingorski, 2001; Hopkins, 2006)*. The following grass components are suitable for mixed cultivation: timothy *(Chourkova, 2007)*, cocksfoot *(Vasilev, 2008)*, smooth bromegrass, meadow fescue *(Norgailine et al., 1994)*. The cultivation of meadow grasses and legumes in mixed stands has a number of advantages over the pure stands. Their coexistence changes the total structure of the stand, improves the vertical distribution of root mass in the soil horizons and layer distribution of vegetative organs, increases the productivity of photosynthesis and increases the yields of obtained biomass *(Zemenchik et al., 2002)*.

The region with its specific soil and climatic conditions also has an important role for the realization of genetic characters of a given variety *(Peev et al., 2000; Slavov and Georgiev, 2000, 2002; Ayres et al., 2007; Marley and Jones, 2006; Pelikan, 2002)*. In the last years the world researches in the field of ecology show that the climate on the earth gradually becomes warmer. That necessitates searching a new approach to the choice of species and varieties of herbaceous forages for study with a view to their cultivation and use under changing ecological conditions.

The objective of this study was to determine the productivity and botanical composition of a local population of bird's foot trefoil in binary mixtures with hybrid ryegrass, smooth brome grass, meadow timothy, Kentucky bluegrass and cocksfoot under the agro-ecological conditions of Troyan.

Material and Methods

During the 2006-2009 period a field trial was carried out in the experimental field of IMSA – Troyan by the block method with 4 replications and size of harvest plot of 5 m^2. The binary herbaceous mixtures of bird's foot trefoil (*Lotus corniculatus L.* -population Troyan) with the following meadow grasses were tested: hybrid ryegrass (*Lolium x boncheanum Kunth*.), smooth bromegrass (*Bromus inermis Leyss*.), meadow timothy (*Phleum pratense L*.), Kentucky bluegrass (*Poa pratensis L*.) and cocksfoot (*Dactilis glomerata L*.). The trial was carried out on light pseudopodzolic soil, supplied slightly with nitrogen and phosphorus and moderately with potassium.

The generally accepted technology of the institute for establishment of an artificial sward was applied. Fertilizing with $P_{0,08}$ and $K_{0,08}$ tha^{-1} was conducted every year in the sowing year together with the basic soil tillage and in the next ones – in autumn, after the end of vegetation. We applied $N_{0,06}$ tha^{-1} during the sowing and early in spring in the next years.

We conducted the sowing by hand broadcast with the generally accepted sowing rates for the different herbaceous species at the ratio of 1:1, recalculated depending on the economic value of seeds.

In the year of the sowing is made a sanitary cut and a dry mass isn't done. In the second year three mowings were conducted and in the third and fourth ones two mowings at hay-cutting ripeness of sward at the stage of budding-early flowering of bird's foot trefoil.

We recorded the following characteristics: botanical composition of sward (in a weight percentage for bird's foot trefoil, grasses and weeds), yield of dry mass (t/ha). We performed mathematical estimation for the productivity by the variance analysis.

The temperature conditions and rainfall during the experimental period were relatively favourable for normal development of the swards in this region.

Results and Discussion

The sward productivity (Table 1) was determined to a great extent by the climatic conditions during the growing season and botanical characteristics of the sown plants. In the second year (2007) the highest dry mass yield was obtained from the pure stand of bird's foot trefoil (17.1 t ha^{-1}) and among the mixed stands, the mixture of bird's foot trefoil with Kentucky bluegrass (16.6 t ha^{-1}) and cocksfoot (16.6 tha^{-1}). They had approximately equal values.

Table 1. Yield of dry mass by years and on average for the period, t ha^{-1}

Variants	2007		2008		2009		In average for the period	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
Bird's foot trefoil	17.1	100.0	16.4	100.0	10.6	100.0	14.7	100.0
Bird's foot trefoil + hybrid ryegrass	14.9	86.9	16.8	102.2	8.7	82.2	13.5	91.4
Bird's foot trefoil + smooth brome grass	16.4	96.1	19.2	117.0	8.9	84.0	14.9	101.0
Bird's foot trefoil + timothy	15.9	93.4	22.0	133.9	9.9	93.1	15.7	106.6
Bird's foot trefoil + Kentucky blue grass	16.6	97.1	20.2	122.7	10.9	102.4	15.9	107.9
Bird's foot trefoil + cocksfoot	16.6	97.2	19.5	118.6	11.3	105.8	15.8	107.3
GD 5%	2.1	12.0	1.6	9.5	1.9	18.0	1.2	8.4
GD 1%	2.8	16.7	2.2	13.2	2.6	24.9	1.7	11.6
GD 0,1%	3.9	23.0	2.9	18.1	3.6	34.3	2.4	16.0

In 2008 the mixed swards of all tested mixtures were superior to the control by 2.2 to 33.9%, respectively. The mixture of bird's foot trefoil with timothy was distinguished for the highest yield of dry mass (22.0 t ha^{-1}). The mixtures of bird's foot trefoil with timothy, Kentucky bluegrass and cocksfoot had very good significance of the differences.

In the fourth year of the experimental period the mixed swards of bird's foot trefoil with cocksfoot and Kentucky bluegrass were superior to the control by

5.8 and 2.4%, respectively. The mixtures of bird's foot trefoil with hybrid ryegrass and smooth brome grass had the lowest yield of dry mass.

On average for the period of study, the mixed stands of bird's foot trefoil with Kentucky bluegrass and cocksfoot were the most productive, exceeding the control by 7.9 and 7.3%. The yield of dry mass of the mixture of bird's foot trefoil and timothy was similar to these values, exceeding the control by 6.6%. A lower yield than that of the pure stand of bird's foot trefoil was recorded for the mixed cultivation of bird's foot trefoil and hybrid ryegrass.

In the second year of the experimental period bird's foot trefoil represented 66.7% (Figure 1) in the pure stand. Its participation in the mixture with smooth brome grass was the greatest (87.96%). Among the grasses, cocksfoot dominated in the sward of primary growth and re-growth (50.0 and 26.47%).

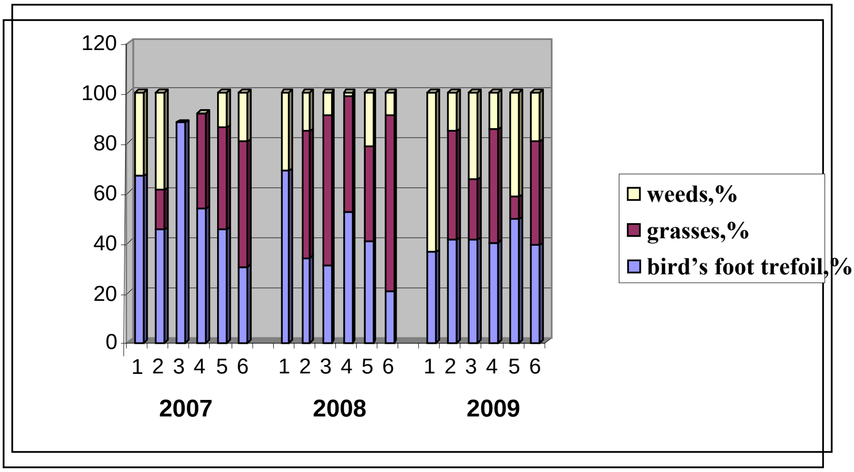

Figure 1. Botanical composition of the sward in a weight percentage

It is evident from the botanical composition of the sward that bird's foot trefoil coexisted well with timothy (52.1%) in the third year. In the mixture of bird's foot trefoil with cocksfoot the share participation of bird's foot trefoil was 20.3% and that of cocksfoot 70.5%. The weeds predominated in the pure sward of bird's foot trefoil (31.5%) and their participation was the lowest in the mixture of bird's foot trefoil with timothy (1.7%).

In the fourth year of the experimental period in the mixture of bird's foot trefoil and Kentucky bluegrass the share participation of bird's foot trefoil was 49.1%. The weeds predominated in the pure swards of bird's foot trefoil, reaching 63.8% respectively.

Conclusions

For the conditions of Troyan the most productive mixtures were those of bird’s foot trefoil with Kentucky bluegrass and cocksfoot, exceeding the control by 7.9 and 7.3%.

The highest relative portion of bird’s foot trefoil in the sward in the second year was shown by the mixture of bird’s foot trefoil and smooth brome grass and in the third year that of bird’s foot trefoil and timothy and in the fourth one that of bird’s foot trefoil and Kentucky bluegrass.

Izučavanje introdukovanih livadskih trava u smešama sa zvezdanom u agroekološkim uslovima Trojana

B. Churkova

Rezime

U toku 2006-2009. godine vršeni su poljski ogledi sa ciljem utvrđivanja produktivnosti i botaničkog sastava lokalnih populacija zvezdana u smešama sa hibridnim ljuljem, bezosnim vlasenom, mačijim repom, livadarkom i ježevicom. Smeše su postavljene po blok metodi sa 4 ponavljanja i na žetvenoj površini od 5 m^2. Korišćene su čiste linije ljulja kao kontrola. Najbolji prinos suve mase, u toku prosečnog perioda istraživanja, je utvrđen kod smeše zvezdana sa livadarkom i ježevicom i bio je viši za 7,9 i 7,3% od kontrole. Smeša zvezdana i mačijeg repa je bila druga po produktivnosti, za 6,6% lošija od kontrolne. Botanički sastav travnjaka je pokazao različit udeo proučavanih trava i zvezdana po godinama i otkosima. U drugoj godini, zvezdan je bio dominantan u travnjaku sa bezosnim vlasenom (87.96%), u trećoj – u smeši sa mačijim repom (52.1%) i u četvrtoj – sa livadarkom (49.1%).

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CONTENT OF HEAVY METALS IN BIOMASS OF NATURAL GRASSLANDS

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Abstract: Natural grasslands and pastures cover more than 1.600.000 hectares of land and represent a large source of livestock feed. In recent times, grasslands have also been exposed to influence of various destructive pollutants. Heavy metals contamination ranks high amongst the most serious eco-toxicological problems. Many of them most part enter the food chain through plants. Determination of heavy metal contents was conducted by utilization of the potentiometric stripping analysis (PSA). It has been determined that the biomass of pastures located higher than 1000 meters above sea level was not contaminated by heavy metals, while the aforesaid content in the biomass of pastures located on lower altitudes, in vicinity of industrial compounds was around or even exceeded the critical concentrations limit.

Keywords: Biomass, natural grasslands, PSA, heavy metals

Introduction

Natural grasslands comprise a significant part in production of livestock feed in Serbia, particularly at high altitudes. Quality of natural grassland biomass is very important factor in development of livestock production *(Vapa, 1993)*. In Kopaonik mountain base, natural grasslands biomasses are almost the only source of food for ruminants. They are characterized by the presence of numerous plant species and, due to exploitation method and inadequate utilization of mineral fertilizers, they result in extremely low crops. Development of all plant species and forming of crops are closely connected with absorption of mineral elements from soil, their repositioning and division within a plant *(Jakovljević and Antić-Mladenović, 2000)*. Intensity of absorption, predominantly transport of mineral elements, does not only depend on type of metal, but also on their participation in biochemical reactions, course of absorption and transport *(Kastori et al., 1997)*.

Heavy metals have influence on photosynthetic activity, mineral nourishment, metabolism of proteins and enzyme activity *(Nikolić, 2009)*, functions of membranes, water regime and some other biochemical processes. Absorption of these elements is mostly conducted by the root which besides its role in absorption of elements from soil, also doubles as a specific filter. Essential elements entering upper parts of plants, while toxic elements are withheld *(Bogdanović et al., 1997)*. It is known that root's epidermis represents a barrier for absorption of lead. Zinc and cadmium is characterized by high mobility through the plant *(Kastori et al., 2000)*. Increased concentration of cadmium leads to standstill or deceleration of plant's growth, and in humans, cadmium causes blood vessel diseases and hypertension. In conditions when soil is highly contaminated, lead enters the human body through via plants and aggregates in liver, kidneys and bones. Zinc belongs to the category of essential elements but becomes toxic if the concentrations are high enough.

Materials and Methods

Plant material was sampled from natural grasslands located in the southern part of Kopaonik Mountain, on altitudes of 512, 466 and 1040-1100m, in localities of Kosovska Mitrovica, Leposavić and sub-Kopaonik villages of: Belo Brdo, Planinica, Marušiće. Grasslands from which the samples were collected are used in traditional manner, i.e. first harvest is conducted by means of mowing and then followed by grazing. Sampling was conducted from mowed biomass (from hay bales) in September 2009. Collection of individual samples was conducted according to the principle of concentric circles, from top to bottom *(Kastori et al., 2006)*. A representative sample weighing 2,5 kg was made of individual samples. Again, another representative sample was made of representative samples from neighboring villages representing a biomass for altitude of approximately 1100m. On altitude of 446 meters, samples were collected at different distances from active Trepča landfill (Bostanište, Leposavić): 500 m, 750 m, 1000 m, 1500 m and 3000 m. Sampling of biomass on altitude of 512 m was conducted in Žitkovac village (northern Kosovska Mitrovica) in immediate vicinity of passive Trepča landfill. Samples of plant material were taken along Lešak-Kosovska Mitrovica main road, 1-2m away from the road, depending on the terrain's ease of access. After sampling, preparation of plant material for analysis took place, commencing with washing with pipe, distilled and then deionized water, until the desired results were achieved. Washed samples were dried at 105°C until a continuous mass was obtained, and then burned at 500°C. A gram of resulting ashes was moistened with 5 cm^3 conc. HNO_3. Obtained solvate was steamed to dry in sand bath. Steaming process was repeated after several drops of conc. HCl were added. Resulting white mass, dissolved with 5 cm^3 2 % HCl, was transferred into a normal dish of 100 cm^3

and obtained solvent was diluted up to the line *(Babincev and Rajaković, 2009)*. Resulting solvent was analyzed by utilization of M1 stripping analyzer, made by RO ELU Leskovac, which functions with deployment of three-electrode system in the pre-electrolyses phase *(Suturović and Marjanović, 1993).* Content of lead, cadmium and zinc from previously razed and prepared biomass samples was determined by concentrating (reducing) in the working electrode under the depositing potentials characteristic for each element in particular. Quantitative characteristic of this determination is duration time of element's oxidation and quantity of elements was determined by method of standard supplements.

Results and Discussion

Statistical processing of results, obtained by PSA-method was performed using the q-test. In Table 1, are shown: the value of added standard, X_S, average value of determining, $\overline{X}$, expressed in µg/dm^3, value of standard discrepancy, *S*, expressed in µg, as a measure of precision of obtained results, coefficient of variation K_v in %, as a measure of re-productiveness and percentage error Er, %. The smallest amounts of the aforementioned metals determined within limits of accuracy of ± 2% were for: Pb (24,98), Cd(13,25) and Zn (19,09) µg/dm^3.

Table 1 Sensitivity of PSA-method for determination of lead, cadmium and zinc

Elements	µg/dm^3		S (µg)	Kv(%)	Er(%)
	X_s	$\overline{X}$			
Pb	2,25	2,38	0,30	12,60	5,78
	8,95	9,35	1,15	12,30	4,47
	22,48	24,98	2,52	11,34	1,12
	890,76	880,58	99,15	11,26	-1,14
Cd	3,25	3,45	0,75	21,74	6,15
	12,99	13,25	2,18	16,45	2,00
	32,47	33,09	4,15	12,54	1,90
	97,30	99,11	12,46	15,57	1,86
Zn	3,75	3,97	0,46	11,59	5,86
	18,75	19,09	1,99	10,42	1,81
	37,50	38,21	3,96	10,36	1,89
	187.50	191,15	19,78	10,35	1,95

Plants absorb heavy metals by means of water, air and most regularly soil. Source of these elements in soil are atmospheric pollutants from motor vehicles, burning of fossil fuels, mineral and organic fertilizers, metallurgic industry, mines and foundries of colored metals, as well as atmospheric deposits *(Bogdanović et al., 1997).* Accumulation of lead in plants along main roads depends on distance, degree of how much the soil is covered with plant life, direction and velocity of wind, frequency of vehicle traffic and duration time of vehicles stopping by

(Filipović-Trajković et al., 2001). Foliar absorption of heavy metals depends on type of plants, thickness of epidermis, age of leaves, amount of humidity and primarily on concentration of all them in soil. *Kadović and Knežević (2002)* determined that concentration of lead in active growth grass was 0,3-15 mg/kg of dry matter, increased by the end of summer to 10 mg/kg and even 30 mg/kg by the end of winter. Vegetation of biomass that we examined was completed with the first mowing (summer), so it could have been foreseen that the content in the aforesaid would be approximately 10 mg kg^{-1} of dry matter.

Table 2. Content of heavy metals in biomass of natural grasslands from northern Kosovo-Metohija

Location	µg g^{-1} SM		
	Pb	Cd	Zn
On altitude higher than 1000 m	2,35	-	55,40
	4,56	-	78,32
	8,95	0,22	93,71
Along Lešak- Kos. Mitrovica main road	10,32	1,62	130,24
	11,15	3,25	141,01
	12,39	6,49	135,49
In the area of passive Trepča landfill Žitkovac	17,48	5,09	206,71
	32,94	10,12	158,55
	27,32	8,45	116,17

Kabata-Pendias and Pendias (1989), determined the values 0.19-9 mg kg^{-1} of dry matter in the upper parts of grass in unpolluted areas, while the content of lead in polluted areas was 63-232 mg kg^{-1}. Same authors stated that natural concentrations of lead in plants are in 5-10 mg kg^{-1} range. Determined content of lead in biomass of natural grasslands, on altitude higher than 1000m, was in 2.35-8.95 µg g^{-1} of dry matter range (Table 2). In polluted areas, content of lead in biomass along the main road was 10.32-12.39 µg g^{-1} of dry matter. Content of lead in biomass in immediate vicinity of passive Trepča landfill Žitkovac amounted to as much as 27.32 µg g^{-1} of dry matter. Significantly more alarming situation was in pasture area within active Trepča landfill (the village of Krnjin) Leposavić. Based on the presented results (Table 3) it can be concluded that even 3000 m away from the landfill's borderline, the biomass was contaminated. Critical concentration for lead was exceeded even in the biomass which was 3km away from the landfill. In closer areas the content of lead was greater and biomass of pastures located 500 m away from the landfill was almost five times richer than the upper limit of critical concentration. Since the absorption of lead is strongest in the root part of the plant *(Bogdanović et al., 1997)* it is possible to expect significantly higher amount of lead in that part of biomass which was not been subject to analysis at this point. Physiological role of cadmium has not been discovered in plant life. Hence, it is less likely that there are membrane transports specific for this ion *(Clemens, 2001*), instead its absorption is most probably conducted by means of conveyers that transport other ions. Based on the aforementioned results (Tables 2 and 3) it can be noticed that cadmium is present in biomasses of almost all localities on altitudes lower than 1000m. It is present in soil along roads where, on particular locations, it exceeds critical content concentration wise. And it can penetrate soil by means of city waste, compost and mud. Based on those results it can be concluded that the main sources of cadmium are flotation and metal foundry. Not on a single measuring locality has cadmium shown toxic level in biomass and it was on toxicity limits in biomass from immediate vicinity of passive landfill in Žitkovac. Critical content of cadmium was evident even in biomass from the first sections of pastures besides the active landfill (Bostanište) in Leposavić.

Table 3. Content of heavy metals in natural grasslands biomass on various distances from active Trepča landfill - Bostanište, Leposavić

Con. Eleme. µg/g SM	Distance from flotation landfill, m				
	500	750	1000	1500	3000
Pb	135,65	101,98	76,48	57,23	21,62
Cd	3,90	2,11	1,96	1,18	0,74
Zn	717,01	539,43	227,11	144,86	116,26

Pollution of living environment with chemical elements: lead, cadmium and zinc have particular ecological, biological and health importance *(Kadović and Knežević, 2002)*. Zinc is from the group of microelements which is, in low concentrations, essential while in high concentrations can become toxic. Content of lead from 150-200 mg kg^{-1} of dry matter of biomass represent the range of critical concentration. Each content greater than the upper critical limit for biomass is toxic. This research has determined that zinc in toxic content is present in biomass from immediate vicinity of landfills regardless if they were passive or active. Beside the active landfill toxic content of zinc was determined in biomass from pastures located several kilometers from the stated one.

Conclusion

Based on the results on the content of heavy metals and their accumulation in pastures biomass we can conclude as follows: The largest polluter of pastures biomass in Kopaonik Mountain base is the flotation of lead-zinc mine with its landfill (Bostanište) in Leposavić as well as the lead foundry with its landfills in Zvečan; Lešak-Kosovska Mitrovica main road represents a major burden in terms of content of heavy metals in biomass along the road itself which is frequently used for livestock grazing; The smallest quantity of heavy metals in biomass was found in locations outside the effect of traffic and process of production of heavy metals and depositing of their waste residues; Concentration of heavy metals in biomass decreases with separation from the source of pollution; Increased content of heavy metals in biomass from natural habitats is not of biotic but non-biotic nature; Implementation of potentiometric stripping analysis for determination of content of micro and toxic elements shown that the said analysis can be applied to determine the content of lead, cadmium and zinc in the biomass from pastures; In the following researches, content of the aforementioned elements should be determined in the soil of the said locations, as well as the level of absorption of these metals in relation to pH-value of the soil.

Sadržaj teških metala u biomasi prirodnih travnjaka

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Rezime

Veliki izvor stočne hrane predstavljaju prirodne livade i pašnjaci koji se u Srbiji prostiru na preko 1.600.000 hektara. U poslednje vreme i travnjaci su izloženi uticajima različitih štetnih polutanata. Kontaminacija teškim metalima je jedan od ozbiljnijih ekotoksikoloških problema. Mnogi od njih preko biljaka ulaze u lanac ishrane. Određivanje sadržaja teških metala vršeno je Potenciometrijskom striping metodom (PSA). Utvrđeno je da biomasa pašnjaka koji se nalaze iznad 1000 m nadmorske visine nije kontaminirana određivanim teškim metalima, dok sadržaj istih u biomasi pašnjaka na nižim nadmorskim visinama, u blizini industrijskih postrojenja se približava ili, čak prekoračuje granicu kritičnih koncentracija.

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EFFECTS OF DROUGHT ON LEAF AREA AND NITROGEN PARTITIONING IN *Lolium multiflorum* L.

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Abstract: Forage quantity and quality are important factors for livestock production in grazing lands. Identification of forage species that can maintain both their forage quality and quantity by developing adaptation mechanisms under drought conditions becomes increasingly important under the imminent climatic changes in Mediterranean grasslands. We studied the effects of water stress on leaf area and nitrogen partitioning of *Lolium multiflorum* L. Seeds from *L. multiflorum* were sown in pots under a transparent shelter. After their establishment, the plants were subjected to three water treatments: a) well watered, b) water stressed and c) re-watered. Leaf area and nitrogen content were measured under the above treatments during the growing season. The water regime significantly affected both leaf area and nitrogen content. After seventeen days of water stress, nitrogen loss increased in leaves, stems and roots of well watered plants, while it significantly decreased in stems of water stressed and re-watered plants. Negative correlation between leaf area and nitrogen content was found, suggesting that *L. multiflorum* could develop specific mechanisms of adaptation under drought conditions.

Key words: water stress, *Lolium multiflorum*, nitrogen content

Introduction

Forage quantity and quality are important factors for livestock production in grazing lands. Identification of forage species that can maintain both their forage quality and quantity by developing adaptation mechanisms under drought conditions becomes increasingly important under the frame of the future climate change, especially for areas such as the Mediterranean. Leaf area could be regarded as a useful index of forage quantity, especially under drought conditions. Reduction of leaf area growth is regarded as a common response of plants to water stress *(Belaygue et al., 1996; Pettigrew, 2004)*; in this way plants limit the development of transpiration surface during water deficit, keeping sink demand of growing leaves in balance with the supply of photo assimilates *(Alves and Setter, 2000)*. After the release from stress this response is rapidly reversed *(Palta, 1984; El-Sharkawy and Cock, 1987; Alves and Setter, 2000)*.

In addition, leaves are the main nitrogen (N) reservoir during vegetative growth *(Sheehy et al., 2004; Xu et al., 2006)*. Leaf N content has been proposed as an indicator of forage quality, especially in semiarid areas *(Huston and Pinchak, 1991; Heitschmidt et al., 1995)*. Nitrogen levels are greatest in metabolic active tissues of plants. The level of metabolic activity within plants is, in turn, largely affected by the environment *(Angell et al., 1990; Ganskopp and Bohnert, 2001)*. In semiarid regions, soil water availability plays a crucial role on soil N availability *(Tsialtas et al., 2001; Jankju-Borzelabad and Griffiths, 2006)*, which decreases with decreasing soil moisture *(Stanford and Espein, 1974)*. In Mediterranean regions, high irradiance, coupled with water stress, usually promotes leaf senescence in late summer, which could imply great N canopy losses from plants *(Sanz Perezr et al., 2009)*. Water stress induced movements of N from shoots to roots in grasses may have important consequences for growth and survival during and after drought *(Heckathorn and Delucia, 1994)*. On the other hand, N distribution in vegetative and reproductive organs of plants is an important factor determining grain yield and quality *(Xu et al., 2006)*.

The aim of this study was to investigate the effects of different water treatments on leaf area development and nitrogen partitioning of *Lolium multiflorum* L. during the vegetative growing stages.

Materials and Methods

The research was conducted in spring 2007 at the farm of the Laboratory of Range Science of Aristotle University of Thessaloniki (longitude: 40^o 34′, latitude: 23^o 43′), Northern Greece at an altitude of 10 m above sea-level. The climate of the area could be characterized as Mediterranean semiarid with cold winters. The mean annual precipitation for the period 1978-2007 was 400 mm. The mean monthly precipitation (mm) and temperature (^{o}C) during the experimental period are presented in Table 1.

Table 1. Mean monthly precipitation (mm) and temperature (^{o}C) during the experimental period

	January	February	March	April	May	June
Precipitation (mm)	24.4	14.2	9.2	67	20	21
Temperature (^{o}C)	9	12	17.1	19.2	25	31

Seeds of *L. multiflorum* L. were individually sown in pots (9 cm x 9 cm x 10 cm) filled with peat. After seedlings emergence and establishment, plants were transplanted in 56 plastic pots (28 cm x 28 cm, one plant per pot), filled with soil (medium texture) from the farm. Then the pots were transferred to a permanent rain shelter with a transparent plastic cover on top. The rain shelter was designed in such a way as to avoid rainfall reaching the pots, while keeping the atmospheric conditions unchanged. During the late vegetative growing stage plants were subjected to three water treatments: a) well watered (W) (up to field capacity), b) water stressed (S) for seventeen days, and c) re-watered (RW); water stressed for seven days and re-watered up to field capacity for the remaining period. Plants from each treatment were harvested and separated into stems, leaves and roots. Leaf area (LA) was measured using an area measurement system (Delta-T Devices, Cambridge, UK). All plant samples were then oven dried at 60°C for 48h in order to assess their dry weight. Dried samples were ground through a 1 mm screen and analyzed for N using a Kjeldahl procedure *(AOAC, 1990)*.

Data were analyzed by ANOVA (following the general linear models procedure; SPSS 14 for Windows). The LSD test *(Steel and Torrie, 1980)* at 0.05 probability level was used to detect differences among means.

Results and Discussion

Plants under well watered conditions presented higher leaf area compared to the water stressed ones during the experimental period (Figure 1). As the growing season proceeded, leaf area of well watered plants presented no significant differences, while it continued to decrease in water stressed plants. However, re-watered plants (RW) seven days after re-watering had higher leaf area than the stressed ones, but significantly lower leaf area than the well watered plants.

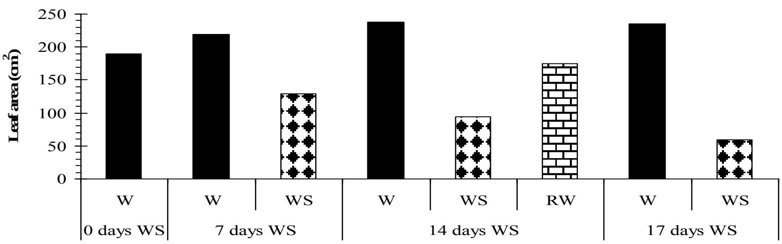

Figure 1. Mean leaf area of *L. multiflorum* in the three water treatments: water (W), water stress (S) and re-watering (RW) 7, 14 and 17 days after the onset of water stress (WS)

This probably means that *L. multiflorum* has the possibility to recover after small periods of water stress, and continues to produce leaves, leading consequently to higher quantity of forage production *(Heitschmidt et al., 1995)*. These results are in agreement with those reported by *Holechek et al. (1989)* for other species. On the other hand, the longer periods of water stress significantly decreased the leaf area of this species. It seems that, after seven days of water stress plants reduced their leaf area by about 40 %, while after seventeen days by 75% (Figure 1). At the same time leaf dry weight of watered stressed plants was reduced by 80% (data not shown). Therefore, by decreasing the quantity and the size of its leaves, this species decreased its forage production under water stress.

However, in grasslands apart from forage quantity, forage quality is equally important for livestock production. As already mentioned earlier, a good indicator of forage quality is the leaf nitrogen content *(Heitschmidt et al., 1995)*. The drought conditions during the experiment affected the nitrogen content of plant tissues (Figure 2). Leaf N content decreased significantly after 14 days of well watered conditions and remained at this level until the end of the experiment. As far as the water stressed plants are concerned, leaf N content was significantly lower ($P<0.05$) in relation to well watered plants seven days after the onset of water stress. The nitrogen content of water stressed plants remained constant fourteen days after the onset of water stress, while there was a significant increase after seventeen days. This could be due to the fact that under water stress the leaf-stem ratio becomes higher with less fiber as the internodes become shorter *(Kugler, 2004)*. Drought stress may delay maturity, thus improving forage quality *(Cherney, 2008)*. However, the re-watered plants presented higher leaf nitrogen content than well watered and water stressed plants after 14 days of water stress.

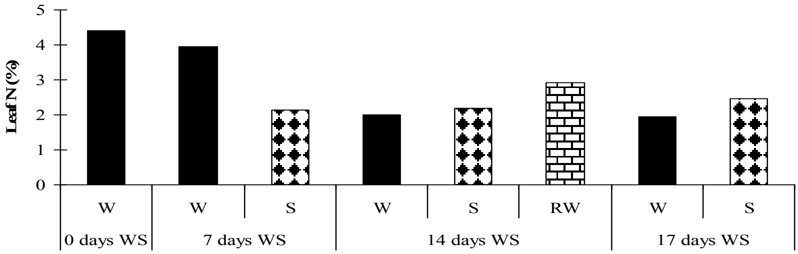

Figure 2. Leaf nitrogen content (%) of *L. multiflorum* plants in three different water treatments: water (W), water stress (S) and re-watering (RW) 7, 14 and 17 days after the onset of water stress (WS)

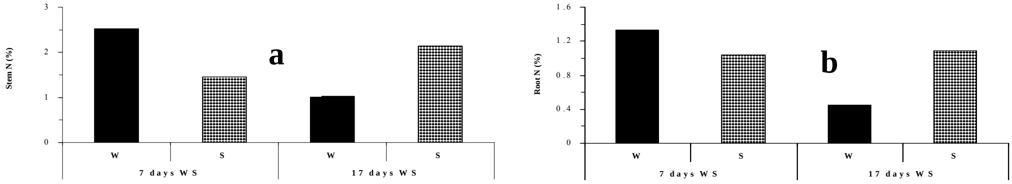

Figure 3a,b. a) Stem nitrogen content and b) Root nitrogen content of *L. multiflorum* in two different water treatments: water (W) and water stress (S) 7, 14 and 17 days after the onset of water stress (WS)

Well watered plants had significantly higher nitrogen content in stems and roots compared to the water stressed ones after seven days of water stress (Figures 3a, b). However, stems and roots of water stressed plants had significantly higher nitrogen content ten days later (day 17). In addition, N content of stems and roots of well watered plants decreased significantly as the growing season proceeded. On the other hand, stem N content of water stressed plants increased, while that of roots remained unchanged. The trend of watered stressed plants of *L. multiflorurm* to decrease their leaf area and to increase the leaf N content was verified by the negative correlation (r= -0.875, P<0.01) found between these two parameters, which is also reported for other species by *Farquhar et al. (2002). L. multiflorum* could probably decrease its leaf area and increase its leaf nitrogen content as an adaptation mechanism in order to survive under drought conditions.

Conclusions

The water treatment significantly affected both leaf area and nitrogen content in plant tissues of *L. multiflorum*. The nitrogen content decreased in leaves, stems and roots of well watered plants, while it increased significantly in stems of water stressed and in leaves of re-watered plants. At the same time, the leaf area of water stressed plants decreased. Consequently, under drought this species seems to balance lower quantity with higher quality of forage production.

Efekti suše na površinu lista i sadržaj azota kod *Lolium multiflorum* L.

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Rezime

Identifikacija krmnih vrsta koje mogu održati kvalitet i kvantitet krme sa travnjaka razvijanjem mehanizama adaptacije u sušnim uslovima postaje sve važnija zbog klimatskih promena koje utiču na mediteranske travnjake. U radu su proučavani efekti suše na površinu lista i na udeo azota kod *L. multiflorum* L. Setva je obavljena u sudove, a biljke su izložene sledećim tretmanima: a) dobro zalivane biljke, b) ne zalivane biljke i c) naknadno zalivane biljke. Vodni režim je značajno uticao na površinu lista i na sadržaj azota. Nakon sedamnaest dana, gubitak azota se povećao u listu, stablu i korenu dobro zalivanih biljaka, dok se značajno smanjio u stablu biljaka pod vodnim stresom i ponovo zalivanih biljaka. Utvrđena je negativna korelacija između površine lista i sadržaja azota, što upućuje na to da *L. multiflorum* može da razvije specifične mehanizme za adaptaciju na sušne uslove.

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THE USE OF A SILAGE INOCULANT IN SILAGES OF GRAINS OF *Pisum sativum*

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Original scientific paper

Abstract: A silage trial under laboratory conditions with mini silos was conducted with forage pea grains (*Pisum sativum*). Different biological silage inoculants (Biomin® BioStabil Plus and Mays, *L. plantarum, L. brevis and E. faecium*, 1 x 10^5 or 1x 10^6 cfu g^{-1} silage) to improve the silage quality, enhance the aerobic stability and to lower the condensed tannin (CT) content. The silages were open after 3, 7, 49 and 91 days. DM content as well as the fermentation profile, the pH value, the aerobic stability *(Honig, 1990)* and the content of CT was analyzed. The inclusion of the silage inoculant had a positive effect on the acidification and the fermentation profile, showing lower DM losses, more lactic and acetic acid, as well as lower content of butyric acid. The compared aerobic stability was excellent in all treatments. After 91 days of ensiling, no CT was found in the silages.

Key words: *Pisum sativum*, inoculant, silage, quality, tannins

Introduction

Leguminous silages have a high buffering capacity. Therefore their acidification is slow and very often insufficient to guarantee a stable preservation. High protein silages do not readily acidify because of high buffering capacities. Consequently, if the pH value does not drop within the first days of ensiling, anaerobic spoilage (e.g. by *Clostridia*) is taking place and silage will not be suitable for feeding, which is an economic loss.

Therefore it is crucial to support the acidification process – either by addition of chemical silage additives, or the natural way by adding additional lactic acid bacteria, which are able to rapidly produce large amounts of lactic acid and thus can improve fermentation quality.

The goal of this test was focused on checking the efficiency of two biological silage inoculants (Biomin® BioStabil Plus in different concentrations and

Biomin® BioStabil Mays, both blends of *L. plantarum, L. brevis* and *E. faecium,* also the first one as granulate) on the quality of grain silages of *Pisum sativum,* on the aerobic stability and the content of condensed tannins.

Material and Methods

Dried grains of *Pisum sativum* were used for the experiment and moistened to adjust their moisture content to 70% adding water. The material was ensiled using the treatments as shown in Table 1, in buckets with a capacity of 5 litters.

Table 1. Treatments for the trial

Treatment	Additive	Concentration of the biological inoculants (cfu g^{-1} silage)
BS2x5	Biomin® BioStabil Plus	2×10^5
BS1x6	Biomin® BioStabil Plus	1×10^6
BSG	Biomin® BioStabil Plus Granulate	1×10^5
BSM	Biomin® BioStabil Mays	1×10^5
C	Control	-

The additives were sprayed on the material and well homogenized. The material was put in buckets and well compacted and sealed for reaching anaerobic conditions. The mini silos were opened at the days 0, 3, 7, 49 and 91. Each treatment had 3 replicates. These parameters: DM, pH, fermentation products (HPLC), condensed tannins and the aerobic stability were studied during a week after the opening of the mini silos *(Honig, 1990)*, comparing the differences between the room and the silage temperature. Differences over 2 °C were considered as a sign of instability.

Results and Discussion

The higher DM losses (Figure 1) were found in the control treatment without additives (after 49 and 91 days, more than 6%). The treatments BS2x5 and BS1x6 had middle values (losses between 4.0 and 6.0%). Treatment with BSM had the lowest losses with less than 0.5 and 4.0% after 7 and 49 and 91 days respectively.

All treatments in acidification (graph 2) had pH values near to 4.5 after 3 and 7 experimental days. Nevertheless, the control treatment only reached pH values between 4.7 and 4.8.

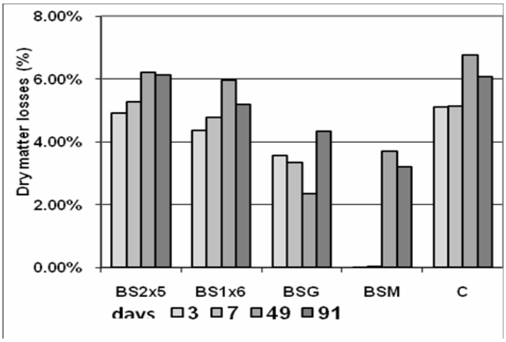


Figure 1. Dry matter losses in silages of *Pisum sativum* grains

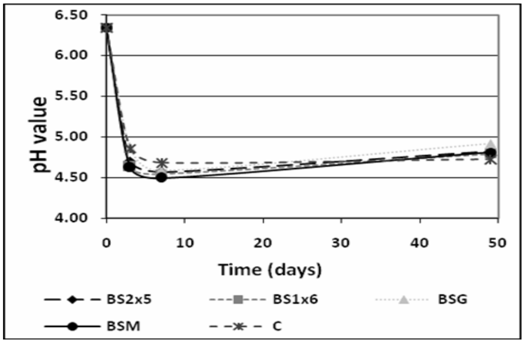
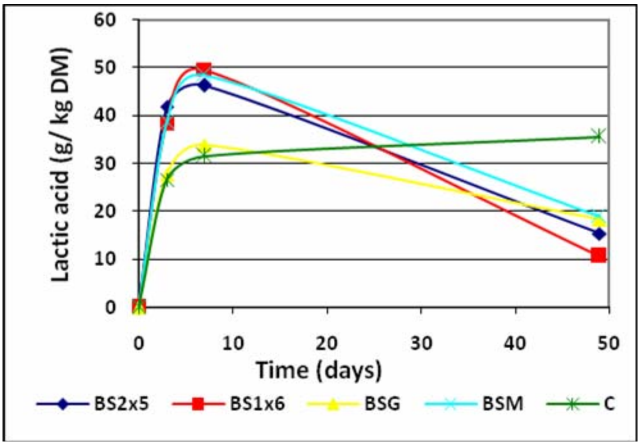


Figure 2. pH decrease in silages of *Pisum sativum* grains

Figure 3 and 4 show the contents of lactic and acetic acid after different opening times of the silages up to 49 days.



Graph 3. Lactic acid production in silages of *Pisum sativum* grains

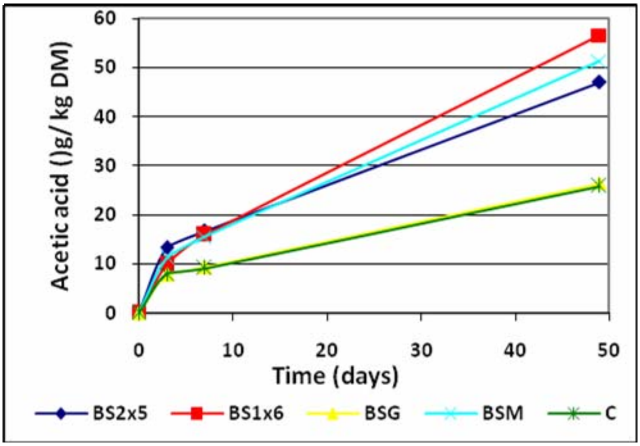


Figure 4. Acetic acid production in silages of *Pisum sativum* grains

The lactic acid production was lower in the control treatment and BSG at the beginning of the test (approx. 30 g kg^{-1} DM at the days 3 and 7, Figure 3). However, after the 49th day the lactic acid content in the control treatment increases slightly (up to 35 g kg^{-1} DM), considering that in other treatments the lactic acid content went down abruptly from 45-50 g kg^{-1} DM (after 3-7 days) to 10-20 g kg^{-1} DM at the day 49. The answer to this performance is reflected in the acetic acid production (Figure 4): the treatments BS2x5, BS1x6 and BSM produced higher acetic acid getting productions about 50-60 g kg^{-1} DM, while the control treatment did not surpassed 30 g kg^{-1} DM. Meaning that certain amount of lactic acid was degraded to acetic acid by the hetero fermentative bacteria.

The ethanol content (Figure 5) was relatively high in all treatments (between 8 and 10 g kg^{-1} DM). The most affected treatments by the high ethanol production were BSM and BSG. High ethanol content in the silage can be related to a higher contamination with yeasts.

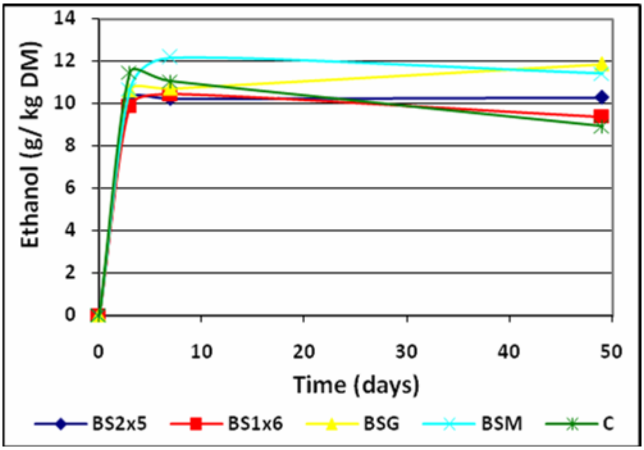


Figure 5. Ethanol content in silages of *Pisum sativum* grains

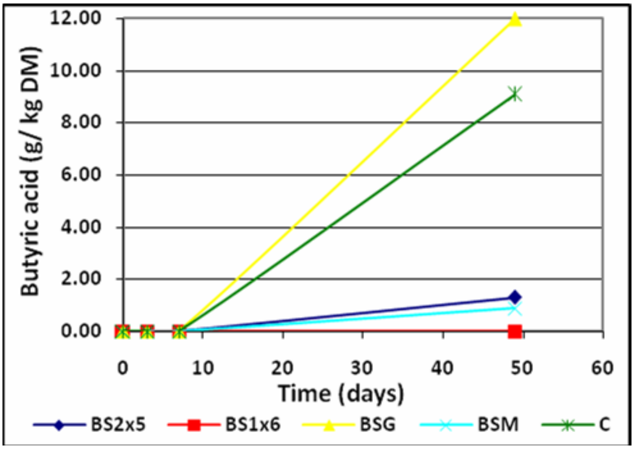


Figure 6. Ethanol content in silages of *Pisum sativum* grains

Butyric acid fermentation was found in all treatments (Figure 6), nevertheless its presence was higher in the treatments control and BSG after 49 days (9 and 12 g kg^{-1} DM respectively). These values are way higher than those recommended by the DLG (2006) of 3 g kg^{-1} DM. All silages remained stable after 192 hours (8 days).

An interesting result was related to the condensed tannin content. The raw material contained 6.67 mf g^{-1} DM. After ensiling (90 days), no condensed tannins were found in any of the silages. It corroborates the results of *Acosta Aragón (2004)*, who worked with sorghum grains with different tannin contents.

Conclusion

The use of inoculants for silages of wet grains of *Pisum sativum* had a positive effect on the acidification and fermentation profile. The dried matter losses were lower, the lactic acid production was higher as well as the acetic acid production in the inoculated silages. A concomitant positive effect of the inoculation was the lower butyric fermentation, as a sign of *Clostridia* inhibition.

Apparently the aerobic stability is not a crucial problem in this kind of silages. The ensiling process has a positive effect on the reduction of condensed tannins.

Korišćenje silažnih inokulanata u silažama zrna stočnog graška (*Pisum sativum)*

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Rezime

Vršeno je ispitivanje silaže stočnog graška *Pisum sativum* u laboratorijskim uslovima korišćenjem mini silosa. Različiti biološki silažni inokulanti (Biomin® BioStabil Plus and Mays, *L. plantarum, L. brevis* y *E. faecium*, 1 x 10^5 or 1x 10^6 cfu g^{-1} silaže) su dodavani radi poboljšanja kvaliteta silaže, povećanja aerobne stabilnosti i radi smanjenja količine kondenzovanih tanina (KT). Silaže su otvarane posle 3, 7, 49 i 91 dana. Analizirani su sadržaj SM, kao i fermentacioni profil, pH vrednost, aerobna stabilnost *(Honig, 1990)* i sadržaj KT. Primena silažnih inokulanata je imala pozitivan efekat na zakišeljavanje i na fermentacioni profil, smanjujući gubitke SM, povećavajući sadržaj mlečne i sirćetne kiseline, kao i smanjujući sadržaj buterne kiseline. Upoređivana aerobna stabilnost je bila odlična u svim tretmanima. Posle 91 dana nakon siliranja, nisu pronađeni KT u silaži.

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PHYSICALLY EFFECTIVE FIBRE IN DAIRY COWS NUTRITION AND METHODS FOR DETERMINATION

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Review paper

Abstract: The content of physically effective fibres in feeds and rations is a function of NDF concentration and physical effectiveness factor (pef). Feeds and dietary content of peNDF depend on NDF concentration and particle length. Total chewing activity (eating and ruminating time) is affected by peNDF content. Dietary physically effective fibre concentration affects animal health, ruminal pH and milk fat content. Practical system for determination of peNDF content in forages (corn silage and alfalfa haylage) and in total mixed ration (TMR) for lactating cows is based on using Penn State Particle Separator (PSPS) sieve system, both, the original PSPS with 2 sieves (19 and 8 mm) as also modified PSPS with 3 sieves (19, 8 and 1.18 mm). Methods for the peNDF content determination of forages and TMR were considered: procedure based on measuring of the proportion of DM retained on two or three, and on estimating physical effectiveness factors (pef_{2s} and pef_{3s}) used for multiplying NDF concentration of original sample; procedure based on determination of the NDF content of the original sample and all materials retained on the sieves, and estimating the proportion of NDF retained on two or on three sieves of the PSPS. Using the original PSPS with two sieves greatly differentiate the peNDF content of the corn silage, alfalfa haylage and TMR, expressed by the proportion DM or NDF retained on sieves. The original PSPS with two sieves provided a better description of the variation in physically effectiveness of forages and TMR. The peNDF contents estimated using modified PSPS with 3 sieves were markedly higher than those estimated with original PSPS. The most adequate and accurate method for determination of the peNDF content in corn silage, alfalfa haylage and TMR for lactating cows is procedure based on multiplying NDF concentration with previously estimated physical effectiveness factor, using the original PSPS with two sieves.

Key words: nutrition, cows, corn silage, alfalfa haylage, physically effective fibres

Introduction

Increased energy requirements of high-yielding lactating cows demands formulation in dairy rations with higher proportion of concentrate, lower proportion of forages and decreased dietary content of NDF. Although maximal milk production is provided, a series of metabolic disturbances are caused, including: sub acute ruminal acidosis, milk fat depression, ruminal parakeratosis, dislocation of abomasum, lameness and fat cow syndrome *(Stojanović and Grubić, 2008)*. Using dietary NDF concentration for regulation concentrate to forage ratio in diets for lactating cows appeared to be appropriate for diet formulation. However, using of dietary NDF concentration as chemical parameter for fibres content in diet does not recognize the subtle characteristics of the diet and its importance for digestion kinetics, passage of digesta, and more exactly on their physical characteristics. Physical characteristics of fibres became particularly important when there is a need for diet formulation with forage to concentrate ratio close to the lowest acceptable level. Dietary concentration of NDF is a chemical parameter of fibres content, but not a parameter of physical characteristics, such as particle length. Possibility that formulated diet meets requirements of high-yielding dairy cows depends of its chemical as also physical characteristics *(Stojanović et al. 2002; Grubić et al. 2000)*. Physical characteristics of diet for lactating cows are defined with forage to concentrate ratio, type of forage and concentrate feeds, and mean particle length of feeds. Appropriate particle length of forages in TMR is significant parameter for diet formulation that should be considered equally with diet chemical composition *(Stojanović et al. 2008)*. The concept of physically effective fibre incorporates the chemical characteristics (NDF content) of forages and diet, and particle length, expressing their value for chewing activity and ruminal function *(Mertens, 1997)*. The physically effective fibres (peNDF) are fibres that effectively stimulate salivation and rumination (*Mertens, 1997*). Content of peNDF is function of NDF concentration and physical effectiveness factor (pef). The range of pef values is between 0 when NDF is not effective in stimulating chewing activity, and 1 when feed or dietary NDF is maximally effective in inducing chewing activity. Because the forage and dietary content of peNDF is defined with their NDF concentration and particles length, the peNDF is connected with forming of rough and dry phase of ruminal content that influences on selective retention of fibres in rumen, with dynamic of ruminal fermentation and passage, and with stimulating of rumination *(Stojanović et al. 2008a)*. Dietary peNDF content affect animal health and milk fat content, considering that ruminal pH as also extent and rate of ruminal fermentation depend of saliva and saliva buffer ($NaHCO_3$) production, during the eating and rumination. Feed intake and rumination increase salivation. The minimal content of effective fibre is necessary in cows' rations. Cows that consume diets with sufficient concentration of NDF from fine chopped forages, express the similar metabolic disorders like cows on diets insufficient in fibres *(Grubić et al. 1999)*. The adequate method for determination of physical effectiveness factor of NDF for forages and TMR still has not been defined. The exact recommendations for peNDF content in diets for high-producing dairy cows are not yet established. Practical system for determination of particle length and physical effectiveness of forages (corn silage and alfalfa haylage) and TMRs for lactating cows, as also for determination the peNDF content, is based on application of Penn State Particle Separator (PSPS) system of sieves, using the original PSPS with 2 sieves (19 and 8 mm) and also using new PSPS with 3 sieves (19, 8 and 1.18 mm). In our production conditions, this is a new method and approach in formulating diets for high-yielding dairy cows that still has not been applied. According to this, the TMRs for dairy cows are formulated *ad hoc,* not considering their physical form that affects cows' health, production performances, productive life, and economical efficiency of milk production.

The effects of physically effective fibre concentration in diet on physiological and production characteristics of lactating cows

Source of the NDF in ration is the most significant for fibres effect in rumen. *Stojanović et al. (2009)* found high correlations between cut length of forages (corn silage and alfalfa haylage) and mean particle length of TMR. Forages with adequate physical form supply fibres in form that is considerably different from NDF found in concentrates. Minimal recommended value for fibre content in TMR for lactating cows should be 25% (DM basis), of which 19% should be from forages (*NRC, 2001*). This value for fibre concentration is increased with lower content of NDF from forages and with shorter chop length of forages. *Mertens* (*1997*) reported that concentration of peNDF in rations for cows at early and mid lactation should be 20% in DM, for maintaining milk fat content at 3.40%. The peNDF concentration of 22% in ration (DM base) maintains mean ruminal pH at 6. Diets with the content of physically effective fibres lower than 25% (DM) and with less than 16% of NDF from forages induced milk fat depression *(Depies and Armentano, 1995)*. In addition to milk fat content issue, significant parameters for adequacy of dietary peNDF concentration are ruminal acetate to propionate ratio, ruminal pH and chewing activity. *Norgaard (1986)* found that ration for lactating cows should provide chewing activity of 30 min/kg of consumed DM in the purpose of maintaining desirable milk fat percent and the optimal ruminal fermentation. Providing for 3.6% milk fat, requires 744 min/day or 36.1 min/kg DM of chewing time, that is supplied with 5.01 kg peNDF/day, in other words with 24% of the peNDF in DM of lactating cows diet *(Mertens, 1997)*. Increasing the corn silage cut length (4.8, 15.9 and 28.6 mm) in TMR for lactating cows, increased CP and fibre (NDF, ADF) total tract digestibility *(Yang and Beauchemin,* *2006)*. Cows fed diet with higher peNDF content expressed tendency for higher milk production, there were no effects on milk fat content, while total chewing time (intake and rumination) was significantly increased. Lack of the effect of different peNDF content on milk fat and protein percentage, can be explain with sufficient concentration of NDF from forages in diet, for maintaining milk fat content above 3.50%. *Zebeli et al. (2006)* found that an increase of the peNDF content in TMR by 1.0% increase the NDF digestibility by 0.41%. Milk fat content was linearly higher with higher dietary concentration of NDF and digestible OM from forages. Milk to protein ratio was greater linearly too, with higher dietary concentration of NDF, peNDF and digestible OM from forages. Content of the peNDF determined using Penn State Particle Separator with 3 sieves ($peNDF_{1.18}$) is adequate parameter of ruminal pH (r=0.67) and NDF digestibility (r=0.56). Lower cut length of alfalfa haylage (22.3 to 4.8 mm) in the diet for Holstein cows in early lactation increases DM intake. Digestibility of the DM, OM, crude protein, fibres (NDF and ADF), NFC and ether extract are also higher. Acetate to propionate ratio is linearly lower with shorter chop length of alfalfa haylage, and total chewing time is decreased too *(Kononoff and Heinrichs, 2003a)*.

Table 1. Effects of length of alfalfa haylage cut in TMR on cows performances in early lactation (*Kononoff and Heinrichs, 2003a*)

Parameter	Cut length			
	Long	Medium long	Medium short	Short
Intake, kg/day				
DM	20.1	20.7	21.8	23.4
Digestibility, %				
DM	63.1	64.4	63.7	66.5
OM	65.4	66.9	66.0	68.4
Crude protein	53.2	53.6	54.0	58.6
NDF	44.7	46.9	45.6	48.1
ADF	52.2	52.8	50.4	54.0
NFC	86.5	87.5	87.5	88.4
Ether extract	52.9	53.5	54.1	58.4
pH	6.09	6.13	6.15	6.04
Volatile fatty acids, mM/l				
Acetate	85.9	88.1	88.3	92.2
Propionate	29.8	31.0	31.5	33.9
A:P ratio	2.92	2.88	2.86	2.75
Milk yield and chemical composition				
Milk yield, kg/day	36.0	34.8	35.3	36.0
Milk fat, %	3.31	3.31	3.27	3.38
Milk protein, %	2.82	2.93	2.91	2.90
Total chewing time				
min/day	776.7	768.3	758.9	723.4
min/kg consumed DM	37.9	37.5	35.2	31.2
min/kg consumed NDF	120.8	119.2	112.1	99.7

Yang and Beauchemin (2007a) emphasized the importance of intake of peNDF and NDF from forages, compared to total dietary NDF intake for optimal ruminal fermentation. Longer particles of forages form ruminal mat that stimulates contractions of reticulo-rumen. Without these movements, rumen becomes less dynamic system, with lower efficiency for volatile fatty acid elimination through absorption and fluid passage, and increasing risk for occurrence of acidosis. Intake of longer particles of forages decreases ruminal starch fermentation increases the extent of starch digestion in the intestines, and that way decreases ruminal concentration of volatile fatty acids and increases acetate to propionate ratio. There is a greater possibility for expression of milk fat depression when dietary concentration of the NDF and peNDF is under required value. Chop length of forages in TMR for lactating cows doesn't affect DM intake when dietary content of concentrate is above 40% (DM base). Decreased cut length of forages increases DM intake in cows fed high-forage diets or diets with low-quality forages (*Krause et al. 2002*). *Krause and Combs (2003)* analyzed the effects of alfalfa chop length (5.3 and 2.7 mm) and corn silage (5.6 and 2.8 mm) in TMR. Percentage of milk fat was decreased (3.07 and 2.90 %) with lower cut length of forages. Period when ruminal pH was under 5.8 was longer. Efficiency of milk production expressed as kg of milk per kg of consumed DM was improved with reduced cut length. Chewing time during eating and rumination was also decreased, from 11.0 to 9.9 min/kg DM and from 19.2 to 16.3 min/kg DM, respectively. Ruminal acetate to propionate ratio was decreased with reduction of forages cut length. Authors defined that 2.0 is minimal value for acetate to propionate ratio, in connection to appearance of acidosis. In study of *Woodford and Murphy (1988)* total chewing activity of 28.2, 24.1 and 20.0 min/kg of consumed DM, resulted in milk fat content of 3.0, 2.9 and 2.6 %, respectively, for cows producing between 31.8 and 35.5 kg per day. Using finely chopped alfalfa haylage (3.02 mm) compared to longer chopped alfalfa haylage (9.57 mm) in diet for primiparous cows at early lactation (first 8 weeks of lactation) decreased milk fat percentage (3.73 and 3.41 %), and yield of 4% FCM (26.54 and 24.81 kg/day). Multiparous cows also expressed lower milk fat content (3.69 and 3.49 %), *(Fischer et al. 1994)*. Lower dietary concentration of the peNDF induces higher decrease in milk fat content if dietary content of NDF is smaller and ruminal pH is lower *(Mertens, 1997)*.

Methods for determination of physically effective fibres

System for the peNDF content determination based on chemical and physical analysis consists of measuring the NDF concentration in feeds and diet, and measuring the proportion of particles retained on screen with 1.2 mm in diameter *(Mertens, 1997)*. Physical effectiveness factor (pef) is equal to the proportion of particles larger than 1.2 mm, and content of physically effective fibres - peNDF calculated by multiplying concentration of NDF in feeds or diet with pef. *Lammers et al. (1996)* developed equipment Penn State Particle Separator (PSPS) that consists of 2 sieves with holes of 19 and 8 mm in diameter. This system is practical and efficient method for determination distribution of TMR particles in forages. It is suggested that PSPS system of sieves is more practical version than laboratory particles fraction separator that is specified by standard S424 (American Society of Agricultural Engineers). Modified version of PSPS contains additional third sieve with hole of 1.2 mm in diameter *(Kononoff et al. 2003)*. *Buckmaster et al. (1997)* defined a system for determination of consumed peNDF, based on measuring mass of particles retained on sieves of original PSPS (19 mm and 8 mm) and chemical analyses for NDF content in each fraction of particles. In study of *Yang and Beauchemin (2007)* the effect of dietary concentration of peNDF was analyzed using different cut length of alfalfa haylage (7.9 and 19.1 mm). Physically effectiveness factor - $pef_{8.0}$ for TMR estimated using the original PSPS with 2 sieves, ranged between 0.31 and 0.54 for treatments, and determined $peNDF_{8.0}$ ranged between 9.6 and 19.8%. While values for $pef_{1.18}$ estimated using new PSPS with 3 sieves, were between 0.93 and 0.94, and values for $peNDF_{1.18}$ were between 28.6 and 34.0%. This indicates lack of sensitivity of new PSPS for changing of cut length of forages and for changing of forage to concentrate ratio. *Beauchemin et al. (2003)* reported an increase of the physically effective fibres content in TMR by 50% when peNDF content was determined as $peNDF_{1.18}$ compared with $peNDF_{8.0}$. Study of *Kononoff and Heinrichs (2003b)* indicates that proportion of particles >1.18 mm does not differ markedly for diets based on corn silage with different cut length, which indicates that using new PSPS is not the best method for determination of effective fibres. Measuring the proportion of larger fractions (>19 and 8 mm) is better parameter of physically effectiveness of fibres. Proportion of TMR fraction retains on three sieves is about 30-50%. *Stojanović (2010)* analyzed effects of reduction of cut length of corn silage and alfalfa haylage (theoretical cut length 22.3, 19.1, 15.9 and 7.9 mm) on content of the peNDF in forages and TMR for high-producing cows at early lactation, and the results are shown in Table 2.

Table 2. Physically effective fibre (peNDF) content of the corn silage, alfalfa haylage and TMRs (% DM), ***(Stojanović, 2010)***

Parameter	Treatment			
	Long	Medium-long	Medium-short	Short
Corn silage				
pef_{3s}	0.98	0.98	0.97	0.97
pef_{2s}	0.71	0.69	0.68	0.61
$peNDF_{3s}$	46.09	46.09	45.62	45.62
$peNDF_{3s-ndf}$	46.46	46.42	46.13	46.10
$peNDF_{2s}$	33.39	32.45	31.98	28.69
$peNDF_{2s-ndf}$	36.06	33.78	33.00	29.70
Alfalfa haylage				
pef_{3s}	0.90	0.91	0.89	0.89
pef_{2s}	0.61	0.56	0.54	0.51
$peNDF_{3s}$	39.27	39.70	38.83	38.83
$peNDF_{3s-ndf}$	40.42	41.12	40.22	40.38
$peNDF_{2s}$	26.61	24.43	23.56	22.25
$peNDF_{2s-ndf}$	29.01	26.28	25.81	23.92
TMR				
pef_{3s}	0.96	0.93	0.93	0.84
pef_{2s}	0.65	0.61	0.60	0.47
$peNDF_{3s}$	29.76	28.83	28.83	26.04
$peNDF_{3s-ndf}$	30.35	29.43	29.26	28.23
$peNDF_{2s}$	20.15	18.91	18.60	14.57
$peNDF_{2s-ndf}$	21.41	20.22	19.21	16.8

Particle size distributions of the forages and the TMR were measured using Penn State Particle Separator.

pef_{3s} and pef_{2s} – Physical effectiveness factors determined as the proportion of particles retained on two sieves and three sieves, respectively.

$peNDF_{3s}$ and $peNDF_{2s}$ – Physically effective fibre determined as NDF content multiplied by pef_{3s} or pef_{2s}, respectively.

$peNDF_{3s-ndf}$ and $peNDF_{2s-ndf}$ – Physically effective fibre determined as the proportion of NDF retained on two or on three sieves of the PSPS.

The determined content of the peNDF for corn silage, alfalfa haylage TMR is considerably higher when method based on new PSPS is used ($peNDF_{3s}$ and $peNDF_{3s-ndf}$), compared with method based on using original PSPS with 2 sieves ($peNDF_{2s}$ and $peNDF_{2s-ndf}$). As the most adequate method for measuring peNDF content in forages and TMRs the original PSPS may be recommended using. Results of measuring content of the peNDF in corn silage (cut length 28.6, 15.9 and 4.8 mm) using PSPS with 2 sieves and the new version with 3 sieves, indicate that application of original version provides the most real parameter about content of physically effective fibres, potentials of diet to stimulate chewing activity, and to prevent ruminal acidosis *(Yang and Beauchemin, 2006)*. Results for peNDF concentration determined using new PSPS are higher compared to those predicted by the original PSPS. The range of values for the peNDF content is significant greater for different cut length of corn silage (peNDF$_{2s}$ 35.2-17.4% and peNDF$_{2s\text{-}ndf}$ 38.2-19.8 %) compared to those determined using the PSPS with 3 sieves (peNDF$_{3s}$ 44.4-47% and peNDF$_{3s\text{-}ndf}$ 50.1-47.3 %).

Conclusion

Appropriate particle length of forages in total mixed ration is significant parameter for diet formulation that should be considered along with its chemical composition. Physical characteristics of diet affects animal health, ruminal fermentation, chewing activity, efficiency of feed utilization, metabolic processes, milk fat content, almost independently from chemical concentration of the NDF. Efficient method for determination physical effectiveness of forages and TMRs for lactating cows is based on using Penn State Particle Separator. Application of the original PSPS with 2 sieves provides the most real estimations of the peNDF content in forages and diets.

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Fizički efektivna vlakna u ishrani krava i metode za determinisanje

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Rezime

Sadržaj fizički efektivnih vlakana u hranivu i obroku je funkcija koncentracije NDF i faktora fizičke efektivnosti (pef). Koncentracija peNDF u hranivu ili obroku uslovljena je koncentracijom NDF, veličinom čestica i stepenom usitnjenosti hraniva. Sadržaj peNDF u obroku uslovljva aktivnost žvakanja pri konzumiranju i preživanju hrane, formiranje grublje, suve faze ruminalnog sadržaja, dinamiku ruminalne fermentacije i pasaže. Od sadržaja fizički efektivnih vlakana zavisi zdravlje životinja, ruminalna pH vrednost, sadržaj masti u mleku. Praktičan sistem za utvrđivanje koncentracije peNDF u kabastim hranivima (silaža kukuruza i senaža lucerke) i kompletnim obrocima za krave u laktaciji zasniva se na primeni sistema sita Penn State Particle Separator (PSPS), i to korišćenjem originalnog PSPS sistema sa 2 sita (19.1 i 8 mm) i modifikovanog sistema sa 3 sita (19.1, 8 i 1.18 mm). Uporedno su razmatrane metode za determinisanje sadržaja

peNDF, i to postupak zasnovan na merenju mase materijala koji ostaje na sitima PSPS-uređaja, i izračunavanju faktora fizičke efektivnosti (pef) kabastih hraniva i kompletno miksovanog obroka, kao i postupak zasnovan na merenju mase i hemijskoj analizi sadržaja NDF u svakoj frakciji. Korišćenje originalnog Penn State Particle Separator sistema sa 2 sita, pruža najrealniju sliku sadržaja fizički efektivnih vlakana, sa znatno većim opsegom variranja utvrđenih vrednosti za sadržaj peNDF za različite stepene usitnjenosti kabastih hraniva i obroka. Vrednosti za sadržaj peNDF dobijene primenom nove verzije PSPS-sistema sita, veće su u odnosu na one koje predviđa originalna verzija PSPS-uređaja. Najpogodniji i najprecizniji metod za determinisanje sadržaja fizički efektivnih vlakana u silaži cele biljke kukuruza, senaži lucerke i kompletnim obrocima za krave u laktaciji, predstavlja postupak koji se zasniva na množenju sadržaja NDF u SM hraniva, prethodno određenim faktorom fizičke efektivnosti hraniva (pef), korišćenjem PSPS uređaja sa 2 sita.

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RELATIVE FEED VALUE OF ALFALFA (*Medicago sativa* L.) AND RED CLOVER (*Trifolium pratense* L.) AT DIFFERENT STAGE OF GROWTH

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Abstract: The effect of the development stage of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) on chemical composition, RFV (relative feed value), DDM (digestibility dry matter) and DMI (dry matter intake) was investigated. Research was conducted in three stages of development of alfalfa, *cv* K-23 and red clover, *cv* K-17 in the second cut. Digestibility dry matter, dry matter intake and relative feed value were calculated according to the appropriate equations adapted from common formulas for forages. The stage of plant development has a significant influence on the chemical composition and the relative feed value of alfalfa and red clover. With growth and development NDF (neutral detergent fibre), ADF (acid detergent fibre) and lignin content increased, whereas CP (crude protein) content, DDM, DMI and RFV decreased. Alfalfa retains high nutritional value from first to third development stage, while RFV of red clover decreases much faster with growth and development.

Key words: alfalfa, red clover, relative feed value, stage of growth

Introduction

The possibility to accurately predict the nutritive value of forage crops is a prerequisite for designing rations and directing forage crops breeding *(Kruse et al., 2008)*. Nutrient deficiencies in low quality roughages affect microbial growth and fermentation in the rumen and result an overall low animal productivity *(Assefa and Ledin, 2001)*. Quality forage promotes good health and better performance *(Harris, 1992)*. Therefore, choosing superior yield and quality forage genotypes is very important for animal health as well as high milk and meat production.

Feed quality of alfalfa and red clover harvested as haylage or hay depend, to a great extent, on the maturity of the stand. With increasing maturity, plant structural carbohydrates, as measured by the ADF and NDF fractions, increase. These fibre fractions represent the more indigestible parts of the plant. As a result, digestibility and energy obtained through fermentation decrease with maturity *(Tavlas et al., 2009)*. RFV has been used for years to compare the quality of legume and legume / grass hays and silages. Having one index to price hay and predict animal performance has been very useful for livestock producers and hay farmers. CP, ADF, NDF and RFV are important criteria for determining hay quality in animal feeding *(Calobro et al., 2007)*.

The main objective of this work was to assess the chemical composition and relative feed value of the alfalfa and red clover in different development stages of the second crop production cycle.

Materials and Methods

The experiment was designed as factorial trials, by randomized block system in three replicates. Soil type was with an organic matter content of approximately 3.5 % and a pH of 6.5. Three stages of growth of alfalfa (*Medicago sativa* L.) *cv* K-23 and red clover (*Trifolium pratense* L.) *cv* K-17 were examined after the first cutting. Samples were hand cut with scissors at 5 cm height. The first stage was cut on June 17th (before flowering), another one on June 24th (in flowering), and a third one on July 1st (at full bloom stage). Dry matter was determined by drying out samples at 65ºC and grinding and sieving them to 1 mm particle size.

CP (crude protein) was computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner (1960). NDF (neutral detergent fibre) and ADF (acid detergent fibre) were determined by the method of *Van Soest (1963)*. Lignin content in ADF was determined as lignin insoluble in 72% H_2SO_4, applying the method of *Van Soest (1963)*. Digestibility dry matter (DDM), dry matter intake (DMI) and relative feed value (RFV) were calculated according to the following equations adapted from common formulas for forages *(Schroeder, 1994)*:

DDM % (Digestibility Dry Matter) = 88.9 – (0.779 × ADF %)
DMI % (Dry Matter Intake (% of Body Weight) = 120 / NDF
RFV (Relative Feed Value) = (DDM % × DMI %) / 1.29

Data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmetic means was tested by LSD test.

Results and Discussion

The maturity stage had a significant effect on the chemical composition and some estimated parameters of alfalfa and red clover. With increasing maturity

NDF, ADF and lignin content increased, and CP content, DDM, DMI and RFV decreased in both investigated forage crops (Table 2 and 3).

The results show that crude protein content decreased in alfalfa from 25.4% of DM (first stage of development) to 19.3% of DM (third development stage). Lower CP content was found in red clover (from 24.9% of DM at the first stage of growth to 16.9% of DM at the third stage of growth). The decline in protein concentration with advancing maturity occurs both because of decrease in protein in leaves and stems, and because stems, with their lower protein concentration, make up a larger portion of the herbage in more mature forage *(Buxton, 1985).* Concentration of NDF and ADF increased significantly as the alfalfa and red clover matured. This result is in agreement with the report of *Kamalak et al. (2005)* who found that NDF and ADF content increased. The highest content of NDF and ADF was recorded in the third stage of development in both investigated forage species (Table 2 and 3). Our findings showed that the lignin content increased with progress of plant development both in alfalfa (from 5.7 to 8.3%) and red clover (from 3.5 to 6.0%).

Significant differences were determined among forage stages of growth in terms of DDM, DMI and RFV for animal feeding. As a result of differences in ADF contents, DDM contents of investigated forage species were also different. The highest DDM ratio was found at the first stage of plant development (66.9 in alfalfa and 65.1% in red clover). The lowest DDM ratio was found at the third stage of plant development (60.8% in alfalfa and 62.7% in red clover). Because NDf contents of the investigated forage crops were different at different stage of growth, DMI ratios were also different.

As a result of differences determined in DDM and DMI values among alfalfa and red clover, RFV values were also different. The RFV of alfalfa and red clover hays harvested at different maturity stages ranged from 177.4 to 132.2 and from 168.2 to 113.0, respectively. As can be seen from the quality standards (Table 1), the grade of alfalfa hay harvested before flowering was prime, whereas those of alfalfa hay harvested at flowering and late maturity were premium-1.

Table 1. Legume, grass and legume-grass mixture quality standards

Quality standard[a]	CP, % of DM	ADF, % of DM	NDF, % of DM	RFV[b]
Prime	>19	<31	<40	>151
1-Premium	17-19	31-35	40-46	151-125
2-Good	14-16	36-40	47-53	124-103
3-Fair	11-13	41-42	54-60	102-87
4-Poor	8-10	43-45	61-65	86-75
5-Reject	<8	>45	>65	<75

[a] standard assigned by Hay Market Task Force of American Forage and Grassland Council

[b] Relative Feed Value (RFV)-Reference hay of 100 RFV contains 41% ADF and 53% NDF

The grade of red clover hay harvested before flowering was prime, as well as alfalfa hay at the same stage of maturity, at flowering it was premium-1, and at full bloom stage it was good-2. These values are in agreement with those reported by *Stallings (2005).* Nutritionally red clover is similar to alfalfa, but generally contains slightly less CP as compared to alfalfa at similar maturity. An advantage of alfalfa over red clover is that feeding value decreases slower with advancing maturity.

Table 2. Nutrient composition and relative feed value of alfalfa, cv K-23 at different growth stages, (% of DM)

Stage of growth	CP	NDF	ADF	Lignin	DDM	DMI	RFV	Description
I stage	25.4	35.1	28.2	5.7	66.9	3.4	177.4	Prime
II stage	21.7	39.5	30.8	7.2	64.9	3.0	152.7	Premium
III stage	19.3	42.8	36.0	8.3	60.8	2.8	132.2	Premium
$LSD_{0.05}$	0.376	0.603	0.829	0.305	0.628	0.097	3.264	
$LSD_{0.01}$	0.570	0.914	1.256	0.462	0.952	0.147	4.945	

Table 3. Nutrient composition and relative feed value of red clover, cv K-17 at different growth stages, (% of DM)

Stage of growth	CP	NDF	ADF	Lignin	DDM	DMI	RFV	Description
I stage	24.9	38.2	25.4	3.5	65.1	3.1	168.2	Prime
II stage	20.8	43.8	30.7	5.0	64.9	2.7	137.9	Premium
III stage	16.9	51.7	33.6	6.0	62.7	2.3	113.0	Good
$LSD_{0.05}$	0.176	2.887	0.365	0.480	0.312	0.115	6.496	
$LSD_{0.01}$	0.267	4.375	0.553	0.728	0.473	0.175	9.843	

The decrease in DDM, DMI and RFV are possibly associated with increased NDF and ADF contents *(Wilson et al., 1991)* and increased lignification and decreased leaf / stem ratio *(Hides et al., 1983)* as the plant matures. At early stages of growth, all parts of plants are highly digestible, but during stem elongation and flowering there is a more rapid decline in the digestibility of stem than of leaf. In representing DMI and DDM as function of NDF and ADF alone, RFV does not explicitly consider plant-related factors that affect DMI and DDM.

Nutrient requirements differ among dairy cows based on production, age, and body condition. Therefore, all cows do not need the same quality of forage. Relative feed value is a way of comparing forage quality and can be an aid in matching forage quality with animal performance. The values are on a relative scale with full bloom alfalfa set at 100. Forages with an RFV greater than 100 are superior to full bloom alfalfa, and forages with an RFV less than 100 are nutritionally inferior to full bloom alfalfa. Early lactation and high producing cows should be fed forages with an RFV of at least 130. Medium producing cows can be fed forages with an RFV of 100-120. Late lactation cows can usually be fed forages with an RFV of around 100.

Conclusion

The choice of the harvesting moment is important insuring the forage crops quality and quantity. Relative feed value might be a relevant indicator supporting the decisions of cutting management, especially when red clover and alfalfa are a component of a legume-grass mixture. This experiment showed a decreasing of the alfalfa and red clover RFV with plant development. Stage of growth showed a significant statistical influence on RFV, DDM and DMI of both investigated forage crops. The most adequate decision of cutting might be in mid-bloom stage, when even the RFV is fast decreasing, the forage yield and crude protein content are high.

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Relativna hranljiva vrednost lucerke (*Medicago sativa* L.) i crvene deteline (*Trifolium pratense* L.) u različitim fazama razvića

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Rezime

Ispitivan je uticaj faze razvića lucerke (*Medicago sativa* L.) i crvene deteline (*Trifolium pratense* L.) na hemijski sastav, relativnu hranljivu vrednost, svarljivost suve materije i konzumiranje suve materije. Istraživanja su obuhvatila tri faze razvića sorte K-23 lucerke i K-17 crvene deteline u drugom otkosu. Svarljivost suve materije, konzumiranje suve materije i relativna hranljiva vrednost su određene računskim putem. Utvrđeno je da faza razvića ima značajan uticaj na hemijski sastav i relativnu hranljivu vrednost lucerke i crvene deteline. Sa rastom i razvićem udeo NDF, ADF i ADL se povećava, dok se udeo sirovih proteina, svarljivost suve materije i konzumiranje suve materije smanjuje. Lucerka zadržava

visoku hranljivu vrednost sve do treće faze razvića, dok se kod crvene deteline ova vrednost mnogo brže smanjuje sa rastom i razvićem.

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TRYPSIN INHIBITORS IN SOME GRAIN LEGUMES

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Review paper

Abstract: One of the limitations for using of grain legumes in animal feeding is the presence of diverse anti-nutritional factors, which decrease nutritive value of grain legumes. Most important thermo-labile anti-nutritional factors are protease inhibitors. Serine protease inhibitors in legume species belong to two different families, referred as Kunitz and Bowman-Birk inhibitor family. There are differences of distribution of these two inhibitors among grain legumes. In soybean, Kunitz type inhibitor is dominant and due to selective soybean breeding major advancements was done by lowering TI activity of raw soybean. The pea seed trypsin inhibitors belong to the Bowman-Birk family of inhibitors and breeding effort is done by decreasing inhibitor activity to low and very low extents.

Key words: grain legumes, pea, soybean, trypsin inhibitors.

Introduction

Grain legumes represent one of the most significant crops in the world. The term *grain legumes* denotes mostly annual crops grown for immature or mature grain, comprising *food legumes*, also known as *edible legumes* or *pulses*, used for human consumption, and *feed* or *fodder* legumes, used in animal feeding *(Mihailović et al., 2004)*. On the other hand, *forage legumes* are used in the form of forage and have an additional role as a source of biomass and green manure *(Mihailović et al., 2007b)*. In animal feeding, generally, nearly all annual legume crops may be used in many ways, such as green forage, forage dry matter, forage meal, silage, haylage, immature grain, mature grain and straw *(Mikić et al., 2006)*.

Grain legumes have a special importance in animal feeding as one of the most quality and least expensive answer for a constant demand for plant protein. Grain legumes have a rather high crude protein content, ranging from about 270 g kg^{-1} in pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) grain dry matter, over nearly 400 g kg^{-1} in white lupin (*Lupinus albus* L.) to more than 500 kg^{-1} in soybean (*Glycine max* (L.) Merr.) meal *(Đorđević, et al. 2005; Mihailović et al., 2007a)*.

One of the limitations to a non-limited use of grain legumes in animal feeding is the presence of the diverse compounds known as anti-nutritional factors, that both decrease nutritive value of grain legumes and, if taken in larger amounts, cause a potentially fatal outcome for both humans and the animals. That is the reason why one of the goals of diverse breeding programmes in grain legumes is to decrease the content of anti-nutritional factors to a safe extent, leading to an increased proportion of grain legumes in diets for all species and categories of animals *(Đorđević et al., 2008a)* One of the most widely distributed anti-nutritional factors are trypsin inhibitors *(Mikić et al., 2009)*.

Biochemistry of trypsin inhibitors

Serine protease inhibitors in legume species belong to two different families, referred as Kunitz (Fig. 1) and Bowman-Birk (Fig. 2) inhibitor family. Kunitz type inhibitors (KTI) have a molecular mass of approximately 20 kDa, with two disulfide bridges. Only single disulfide bond is enough for stabilities of the reactive site of inhibitor. The reactive site loop of KTI adopts a canonical conformation similar to those in other substrate-like inhibitors. Inhibitor reacts with trypsin in 1:1 ratio while with chymotripsin in 1:2 ratios *(Rawlings et al., 2004)*. Bowman-Birk inhibitors (BBI) have a molecular mass in the range of 8-10 kDa, with seven disulfide bridges. BBI are double-headed serine protease inhibitors with a network of highly conserved disulfide bridges. Single BBI polypeptides comprise an arrangement of two sub-domains with conserved array disulfide bridges, which play a important role in the stabilization of their reactive site configuration *(Qi et al., 2004)*. These inhibitors interact simultaneously and independently with two (not necessarily identical) molecules of protease. Partial peptic digestion yields two active fragments, by splitting molecules on two homologous regions. The first reactive site of most legume inhibitors inhibits trypsin, whereas the second one inhibits trypsin, chymotrypsin and elastase.

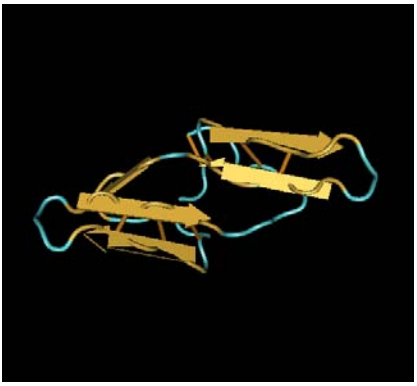
 (a)  (b)

Figure 1. Structure of Kunitz (a) and Bowman-Birk (b) inhibitors

Kunitz and Bowman-Birk inhibitors cause enlargement of the pancreas in rodents and birds, and hyper secretion of digestive enzymes. This leads to a loss of sulphur-rich endogenous proteins, trypsin and chymotrypsin. Both inhibitors act by suppressing negative feedback regulation of pancreatic secretion through increased release of the hormone cholecystokinin from the intestinal mucosa *(Lajolo and Genovese, 2002)*.

KTI family is widely spared over living organism, while BBI proteins were found in only in *Fabaceae* and *Poaceae*. Evolution of KTI and BBI among legume species suggests that the family of KTI is gradually replaced with BBI in the process of evolution of legume species *(Norikoa et al., 1988)*.

Trypsin inhibitors in soybean

The main protease inhibitors in soybean - Kunitz trypsin inhibitor (KTI) and the Bowman–Birk inhibitor (BBI) constitute at least 6% of the protein present in soybean seed *(Ryan, 1973)*. Trypsin inhibitors are responsible for the reduced digestibility of seed proteins, and for this reason, constitute the main anti-nutritional factors of soybeans. About 80% of the inhibition of the tryptic activity in soybean seed is caused by Kunitz-trypsin inhibitor *(Brandon, 1993)*. Many breeding programs have a goal to lower level of KTI in soybean seed *(Đorđević et al., 2009)*. Five electrophoretic forms of SKTI have been discovered. The genetic control of four forms, *Ti a, Ti b, Ti c*, and *Ti d*, has been reported as a codominant multiple allelic series at a single locus *(Orf and Hymowitz, 1979)*. The same authors found that the fifth form does not exhibit a soybean trypsin inhibitor and is inherited as a recessive allele designated *ti*. The Ti locus has been located on linkage group 9 in the classical linkage map of soybean *(Kiang, 1987)*. Recessive form was found in soybean germplasm samples PI 157440 and PI 196168, lacking

KTI. The germlasm PI 157440 was a parent of the first registered Kunitz-free genotype named Kunitz. This variety was developed by backcrossing and is the progeny of an F_2 plant selected from the fifth backcross, Williams 82 and PI 157440 *(Bernard et al., 1991)*.

The major advancements in lowering TI activity of raw soybean in the breeding program of Maize Research Institute Zemun Polje *(Srebrić and Perić, 2009)* are achieved by crossing parent donor of desirable character (*titi* line) with high yielding varieties (*Ti* line). Identification of lines lacking KTI is done by protein electrophoresis of mature seed from the individual plants in several segregating generations *(Đorđević et al. 2008b)*. After field trials and yield testing, two varieties lacking KTI – Lana and Laura, were released *(Srebrić at al., 2008)*. Trypsin inhibitor content in this varieties ranges from and 15,01 mgg^{-1} for Laura to 15,35 mgg^{-1} for Lana which was about 50% reduced as compared with the genotypes of standard grain type *(Perić at al.,2009)*. This type of varieties is suitable for small farms with direct feed production and animal growing *(Đorđević, 2009)*. Recently, indirect selection based on DNA marker linked to *Ti* locus became an easier and more efficient method than protein electrophoresis for detecting genotypes of interest. Several studies reported DNA markers (RAPD and SSR) tightly linked to *Ti* locus *(Kim et al., 2007)*. For markers to be most useful in breeding programs, they should reveal polymorphism in different genetic backgrounds, which is referred to as marker validation.

The Bowman-Birk protease inhibitor genes in wild and cultivated soybean are a multigene family with at least 7 family members, and genetic control is presumed to be similar to KTI *(Wang, 2008)*. Removal of KTI from seed without any intervention upon BBI family, although reducing the processing costs, still does not solve a problem of direct livestock nutrition. Unfortunately, nulls for the BBI were not found in *G.max* or *G.soja (Domagalsky et al., 1992)* but only in perennial species, for which problems of crossability and fertility occur. Due to previous reasons, breeding for this trait is more difficult and complex.

Trypsin inhibitors in pea and some other grain legumes

Along with lectins, trypsin inhibitors are the main anti-nutritional factors in pea grain *(Mihailović et al., 2005)*. Their activity is controlled by the gene *Tri* that has been mapped on pea linkage group 5 *(Domoney et al., 1994)*. As in other grain legumes, these factors have a proteinaceous nature, belonging to albumins, and are inactivated by high temperatures or soaking in formaldehyde. The purification of the pea seed trypsin inhibitors revealed these belong to the Bowman-Birk class of inhibitors *(Domoney et al., 1993)*. According to their trypsin inhibitor activity, expressed by trypsin inhibitor unit (TIU) per grain dry matter (DM), feed pea cultivars are classified into four groups: 1) very low activity (2-4 TIU mg^{-1} DM), low activity (4-7 TIU mg^{-1} DM), medium activity (7-10 TIU mg^{-1} DM) and fairly high activity (10-13 TIU mg^{-1} DM). Due to achievements in breeding, responding to the demands by animal husbandry, all modern feed pea cultivars have low or very low trypsin inhibitor activity, making farmers independent from processing industry and providing them with an excellent source of quality plant protein. Although it is confirmed that environment may influence trypsin inhibitor activity *(Bacon et al., 1995)*, it is certain that genotype remains the most important factor in its expression and thus underlines the role of breeding.

The activity of trypsin inhibitors in grass pea (*Lathyrus*) is about 28 TIU mg^{-1} DM and is not correlated to environment and grain yield *(Wang et al., 1998)*. In the genus *Vigna* Savi, the trypsin inhibitor present in cowpea (*V. unguiculata* (L.) Walp.) is of the Bowman-Birk type and is controlled by the gene *CpTI* (*Xu et al., 1996*), while the trypsin inhibitor in mung bean (*V. radiata* (L.) R. Wilczek) resembles one in the former but is unlike the trypsin inhibitor of soybeans *(Chrispeels and Baumgartner, 1978)*.

Conclusions

The research on the trypsin inhibitors in grain legumes is one of the most important milestones in the breeding these species and is indispensable from other crucial goals such as yield and biofortification. Only the cultivars with known and desirable content of trypsin inhibitors may represent a solid basis for the further improvement of grain legumes and their wide use in human consumption and animal feeding.

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Inhibitori tripsina nekih zrnenih mahunarki

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Rezime

Jedno od ograničenja korišćenja zrnenih mahunarki u ishrani domaćih životinja jeste prisustvo raznih antinutritivnih činilaca, koji snižavaju hranljivu

vrednost zrnenih mahunarki. Najvažniji termolabilni antinutritivni činioci su inhibitori proetaze. Inhibitori serin proteaze zrnenih mahunarki pripadaju dvema različitim familijama, uobičajeno označenih kao Kunic i Baumen-Berk familije. Postoje razlike u rasprostranjenosti ova dva tipa inhibitora kod zrnenih mahunarki. Kod soje, preovlađuje Kunic tip inhibitora, čijim je smanjenjem ostvaren veliki pomak u oplemenjivanju. Inhibitori tripsina graška pripadaju Baumen-Berkovoj familiji, na čije je smanjenje do niskog i vrlo niskog nivoa usmereno oplemenjivanje.

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ENERGY VALUE OF MEADOW HAY FROM DIFFERENT GRASSLAND REGIONS OF REPUBLIC OF MACEDONIA

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Original scientific paper

Abstract: In order to determine the energy value of the meadow hay, 10 samples originating from the Skopje and Males region have been analysed. The productive energy of hay fluctuates above and below the limit of the average values of hay on the territory of Macedonia. Hay from the Males region can be classified as good and hay from the Skopje region can be classified as medium meadow hay. The increase of the crude fibre content followed by simultaneous and proportional decrease of the protein content and NFE matters during storage has negative influence on the energy value of the hay. Nevertheless the application of the different formulas for computing the energy value represents one applicative solution for practice, in any case it is not enough coherent and realistic. For purpose of objective estimation of energy value of hay in different regions, it is necessary to choose and apply only one of the computing methods.

Key words: meadow hay, energy value, nutrition

Introduction

The energy value of the meadow hay is one of the important indicators of quality and the total nutritional value. The nutritional, i.e. the energy value depends mostly on the type of grass *Kolarski et al. (1990)*. *Todorov (1995)*, in addition to other data, states that the amount of the gross energy (GE) in the meadow hay is 17.09 – 18.40 MJ/kg DM. The amount of the metabolic energy (ME) in the meadow hay is from 7.07 MJ/kg DM after flowering to 9.04 MJ/kg DM at the beginning of forming of ears. The productive energy of the meadow hay for lactation (NEL) is highest in the hay at the beginning of forming ears (5.10 MJ/kg DM), and lowest after the flowering of the hay (3.84 MJ/kg DM), whereas the one for growth and fattening (NEGF) is lowest after the flowering of the hay, and highest in the beginning of forming ears (4.92 MJ/kg DM). *Grbesha (1993)* also stated similar data. *Stekar et al. (1996),* based on 864 performed analyses, determined the average energy value of the meadow hay from different cutting. In addition they state that the meadow hay in average contains 0.350 SU/kg, whereas the productive energy of the meadow hay for lactation (NEL) was on level of 4.59 MJ/kg DM. Similar researches performed on 693, i.e. 655 samples affirm that the meadow hay contains 0.313 SU/kg *(Stekar et al. 1994)*, i.e. 0.339 SU/kg *(Stekar et al. 1995)*. *Obračević (1984)***,** in his assessment tables of the energetic value in the fodder states that the net energy of the meadow hay for lactation (NEL) is highest in the highland hay when flowering (4.34 MJ/kg DM), and lowest in the lowland hay when flowering (4.33 MJ/kg DM), while the one for growth and fattening (NEGF) is lowest in the lowland hay when flowering (3.98 MJ/kg DM), and highest in the highland hay when flowering (3.99 MJ/kg DM). *Bogdanov (1997)* states similar data where in the lowland hay is 3.57-4.72, and in highland hay 3.57-5.07 MJ/kg ADM. In the lowland hay, i.e. highland hay the amount in NEGF is 3.25-4.70, i.e. 3.26-5.09 MJ/kg ADM. The nutritional value of the fodder, and the meadow hay, was subject of research of individual researchers in Macedonia *(Vaskov et al. 1996; Shokarovski et al. 1999)*. Examining the 45 samples of hay from natural and artificial meadows from different locality in Macedonia *(Vaskov et al. 1996)* the average gross energy value is determined to be 17.85 MJ/kg DM and average content of the metabolic energy to be 8.59 MJ/kg DM. The productive energy of the meadow hay for lactation (NEL) is 4.97 MJ/kg DM, whereas for growth and fattening (NEGF) is 4.66 MJ/kg DM. Similar but lower values are those from examination of 70 samples of hay from natural and artificial meadows from different localities in Macedonia *(Shokarovski et al. 1999)*. The average gross energy value of the meadow hay is 17.74 MJ/kg DM with average content of the metabolic energy of 8.05 MJ/kg DM. The productive energy of the meadow hay for lactation (NEL) is 4.61 MJ/kg DM, whereas the one for growth and fattening (NEGF) is 4.24 MJ/kg DM. Based on the results from the researches, as well as the literature data from different authors the division of the meadow hay according to energy value is accepted like a bad meadow hay (7.16 ME, MJ/kg ADM and 0.27 SV, SU/kg ADM), middle meadow hay (7.40 ME, MJ/kg ADM and 0.30 SV, SU/kg ADM), good meadow hay (7.69 ME, MJ/kg ADM and 0.34 SV, SU/kg ADM) and very good meadow hay (8.37 ME, MJ/kg ADM and 0.40 SV, SU/kg ADM) *(Mc Donald et al. 1988; Ševković et al. 1986; Ševković et al. 1991; Sinovec and Ševković, 1995)*.

Materials and Methods

In order to examine the meadow hay energy value a net energy is determined as productive energy measure, i.e. the productive action of the food. In the study of meadow hay energy value from different grassland regions in

Macedonia the results for the basic chemical composition are given. Three methods for computing the productive activity are used. The following mathematic expression is used for calculating the net energy as measure of the productive value of the food (NEL, net energy for lactation, $MJkg^{-1}$):

$$NEL\ [MJ/kg] = ME \times [\ 0.6 + 0.24 \times (q - 0.57)] \quad (1)$$

Where q (metabolics of the energy) = BE/ME *(Grbesha, 1993)*

$$BE = 0.0226\ x_1 + 0.0407\ x_2 + 0.0192\ x_3 + 0.0172\ x_4$$

x_1 crude proteins (N x 6.25 g/kg ADM), x_2 crude ether extract (gkg^{-1} ADM), x_3 crude fiber (g/kg ADM) and x_4 NFE matter (gkg^{-1} ADM).

$$ME = 0.0152\ x_1 + 0.0342\ x_2 + 0.0128\ x_3 + 0.0159\ x_4$$

x_1 digestible protein (N x 6.25 g/kg ADM), x_2 digestible ether extract (gkg^{-1} ADM), x_3 digestible fiber (g/kg ADM) and x_4 digestible NFE matter (gkg^{-1} ADM).

For calculating the metabolic energy digestibility coefficient of 58, 48, 60 and 64% was used for crude proteins, ether extract, fibre and NFE matters.

$$NEL\ [MJ/kg] = ME \times [0.6\ (1 + 0.004 \times (q - 0.57) \times 0.9752] \quad (2)$$

Where q (metabolic of the energy) = BE/ME x 100 (*Obračević, 1984*)

$$BE = 0.02414\ x_1 + 0.03657\ x_2 + 0.02092\ x_3 + 0.01699\ x_1 - 0.00063\ x_5$$

x_1 crude protein (N x 6.25 g/kg ADM), x_2 crude ether extract (gkg^{-1} ADM), x_3 crude fibre (gkg^{-1} ADM), x_4 NFE matter (gkg^{-1} ADM) and x_5 total glucose (gkg^{-1} ADM).

$$ME = 0.01715\ x_1 + 0.03766\ x_2 + 0.0138\ x_3 + 0.01464\ x_4 - 0.00063\ x_5$$

x_1 digestible protein (N x 6.25 g kg^{-1} ADM), x_2 digestible ether extract (g kg^{-1} ADM), x_3 digestible fibre (g kg^{-1} ADM), x_4 digestible NFE matter (g kg^{-1} ADM) and x_5 digestible glucose (if they are > 80 g kg^{-1} ADM)

For calculating the metabolic energy digestibility coefficient of 58, 48, 60 and 64% was used for crude proteins ether extract, fibre and NFE matter.

$$NEL\ [MJ/kg] = 1.6276 + 0.1281\ x_1 + 0.0898\ x_2 + 0.0538\ x_3 - 0.0365\ x_4 \quad (3)$$

Where *(Stekar et al. 1993, 1994, 1995)*

x_1 crude protein (N x 6.25 g kg^{-1} ADM), x_2 crude ether extract (g kg^{-1} VSM),x_3 crude fibre (g kg^{-1} ADM) and x_4 NFE matter (g kg^{-1} ADM).

Computing the material units of the net energy as a measure of the productive energy of food (starch value, SU/kg) coefficients of digestibility from

58, 48, 60 and 64% are used for crude proteins, ether extract, fibre and NFE matter. For computing of the starch value as measure of the productive action of the food the following mathematic expressions are used:

$$SV\ [SU/kg] = 16.5855 + 1.2750\ x_1 + 0.9241\ x_2 - 0.7965\ x_3 + 0.5928\ x_4 \quad (4)$$

Where *(Stekar et al. 1996)*
x_1 crude protein (N x 6.25 g/kg ADM), x_2 crude ether extract (gkg^{-1} ADM), x_3 crude fibre (gkg^{-1} ADM) and x_4 NFE matter (gkg^{-1} ADM).

$$SV\ [SU/kg] = 23.70 + 1.014\ x_1 + 0.810\ x_2 - 0.734\ x_3 + 0.415\ x_4 \quad (5)$$

Where *(Cmok et al. 1987)*
x_1 crude protein (N x 6.25 gkg^{-1} ADM), x_2 crude ether extract (gkg^{-1} ADM), x_3 crude fibre (gkg^{-1} ADM) and x_4 NFE matter (gkg^{-1} ADM).

$$SV\ [SU/kg] = 0.94\ x_1 + 1.91\ x_2 + 1.00\ x_3 + 1.00\ x_4 - 0.58\ x_3 \quad (6)$$

Where *(Sinovec and Ševković, 1995)*
x_1 crude protein (N x 6.25 gkg^{-1} ADM), x_2 crude ether extract (gkg^{-1} ADM), x_3 crude fibre (gkg^{-1} ADM) and x_4 NFE matter (gkg^{-1} ADM).

Results and Discussion

Table 1 shows the energy value of the hay obtained by application of different methods of research from different regions in Macedonia.

Table. 1. Energy value of hay from different grassland regions of Macedonia

Region	Measures of variation					
(formula*)	X	±	Sx	Sd	Cv	Iv
	Net energy of lactation NEL (MJ kg^{-1})					
Skopje (1)	4.482		0.028	0.088	1.964	4.364-4.603
Males (1)	4.479		0.013	0.040	0.888	4.396-4.511
Skopje (2)	4.357		0.027	0.084	1.928	4.210-4.458
Males (2)	4.334		0.010	0.032	0.736	4.268-4.360
Skopje (3)	4.217		0.072	0.228	5.407	3.818-4.571
Males (3)	4.421		0.078	0.247	5.579	3.982-4.851
	Starch value SV (SU kg^{-1})					
Skopje (4)	0.312		0.011	0.033	10.728	0.253-0.368
Males (4)	0.345		0.011	0.035	10.072	0.286-0.405
Skopje (5)	0.299	a	0.009	0.028	9.442	0.249-0.348
Males (5)	0.327	b	0.009	0.029	8.889	0.279-0.378
Skopje (6)	0.340	a	0.005	0.015	4.544	0.319-0.370
Males (6)	0.352	b	0.004	0.012	3.471	0.329-0.372

*according to the formulas given in the chapter Material and Methods of work [a, b] $p<0.05$

The table shows that the numeric differences in the energy value of hay are brought out, but statistically significant differences in the content of productive energy for lactation of hay from different regions in Macedonia are not determined. On the other hand, examination of starch value as material unit of productive energy significant values are determined ($p<0.05$) in the energetic value of hays from Skopje and Males region. Energy value of meadow hay is among the more significant indicators of the quality and total nutritional value, and mostly depends on type of plants in the hay *(Kolarski et al. 1990)* and the stadium of plants development in the moment of cutting *(Ševković et al. 1986)*. The large quantity of carbohydrates (herbage) and especially fat provides higher energy value, than the presence of proteins (leguminose) considering the low energy values of proteins in unit weight regarding the other organic matter. On the other hand, the hay energy value is inverse from the content of the fibre which is deposited in plants in higher phases of vegetation. Productive energy for lactation *(Todorov, 1995)* of the meadow hay is highest in the hay at the beginning of forming ears (5.10 MJ kg^{-1} DM), and lowest after flowering (3.84 MJ kg^{-1} DM). Data in the literature of the content of meadow hay productive energy is equal. *Grbesha (1993)* considers that, depending on the vegetative stadium, the productive energy for lactation of meadow hay is 3.89-6.16 MJ kg^{-1}, i.e. in average from 4.33-4.59 MJ kg^{-1} *(Obračević, 1984; Stekar et al. 1996)*. Starch value of meadow hay, as material unit of productive energy, is 0.313-0.350 SU/kg *(Stekar et al. 1994, 1995, 1996)*. The energy value of the meadow hay data obtained with the performed researches in Skopje and Males region are equal with literature data. Beside the stated, comparing the results with the data of authors who did their researches on the meadows in the eastern parts of the Balkan Peninsula, especially in Macedonia, is from essential meaning. Based on the researches of numerous samples of hay in meadows of different localities in Macedonia *Vaskov et al. (1966)* consider that productive energy for lactation of hay is on average level of 4.97 MJ kg^{-1}, and *Shokarovski et al. (1999)* give similar, but little lower values of 4.61 MJ kg^{-1}. Regarding these data, the results show that productive energy for lactation of hay from the examined regions moves around and under the lower limit stated as average for the territory of Macedonia. Also, based on the results for energy value, the hay from Males region can be classified as good, and the hay from Skopje region as middle meadow hay *(Ševković et al., 1986)*. Not depending of the way of calculating the average productive energy for lactation of meadow hays from the two localities was 4.217-4.482 MJ kg^{-1}, and the starch value was 0.299-0.352 SU kg^{-1}. It is interesting to mention that, regarding the differences in the average chemical composition of hay from different localities and application of different formula, the numeric differences in the content of productive energy for lactation are not statistically significant, whereas the differences in starch value are significant using the formula given by *Cmok et al. (1987)* and *Sinovec and*

Ševković (1995). Using certain formulas for calculating the energy value of hays, even if it represents an applicative solution in practice, it is not sufficiently coherent and real way. Namely, using the formulas for calculating the productive energy for lactation stated by *Grbesha (1993)* and *Obračević (1984)* give statistically significant higher values than recommended by *Stekar et al. (1993, 1994, 1995)* when it comes to hay from Skopje region, whereas the hay from Males region the had same differences just between the methods used by *Grbesha (1993)* and *Stekar et al. (1993, 1994, 1995)*. Calculating the starch value, significantly higher values are given with using the formula stated by *Sinovec and Ševković (1995)* than using the formula used by *Cmok et al. (1987)* and *Stekar et al. (1996)*. Beside the general differences, there are numeric differences obtained with using the same formula for hay in different localities. So in order to asses the energy value of hay from different regions more objectively, it is necessary to chose and use one method. The eventual choice is made based on comparison with mathematic results obtained from biological and production tests.

Conclusion

Based on the presented the following conclusions are:

1. Productive energy of hay from the examined regions is around and below the bottom limit of average values of hay on the territory of Macedonia. The hay from region in Males can be classified as good, and the one from Skopje region as medium meadow hay.
2. The increase of fibre content with simultaneous and proportional decreasing of the protein content and NFE matter during the storage has negative influence on the energy value of hay.
3. Even if application of different formulas for calculating the energy value of hay represents applicative solution in practice, it is not a credible way. In order to asses the energy value of the hay from different regions it is necessary to choose and apply only one of the methods for calculation.

Energetska vrednost livadskog sena različitih pašnjačkih regiona Republike Makedonije

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Rezime

U cilju ispitivanja energetske vrednosti livadskog sena analizirano je po 10 uzoraka poreklom iz Skopskog i Maleševskog regiona. Produktivna energija sena

sa ispitivanih regiona kreće se oko i ispod donje granice prosečnih vrednosti sena sa teritorije Makedonije. Seno sa regiona Malesa moze se klasifikovati u dobra, a sa Skopskog regiona u osrednja livadska sena. Porast sadržaja sirove celuloze uz simultano proporcionalno smanjenje sadržaja proteina i bezazotnih ekstraktivnih materija u toku skladiranja ima negativan uticaj na energetsku vrednost sena. Iako primena različitih formula za izračunavanje energetske vrednosti sena predstavlja aplikativno rešenje za praksu, nije dovoljno pouzdan način. U cilju što objektivnije procene energetske vrednosti sena pojedinih regiona, neophodno da se izabere i primeni samo jedna od metoda izračunavanja.

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QUALITY PARAMETERS OF ZP HYBRIDS BIOMASS

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Abstract: The aim of the present study was to observe quality parameters of ZP hybrids biomass for silage and to determine the interrelation of these parameters, as well as, their effects on the digestibility of maize biomass dry matter. The whole plants of the following maize hybrids were used in the study: ZP 196, ZP 305, ZP 505, ZP 643, ZP 737 and ZP 753. Yield, structure of the dry matter yield of the observed maize hybrids, as well as the yield of the digestible dry matter was estimated. The content of lignocellulose fibres and the digestibility of dry matter of the whole maize plant were established by chemical analyses. Obtained results showed that the NDF content in the whole maize plant of the observed ZP hybrids varied from 39.87% to 44.93%. The content of NDF and hemicellulose affected the digestibility of the whole maize plants. The difference in the digestibility of the dry matter of the whole maize plant over hybrids amounted to 8.56%. Although the content and the interrelation of remaining lignocellulose fibres affected the difference in the digestibility of the dry matter of the whole plant of the observed hybrids, the coefficient of correlation between these parameters and the digestibility was not high.

Key words: maize hybrids, lignocellulose fibres, dry matter digestibility.

Introduction

Maize is the most important forage plant, because the highest accumulation of solar energy per area unit is accomplished by the silage maize production. Due to quality of biomass, suitability for silage and diversified utilisation as feed maize ranks first among forage plants. A positive correlation between high yields, net energy and the net energy concentration, as well as, between the organic matter digestibility and the net energy concentration is accomplished in the produced maize plant biomass. Although the greater maize maturity is, the greater net energy concentration is, the digestibility of the dry and organic matter of the whole maize plants is not reduced. In contrast to maize, other plants are characterised with the increased net energy concentration with a greater plant maturity, but the digestibility of the dry and organic matter of the whole plant significantly decreases. The yield of the total dry matter of silage maize ranges from 12 to 26 t

ha^{-1} in dependence on the genetic potential and agro-ecological conditions in the full waxy maturity stage for silage (dry matter content of the whole plant ranges from 35 to 42%) *(Pejić et al., 1989).*

It was thought that yielding maize hybrids that were good for the grain production were also good for the silage production *(Zscheischler, 1979).* Recent studies within this field point out to changes in regard to this statement *(Zimmer et al., 1985; Thomer et al., 1986).* It is known that there are significant changes in the content of lignocellulose fibres in the whole maize hybrid plant that considerably affect the digestibility of the dry and organic matter, and therefore on the yield of digestible dry matter *(Terzić, 2006).* All carbohydrates in plant nutrients are grouped into: I structural carbohydrates (carbohydrates of cell walls), which include NDF (neutral detergent fibres-hemicellulose+cellulose+lignin), ADF (acid detergent fibres- cellulose+lignin), ADL (lignin) and II non-structural carbohydrates - NFC (carbohydrates present in the plant cell content) that are made of starch, sugars and pectin *(Jovanović et al., 1993).*

There are numerous confirmations that the feeding ration with a high NCF content ferment and produce a great quantity of propionic acid in the rumen, which results in a higher insulin concentration in blood, i.e. in animal weight gain *(Jovanović et al., 1993).* Feeding rations rich in digestible fibres (hemicellulose and cellulose) produce acetic acid in the rumen and increase the growth hormone level in blood, which results in the increased milk production *(Jovanović et al., 1993).*

Considering all stated, as well as, the fact that the digestibility does not depend on the energy concentration, it is necessary to study the content of lignocellulose fibres and the digestibility of dry matter of the whole maize plant as a primary indicators of silage biomass quality.

Materials and Methods

The hybrids of the FAO maturity group 100-700 (ZP 196, ZP 305, ZP 505, ZP 643, ZP 737, ZP 753) were used in this study. The two-replicate trail was set up according to the randomised complete-block design in the experimental plot of the Maize Research Institute, Zemun Polje. The experimental plot size amounted to $21m^2$, while sowing density was 60,000 plants per hectare.

Plants of each replicate were harvested in the full waxy maturity stage from the area of 7 m^2 (two inner rows), and yield of fresh biomass were estimated. Five average plants per replicate were selected for further tests. Samples of the whole plants, plants without ears and ears were cut and dried at 60^oC for 48^h. In order to determine the content of dry matter the whole plant samples were ground in the 1-mm mesh mill. Then, the analysis of the absolute dry matter was done on the oven dry basis (105^oC for 12 h) in order to estimate the total dry matter. Moreover, the analysis of the content of lignocellulose fibres (NDF, ADF, ADL, cellulose, hemicellulose) was performed by the modified Van Soest detergent method *(1963)*. The method was modified by *Mertens (1992)*. *In vitro* digestibility of the whole maize plant was done by the Aufréré method *(Aufréré, 2006)*. This method is based on the hydrolysis of proteins of the whole plant in the pepsin acid solution (Merck 2000 FIP u/g Art 7190) at 40°C for 24^h, and then on the hydrolysis of carbohydrates in the cellulase solution (cellulose Onozuka R10) in duration of 24 h.

Results and Discussion

Table 1 presents the structure of the dry matter yield of the observed ZP maize hybrids. The yield of the whole ZP maize hybrid plant dry matter in the full waxy maturity stage for silage and with the content of dry matter ranging from 33.82 to 43.37% varied from 14.9 to 25.7 t ha^{-1}. The yield of digestible dry matter of the whole plant of the observed ZP maize hybrids ranged from 8.9 to 16.5 t ha^{-1}. The highest yield of dry matter and digestible dry matter per hectare was detected in the hybrid ZP 505. The share of ear dry matter in the total dry matter yield varied from 49.66 to 54.95%. The differences in yields of dry matter of the whole maize plant of the observed ZP hybrids amounted to 10.8 t ha^{-1}. Then the differences in yields of digestible dry matter amounted to 7.6 t ha^{-1}, while the participation of ear dry matter in the total dry matter yield of the whole plant was 5.9%.

Table 1. Yield structure of ZP maize silage hybrids

Hybrids	Yield of digestible dry matter (t ha^{-1})	Dry matter yield (t ha^{-1})		Participation of ear dry matter (%)
		Whole plant	Ear	
ZP 196	8.9	14.9	7.4	49.66
ZP 305	13.2	20.2	11.1	54.95
ZP 505	16.5	25.7	14.0	54.47
ZP 643	14.6	25.1	12.9	51.39
ZP 737	15.9	25.1	11.3	45.02
ZP 753	15.1	22.7	12.3	54.19

Data on the content of NDF, ADF, ADL, hemicellulose and cellulose are presented in Table 2. The differences in the content of NDF, ADF, ADL, hemicellulose and cellulose among observed ZP hybrids were 5.05%, 4.49%, 0.56%, 4.01% and 3.95%, respectively.

Table 2. Content of lignocellulose fibres of the whole plant of the ZP maize hybrids

Hybrids	NDF (%)	ADF (%)	ADL (%)	Hemicellulose (%)	Cellulose (%)
ZP 196	44.40	23.13	3.50	21.27	19.63
ZP 305	42.29	21.15	3.28	21.14	17.87
ZP 505	39.88	18.64	2.94	21.24	15.68
ZP 643	44.93	20.96	3.41	23.97	17.55
ZP 737	42.90	22.01	3.12	20.84	18.89
ZP 753	39.96	22.00	3.40	19.96	18.60
$LSD_{0.05}$	0.508	0.269	0.220	0.739	0.342

Considering the crucial effect of lignocellulose fibres on the digestibility of the whole maize plant dry matter, the correlation dependence between the content of these components and the dry matter digestibility was observed (Table 3). A significant negative correlation was determined between the NDF content and the dry matter digestibility (r = –0.56) and between the hemicellulose content and the digestibility (r = –0.57). *Wermke (1986)* ascertained a significant negative correlation (r = –0.67) between the digestibility and the ADF content, as well as, between the digestibility and lignin (r = –0.95). *Burritt et al. (1985)* studied three grass species and found high correlation dependence between the dry matter digestibility and the content of lignocellulose fibres. The coefficient of correlation between the digestibility and NDF, ADG, ADL and cellulose amounted to –0.84, –0.93, –0.91 and –0.86, respectively. *Wermke (1986)* established that there were maize hybrids with a low digestibility and a high content of stover cell wall constituents, as well as, there were hybrids with a high digestibility and a low content of cell wall constituents.

Table 3. Correlation dependence between dry matter digestibility and lignocellulose fibres

	ADF	ADL	Hemicellulose	Cellulose	Digestibility
NDF	0.78**	0.55	0.68*	0.47	–0.66*
ADF		0.60*	0.08	0.86**	–0.43
ADL			0.19	0.71*	–0.33
Hemicellulose				–0.25	–0.57*
Cellulose					–0.06

These hybrids were defined as a dislocation type of hybrids. Hybrids with a high digestibility and a low NDF content of stover and a high NDF content of stover and a low ear digestibility are classified into types of hybrids with prolonged stover assimilation.

The Figure 1 presents results of the digestibility of the whole ZP maize hybrid plant dry matter.

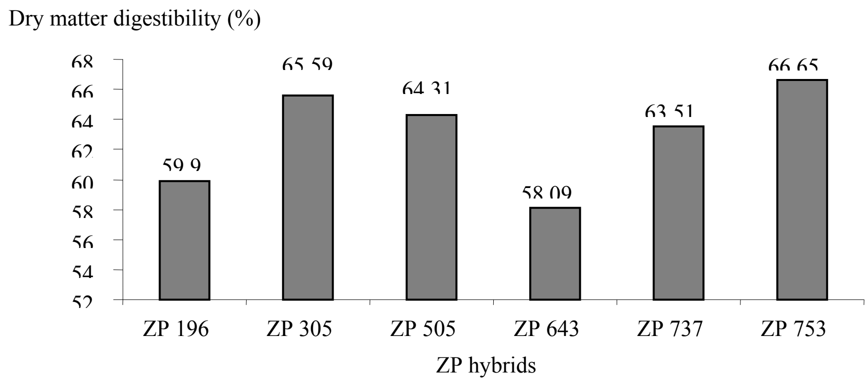

Figure 1. Dry matter digestibility of ZP silage hybrids

The digestibility of the whole ZP maize hybrid plant dry matter ranged from 58.09% to 66.65%. The difference in the digestibility of the whole maize plant dry matter amounted to 8.56%. According to results obtained by *Deinum et al. (1981)* and *Andreu et al. (1974)* this difference varied from 2% to 3%, while Terzić (2006) established the highest difference among observed hybrids of 11.52%. The hybrid ZP 196, among observed hybrids, had the lowest yield of dry matter (14.9 t ha^{-1}), of digestible dry matter (8.9 t ha^{-1}) and the lowest digestibility of the whole plant dry matter (59.90%). Hybrids ZP 643 and ZP 737 had the same dry matter yield (25.1 t ha^{-1}). However, the hybrid ZP 737 had the digestibility of the whole plant dry matter higher by 8.56%, hence the yield of the digestible dry matter was higher by 1.3 t ha^{-1}. As a low digestibility of the whole plant dry matter in hybrids with a high yield of the whole plant dry matter can disguise the actual value of silage maize, the yield of the digestible dry matter is considered the most real indicator of quality.

Conclusion

According to our results, significant differences were determined observed hybrids in contents of NDF, ADF and cellulose in, while the contents of ADL and hemicellulose did not much differ over hybrids. Moreover, a significant negative correlation was determined between the NDF content and the whole plant dry matter digestibility, as well as, between the hemicellulose content and the digestibility. The difference in the digestibility of the whole maize plant dry matter in observed ZP hybrids amounted to 8.65%. The hybrid ZP 753 had the highest dry matter digestibility, while the hybrid ZP 643 had the lowest digestibility. Although these two hybrids had the equal yield of the whole plant dry matter, the hybrid ZP 753 had the yield of the whole maize plant digestible dry matter higher by 1.3 t ha^{-1}.

The evaluation of silage maize hybrids based on the yield of the whole plant digestible dry matter is more reliable indicator of their quality.

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Parametri kvaliteta biomase ZP hibrida kukuruza

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Rezime

Cilj ovog istraživanja bio je da se ispitaju parametri kvaliteta biomase ZP hibrida kukuruza za silažu i da se utvrdi međusobna zavisnost ovih parametara kao i njihov uticaj na svarljivost suve materije kukuruzne biomase. U istraživanjima korišćeni su cele biljke kukuruza hibrida ZP 196, ZP 305, ZP 505, ZP 643, ZP 737 i ZP 753. Određen je prinos i struktura prinosa suve materije ispitivanih hibrida kukuruza, ko i prinos svarljive suve materije. Hemijskim analizama utvrđen je sadržaj lignoceluloznih vlakana i svarljivost suve materije cele biljke kukuruza. Rezultati ispitivanja pokazali su da je sadržaj NDF u celoj biljci kukuruza kod ispitivanih ZP hibrida bio u intervalu od 39,87 do 44,93%. Sadržaj NDF i hemiceluloze najviše je uticao na svarljivost suve materije cele biljke kukuruza. Razlika u svarljivosti suve materije između hibrida kukuruza bila je 8,56%. Iako je sadržaj i međusobni odnos ostalih lignoceluloznih vlakana uticao na razliku u svarljivosti suve materije cele biljke ispitivanih hibrida, koeficijent korelacije između ovih parametara i svarljivosti nije bio visok.

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THE EFFECT OF DIET ON THE *in situ* DRY MATTER DEGRADABILITY OF SOME PLANT-DERIVED FEEDSTUFFS

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Abstract: This paper presents results on the dry matter degradability of maize gluten, sunflower meal, dehydrated alfalfa and soybean meal. The experiments included three fistulated rams fed diets having the roughage to concentrate ratios of 80:20, 50:50 and 20:80. The dynamics of the ruminal dry matter degradability of the above plant-derived feedstuffs was evaluated at six incubation times: 4, 8, 12, 16, 24 and 48 hours. A comparison of the obtained results on the dry matter degradability of the test feedstuffs in the diet containing 20% roughage and 80% concentrate with those of the other diets showed lower values of dry matter degradability. The dry matter degradability of the feedstuffs tested in this study was found to be affected by the diet composition, the variability between the feeds and the incubation period. The highest dry matter degradability was estimated in soybean meal, at all incubation times and in all diets, followed by maize gluten and sunflower meal. The lowest dry matter degradability was exhibited by dehydrated alfalfa, at all incubation times and in all diets.

Key words: rams, nylon bags, DM degradability

Introduction

The degree of the dry matter degradability primarily depends on feedstuffs which are exposed to microorganisms` action in the rumen on one side and on the ratio between the roughage and concentrated part of the meal, on the other. For the calculation of dry matter degradability, Nylon Bag Technique was used. This method was developed by *Mehrez and Orskov (1977)* for the reason of description of degree and speed of degradability of examined feedstuffs incubated in the rumen of experimental animals.

This method is consisting of the incubation of nylon bags with the samples of the examined feedstuffs into the rumen of fistulated animals. We have chosen this method for our research for several reasons and one of most important was that this method was accepted and recognised and technically practical for the conditions in which the experiments were carried out.

Possibility of speed measurement of degradability is very important instance because of its giving information on factors which have an effect on roughage food taking. Easy consumption of food is not the only reason for cud's voluntary taking it but the speed of the process of consumption as well, because both these factors have an effect on the overall food consumption.

The effect of the portion on dry matter and crude protein degradability was pointed out by *Lindberg (1981)*, then *Kristensen et al.(1982), Ilić et al.(2009)* and else.

Materials and Methods

Investigations were carried out in the Institute for Animal Husbrandy, Belgrade-Zemun.

The experiments included three fistulated rams approximately 18 months of age. The animals were of similar weight of about 65 kg, or more precisely, two rams weighed 65 kg each and one 66 kg. Permanent plastic fistulas of 45 mm in diameter were surgically inserted into the rumen of the rams. The rams were placed in individual cages which enabled individual feeding and watering and complete control of the test animals. The animals were fed twice a day, at 7 a.m. and 3 p.m. The rams received about 1 kg dry matter and about 160 gr crude proteins in all experiments.

Four plant-derived feedstuffs were used to determine the effect of the dietary roughage to concentrate ratio on dry matter degradability. Three weight ratios of roughage to concentrate were used: 80:20, 50:50 and 20:80. Each of the three experiments was replicated three times. The preliminary feeding period lasted 15 days.

To evaluate the dynamics of the ruminal dry matter degradability of the above feedstuffs, six incubation times were used: 4, 8, 12, 16, 24 and 48 hours.

Dry matter degradability was estimated by the method designed to interpret the results obtained by the nylon bag technique of *Mehrez et al. (1977).* The nylon bags used in the experiment were 140 x 90 mm in size. They were numbered for identification.

All feedstuffs were treated equally, i.e. ground to a meal through a 2.5 mm screen in a laboratory mill. Subsequently, the bags were weighed on an analytical balance first empty and then filled with the feedstuff samples.

After specific periods of incubation, the bags tied to the plastic fistulas were removed from the rumen and immediately immersed in cold water to prevent further fermentation and remove feedstuff remains. Then, they were machine-washed using a cold rinse cycle for 20 minutes. Following the washing process, the bags were separated from the plastic fistulas and dried in a dryer at a temperature of 60 to 65 C^0 for 48 hours i.e. until a constant weight was achieved.

The data obtained in the experiment were subjected to a three-factor analysis of variance. The three factors were: different roughage to concentrate ratios (factor A), feedstuffs (factor B) and incubation times (factor C). The differences established between the treatment means were analyzed by the LSD test.

Results and Discussion

The dry matter degradability of maize gluten, sunflower meal, dehydrated alfalfa and soybean meal used in the diets containing different roughage to concentrate ratios is given in Tables 1, 2 and 3.

Table 1. Effect of incubation on degradability D.M. (%) of feedstuffs examined on diet 80:20

Incub. periods	Maize gluten	Sunflow. meal	Alfalfa meal	Soybean meal
4	40,10	36,60	33,84	43,37
8	47,34	44,68	40,90	55,42
12	54,45	53,15	48,53	67,18
16	62,77	58,91	55,60	78,62
24	70,41	64,89	63,47	87,61
48	81,40	77,31	72,05	94,13

Table 2. Effect of incubation on degradabilityD.M. (%) of feedstuffs examined on diet 50:50

Incub. periods	Maize gluten	Sunflow. meal	Alfalfa meal	Soybean meal
4	35,53	35,18	30,78	40,96
8	43,64	42,80	37,81	49,14
12	52,57	49,76	44,83	57,89
16	60,22	56,57	52,02	66,45
24	67,30	63,43	59,29	75,99
48	76,33	74,60	67,28	87,16

Table 3. Effect of incubation on degradability D.M. (%) of feedstuffs examined on diet 20:80

Incub. periods	Maize gluten	Sunflow. meal	Alfalfa meal	Soybean meal
4	31,75	33,43	27,76	34,97
8	38,34	39,82	34,17	41,57
12	46,36	46,42	40,67	48,36
16	53,15	52,79	46,88	56,19
24	61,85	59,91	53,21	64,76
48	71,11	68,90	61,20	77,92

The diet composition was found to affect the dry matter degradation rate of the test feedstuffs. The LSD test of the three dietary roughage to concentrate ratios revealed very significant differences (P<0.01). The dry matter degradation rate was also affected by the variability between the feedstuffs tested in the experiment.

The differences in dry matter degradability between maize gluten, sunflower meal, dehydrated alfalfa and soybean meal were statistically very significant (P<0.01).

The dry matter degradability of the feedstuffs was also dependent on the incubation period. The differences established between the incubation time treatment means were statistically very significant (P<0.01), except for the maize gluten and the sunflower meal in the diet containing 50% roughage and 50% concentrate, at incubation times of 4 and 8 hours, and for those in the diet composed of 20% roughage and 80% concentrate at incubation times of 12 and 16 hours, where the established differences were not significant. (Table 4 and 5)

Table 4. All Pairwise Multiple Comparison Procedures (Fisher LSD Method)

Comparison	Inc. periods	Diff of Means	LSD (alpha=0.05)	P	Diff>=LSD
Mg vs. Sm	4	1.320	1.414	0.067	No
Mg vs. Sm	8	0.170	1.414	0.810	No
Mg vs. Sm	12	4.340	1.414	<0.001	Yes
Mg vs. Sm	16	3.490	1.414	<0.001	Yes
Mg vs. Sm	24	4.930	1.414	<0.001	Yes
Mg vs. Sm	48	5.280	1.414	<0.001	Yes

Table 5. All Pairwise Multiple Comparison Procedures (Fisher LSD Method)

Comparison	Inc. periods	Diff of Means	LSD (alpha=0.05)	P	Diff>=LSD
Mg vs. Sm	4	4.210	1.552	<0.001	Yes
Mg vs. Sm	8	2.300	1.552	0.005	Yes
Mg vs. Sm	12	1.540	1.552	0.052	No
Mg vs. Sm	16	0.410	1.552	0.598	No
Mg vs. Sm	24	2.120	1.552	0.008	Yes
Mg vs. Sm	48	5.020	1.552	0.001	Yes

Similar results were obtained by *Madsen et al. (1994), Moloney et al. (2001)*. Recent findings show that the nutritional value is not only dependent on their composition, but also on their digestibility. *(Dhanoa et al. 2000; Lanzas et al. 2007)*.

The roughage to concentrate ratio of the diet had a significant effect on the degradability of dry matter of the test feedstuffs. Similar results were obtained by *Ganev et al. (1979)*, *Lindberg (1981)*, *Kristensen et al. (1982)* and *Setala (1983)*.

Conclusion

The obtained experimental results on the effect of the roughage to concentrate ratio on the dry matter degradability of maize gluten, sunflower meal, dehydrated alfalfa and soybean meal suggested the following:

The dietary roughage to concentrate ratio affected the dry matter degradability of the feedstuffs used in the experiment. The highest dry matter degradability was exhibited by the diet containing 80% roughage and 20% concentrate, followed by the diet having equal parts of roughage and concentrate and the diet with the lowest proportion of roughage, the latter showing the lowest degradability of dry matter. In terms of the dry matter degradability of the test feedstuffs, the highest degradability was exhibited by soybean meal, at all incubation times and in all tested diets.

Lower degradability of dry matter was estimated in sunflower meal and maize gluten, which showed similar degradability at shorter incubation times. However, the prolongation of incubation induced higher degradability of the maize gluten dry matter. The lowest dry matter degradability was found in dehydrated alfalfa, at all incubation times and in all test diets.

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Uticaj obroka na *in situ* razgradljivost suve materije nekih biljnih hraniva

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Rezime

U radu su prezentovani rezultati razgradljivosti suve materije kukuruznog glutena, suncokretove sačme, dehidrirane lucerke i sojine sačme. Ogledi su izvedeni na tri fistulirana ovna. U ispitivanjima su korišćeni sledeći težinski odnosi kabastog i koncentratnog dela obroka: 80:20, 50:50, 20:80. Radi utvrđivanja dinamike razgradnjivosti suve materije navedenih hraniva u buragu izabrana su šest inkubaciona vremena i to 4, 8, 12, 16, 24 i 48 sati. Upoređenjem dobijenih rezultata o razgradljivosti suve materije ispitivanih hraniva na obroku sa 20% kabastog i 80% koncentratnog dela sa ostalim obrocima uočavaju se niže vrednosti razgradljivosti suve materije. Utvrđeno je da je sastav obroka imao uticaja na razgradljivost suve materije ispitivanih hraniva. Osim sastava obroka na stepen razgradljivosti uticala je i varijabilnost koja je utvrđena između ispitivanih hraniva. Na razgradljivost suve materije ispitivanih hraniva takođe je uticalo i vremensko

trajanje inkubacija. Najveća razgradljivost suve materije je utvrđena kod sojine sačme i to u svim inkubacionim vremenima, a ujedno i na svim ispitivanim obrocima. Zatim dolaze kukuruzni gluten i suncokretova sačma. Najmanja razgradljivost suve materije je bila kod dehidrirane lucerke, kako u svim vremenskim inkubacijama, tako isto i na svim ispitivanim obrocima. Utvrđene razlike između sredina tretmana inkubacionih vremena statistički su vrlo značajne (P<0,01), osim za kukuruzni gluten i suncokretovu sačmu na obroku sa 50% kabastog i 50% koncentratnog dela obroka i to u inkubacionim vremenima od 4 i 8 sati, zatim na obroku sa 20% kabastog i 80% koncentratnog dela obroka u inkubacionim vremenima od 12 i 16 sati, gde utvrđene razlike nisu značajne.

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POSSIBILITIES OF PRODUCING HIGH QUALITY FEEDSTUFF USING PLANT RESIDUES OF SWEET MAIZE HYBRIDS

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Original scientific paper

Abstract: The aim of this study was to observe the possibilities of the high quality feedstuff (fodder) production by the use of the processed green residues of sweet maize after harvest of consumer ears. The green residues of the sweet maize hybrids ZP 441su and ZP 531su and the whole plant of the hybrid ZP 544, used as a check, were used in the study. The yield of fresh mass, dry matter and the content of dry matter of green residues of the ZP sweet maize hybrids were determined. The contents of protein, ammonium nitrogen, volatile fatty acids (lactic, acetic and butyric acids), lignocellulose fibres, as well as, the dry matter digestibility and pH values of silage were determined by chemical analyses. According to obtained results the content of ammonium nitrogen, lactic acid, acetic acid and pH values ranged from 0.02% to 0.03%, 2.57% to 3.89%, 0.68% to 0.81% and from 3.74 to 3.88, respectively. Furthermore, the content of protein and NDF ranged from 7.19% to 10.08% and 39.81% do 47.66%, respectively. Processed silages, ZP 441su +PR and ZP 531su +PR, did not significantly differ in the dry matter digestibility in comparison to silage of the hybrid check ZP 544, while other processed silages of residues had a greater digestibility than silage made of the check hybrid. Based on obtained results, the high quality feedstuff can be produced by the use of the processed residues of sweet maize hybrid plants.

Key words: sweet maize, greed residues, silage quality, silage.

Introduction

Sweet maize is present in food mainly due to a high content of proteins, reducing sugars and dietary fibres. For instance, the sucrose content in the standard grain quality hybrids amounts to approximately 4% at the milk stage, while it amounts to approximately 10% in sweet maize hybrids. However, after harvest or delayed harvesting operations, the content of sugar, first of all of sucrose, rapidly decreases as it converts into starch. The sweet maize kernel looses about 50% of the sucrose content for only 24 h *(Amir et al., 1971)*.

Green residues of sweet maize left in fields after harvest can be used in animal feed. The residues make 60-70% of the basic yield and are composed of husk, leaves, cobs, kernel leftovers and a small amount of stalks *(Fritz et al., 2001)*.

Although, the dry matter yield of the whole sweet maize plant, as well as, the yield, of the digestible dry matter are on the average lower by 40% than the corresponding yields of the standard grain quality flint hybrids *(Bekrić, 1997)*, the high quality fodder with enhanced nutritive values can be made of processed residues of sweet maize. Furthermore, the low dry matter content in sweet maize residues is a limiting factor of their storage especially for a longer period of time, as they rapidly decay and rot. Due to high costs of artificial drying, ensiling can be a more favourable option for increasing the economic value of sweet maize residues.

The aim of this study was to prepare silage combinations by processing fresh mass of ZP sweet maize hybrids, to observe their quality and to compare their quality with the quality of silage made of the whole plants of the standard grain quality ZP hybrid.

Materials and Methods

Two ZP sweet maize hybrids (ZP 441su and ZP 531su) were used in this study. The two-replicate trial was set up in the experimental field of the Maize Research Institute, Zemun Polje, according to the randomised complete-block design. The crop density amounted to 60.000 plants ha^{-1}.

After ears were harvested, five average plants of both sweet maize hybrids were taken from each replication. Fresh plant mass without ears was chopped and two samples of 500g were measured. Residue samples were dried at the temperature of 60^{o}C for 48h to the constant weight. The content of the total dry matter and the dry matter yield of sweet maize residues were estimated after 12-h drying of ground samples at 105^{o}C.

In order to prepare silage, the green residues of sweet maize were harvested. Their yield was evaluated and then they are crushed and mixed with the components for processing. Ground maize (PR), commercial mineral additive (MM) and commercial microbiological additive Yuasture (KVS) were used in silage processing. Additives in silages averaged 15-17%. The following three silage combinations were prepared for this study according to the original recipes: I - hybrid residues + ground maize, II - hybrid residues + ground maize + commercial mineral additive, III - hybrid residues + ground maize + commercial microbiological additive. In order to compare results, harvest, crushing and ensilage of the whole plants of the maize hybrid ZP 544 were simultaneously done at the stage of physiological maturity.

When fermentation and stabilisation of silage were completed, the content of ammonium nitrogen and a pH value of silage were observed after the method of the Danish National Committee for Silage *(Obradović, 1965)*. Also, the content of volatile fatty acids (lactic, acetic and butyric acids) was observed and scoring and the silage quality evaluation were done by the Flieg method *(Obradović, 1965)*. At the same time, two replicates of 500 g of silage samples were drawn and dried at 60°C for 48 h to the constant weight. Samples were ground in a mill with 1-mm sieves and then dried at 105°C for 12 h. After drying, the content of the total silage dry matter was determined. Then, the content of lignocellulose fibres were analysed (NDF-fibres insoluble in neutral solutions, ADF-fibres insoluble in acid solutions, ADL-lignin) using the Van Soest detergent method *(Van Soest, 1963)* modified by *Mertens (1992)*. Furthermore, the content of crude proteins was analysed *(Official Gazette of SFRY, 1987),* as well as, the silage dry matter digestibility by the enzymic hydrolysis method *(Aufréré, 2006).* This method is based on the silage protein hydrolysis in the pepsin acid solution (Merck 2000 FIP U/g art 7190) for 24 h at 40°C, and then on the hydrolysis of carbohydrates in the cellulase solution (cellulose Onozuka R10) in duration of 24 h.

Results and Discussion

The yield of fresh mass, dry matter and the dry matter content in residues of ZP sweet maize hybrids are presented in Table 1. The dry matter content in the residues of sweet maize hybrids used for silage was low (20.98% and 24.89%) considering that the optimum dry matter content in fibrous feeds for silage ranges from 30 to 35% *(Dinić and Đorđević, 2005)*. The optimum moisture is necessary for the maximum activity of micro organisms. Although the differences in yields of fresh mass amounted to 9.5 t ha^{-1}, dry matter yields were approximately equal (11.8 and 11.7 t ha^{-1} in ZP 441su and ZP 531su, respectively).

Table 1. Yields of fresh mass, dry matter and the dry matter content of ZP sweet maize plants without ears

Hybrids	Dry matter content (%)	Fresh mass yield (t ha^{-1})	Dry matter yield (t ha^{-1})
ZP 441su	20.98	56.4	11.8
ZP 531su	24.89	46.9	11.7

Table 2 shows data on lignocellulose fibres (NDF, ADF, ADL), proteins and the dry matter digestibility of silages. The NDF content was higher by 3.31-7.85% in all silages of processed residues of sweet maize hybrids than in silages made from the check hybrid ZP 544. The ADF content was higher by 0.43-4.31% in all silage combinations than in silages made from the check hybrid. Silages, ZP 441su + PR and ZP 441su + PR + MM, had a lower ADL content in relation to the silages made from the check hybrid, while the remaining silages had a higher content. Silages of processed residues of the hybrid ZP 531su had approximately the same protein content as silages made from the check hybrid, while this content in processed residues of the hybrid ZP 441su was higher by 1.74-2.00%. According to results obtained by *Mustafa et al.* (*2004*), the content of NDF, ADF, ADL and proteins in ensilaged residues of sweet maize amounted to 58.2%, 28.3%, 2.0% and 9.6%, respectively. The comparison of these results with results of our analysis leads to a conclusion that processing of sweet maize residues affects decreasing of NDF and ADF contents, as well as, increasing of silage nutritive values.

Table 2. Digestibility, contents of NDF, ADF, ADL, hemicellulose, cellulose and proteins in silages after fermentation

Silage	NDF (%)	ADF (%)	ADL (%)	Proteins (%)	Digestibility (%)
ZP 441su +PR	44.61	23.42	2.95	9.61	63.24
ZP 441su + PR + MM	43.12	21.99	2.91	9.46	67.36
ZP 441su + PR + KVS	41.63	23.04	3.29	9.72	67.89
ZP 531su +PR	44.71	25.87	3.93	7.54	62.33
ZP 531su +PR + MM	47.61	25.74	3.75	7.25	64.09
ZP 531su +PR + KVS	47.66	25.08	3.54	7.70	65.43
ZP 544	39.81	21.56	3.04	7.72	63.09

The silage dry matter digestibility of processed residues of sweet maize hybrids ranged from 62.33 to 67.89%. Except in the silage ZP 531su + PR, the silage dry matter digestibility of processed residues of sweet maize hybrids was higher by 0.15-4.80% in comparison with the silage produced from the check hybrid. Based on results previously obtained by *Terzić et al. (1994)* the dry matter digestibility of silage made from the whole plant of sweet maize amounted to 64.70%, while *Mustafa et al.* (*2004*) determined that this digestibility amounted to 60.1%. The dry matter digestibility of silage made from processed residues of male component lines in the seed production ranged from 64.2 to 67.0% *(Semenčenko et al., 2009)*. The silage dry matter content in this study was within the optimum limits for the silage of the whole maize plant and varied from 33.20% (ZP 441su + PR + KVS) to 40.25% (silage of the check hybrid ZP 544). All silages of sweet maize processed residues had almost the same pH value, while the silage of the check hybrid deviated in the amount of ±0.09. The ammonium nitrogen content was low in all observed silages (0.02-0.03%) (Table 3).

Results on the content of volatile acids and their percentile share in the total content are presented in Tables 4 and 5. The lactic acid content was higher in silages of sweet maize processed residues by 0.68-1.32% than in silages made from the check hybrid. The content of acetic acid, i.e. total acids in silages ranged from 0.59 to 0.81%, i.e. from 3.25 to 4.70%, respectively. According to results gained by *Terzić et al. (1994)* the content of lactic, i.e. acetic acid in the silage of the whole sweet maize plant amounted to 3.48%, i.e. 0.84%, respectively. The same authors stated that the ammonium nitrogen content had amounted to 0.04% in sweet maize silage, while the pH value of this silage had been 3.80. The lactic acid content amounted to 5.1%±2.2% in silages of sweet maize residues, while the pH value of silages amounted to 3.45±0.04 *(Mustafa et al., 2004)*. High percentage participation (over 81%) of lactic acid in the total content of acids in silages of sweet maize processed residues was determined; hence, these silages got a maximum score by the Flieg method (100) and a maximum grade (very good). Although the acetic acid content in the total acid content was a somewhat higher (20.98%) in the silage made from the check hybrid and had the score of 95, it also got the highest grade (very good).

Table 3. Contents of dry matter, ammonium nitrogen and pH values in silages after fermentation

Silage	Dry matter (%)	pH	Ammonium nitrogen (%)
ZP 441su +PR	34.17	3.87	0.03
ZP 441su + PR + MM	34.72	3.88	0.03
ZP 441su + PR + KVS	33.20	3.85	0.03
ZP 531su +PR	36.30	3.79	0.02
ZP 531su +PR + MM	36.88	3.74	0.02
ZP 531su +PR + KVS	35.57	3.81	0.02
ZP 544	40.25	3.83	0.02

Table 4. Content of volatile acids in silages after fermentation

Silage	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)	Total volatile acids (%)
ZP 441su +PR	3.25	0.70	-	3.95
ZP 441su + PR + MM	3.60	0.79	-	4.39
ZP 441su + PR + KVS	3.73	0.59	-	4.32
ZP 531su +PR	3.68	0.74	-	4.42
ZP 531su +PR + MM	3.55	0.81	-	4.36
ZP 531su +PR + KVS	3.89	0.81	-	4.70
ZP544	2.57	0.68	-	3.25

Table 5. Percentage participation of volatile acids in the total acid content and the silage quality evaluation

Silage	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)	Scores	Quality evaluation
ZP 441su +PR	88.28	17.72	-	100	very good
ZP 441su + PR + MM	82.00	18.00	-	100	very good
ZP 441su + PR + KVS	86.34	13.66	-	100	very good
ZP 531su +PR	83.26	16.74	-	100	very good
ZP 531su +PR + MM	81.42	18.58	-	100	very good
ZP 531su +PR + KVS	82.77	17.23	-	100	very good
ZP544	79.08	20.92	-	95	very good

Conclusion

According to obtained results it can be concluded that all silages of sweet maize processed residues had the optimum dry matter content (33.20-36.88%).

The digestibility of silages made from processed residues was higher by 0.15-4.80% than of silages produced from the check hybrid ZP 544. According to obtained scores, all observed silages of sweet maize processed residues are of high quality, as well as, it is silage of the check hybrid ZP 544, which indicates to a fact that silage made in such a way from sweet maize processed residues can be fermented feedstuff of high quality. Such silages can be used as a substitute of standard silage made from the whole maize plant.

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Mogućnosti proizvodnje kvalitetne kabaste hrane za životinje korišćenjem ostataka kukuruza šećerca

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Rezime

Cilj ovog istraživanja bio je da se ispitaju mogućnosti proizvodnje kvalitetne kabaste hrane za životinje (silaže) korišćenjem oplemenjenih zelenih ostataka biljke kukuruza šećerca nakon berbe konzumnih klipova. U istraživanju su

korišćeni zeleni ostaci hibrida kukuruza šećerca ZP 441su, ZP 531su i cela biljka hibrida ZP 544 kao standarda. Određen je prinos zelene mase, suve materije i sadržaj suve materije zelenih ostataka ZP hibrida šećerca. Hemijskim analizama utvrđen je sadržaj proteina, amonijačnog azota, isparljivih masnih kiselina (mlečne, sirćetne i buterne), sadržaj lignoceluloznih vlakana, svarljivost suve materije i pH vrednost silaža. Prema našim rezultatima sadržaj amonijačnog azota u silažama bio je od 0,02% do 0,03%, mlečne kiseline od 2,57% do 3,89%, sirćetne od 0,68% do 0,81%, a pH vrednost od 3,74 do 3,88. Sadržaj proteina bio je od 7,19% do 10,08%, a NDF-a od 39,81% do 47,66%. Oplemenjene silaže, ZP 441su +PR i ZP 531su +PR, nisu se značajno razlikovale po svarljivosti suve materije od standardne silaže hibrida ZP 544, dok su ostale oplemenjene silaže rezidua u odnosu na standard imale višu svarljivost. Prema našim rezultatima upotrebom oplemenjenih zelenih ostataka kukuruzne biljke hibrida šećerca može se proizvesti kvalitetna kabasta hrana za životinje.

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CHANGES IN LIGNIN STRUCTURE FROM LEAF AND STEM OF ALFALFA WITH GROWTH AND DEVELOPMENT

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Original scientific paper

Abstract: The changes in the concentration and structure of lignin isolated from leaf and stem of alfalfa, *cv* K-22 at different stages of maturity was investigated. Samples were cut in the seven-day interval in the second cut-22[nd], 29[th] and 36[th] day of vegetation. The lignin content is the largest in alfalfa stem at the full bloom stage. A higher proportion of nitrogen is recorded in lignin from alfalfa leaf. The smallest amount of hydrogen is recorded in alfalfa leaf, at third development stage (8.25%), whereas the amount of hydrogen at first and second development stage were similar. In alfalfa leaf is not estimated clear effect of development stage on sulphur content in lignin, whereas it increased in lignin from alfalfa stem from 4.25 to 4.70%. With the advancement of development, lignin synthesized in the alfalfa leaf does not transform from guaiacyl type into syringyl type of lignin. On the contrary, the lignin from alfalfa stem contains the higher ratio of syringyl units in the later stages of development.

Key words: ATR-IR spectroscopy, alfalfa, lignin, leaf, stem

Introduction

Lignins are polymeric aromatic constituents in plant cell walls. They are traditionally considered to be dehydrogenative polymers from three monolignols: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The hardwood lignins are built from the guaiacyl propane units (G) and the syringyl propane units (S). Lignins obtained from straws are built from the guaiacyl propane units, the syringyl propane units and 4-hydroxylphenyl propane units (H) *(Faix, 1991)*.

The objective of this work is to present the structural information of lignin isolated from leaf and stem of alfalfa at three different stages of plant development. These are relevant with regard to forage research as it has been frequently emphasized that not only the lignin amount, but also the lignin structure influences the forage nutritional value.

Materials and Methods

Three maturities of alfalfa, cv K-22, after the first cut were chosen for this study. The first harvest of forage crops was taken on June 17th at mid-bud stage. The second harvest was taken on June 24th at about 55% bloom. The third harvest was on July 1st in the full bloom. All values are in average.

Lignin was determined as lignin insoluble in 72 % (w/w) sulphuric acid, applying the method of *Van Soest and Wine (1967)*. Concentrations of C, H, N and S were obtained using Vario EL III C, H, N, S/O Elemental analyzer (Elementar). ATR spectra (1700-500 cm^{-1}) were obtained using Nicolet, Model 6700 FT-IR, Crystal-Diamond. Spectra were obtained with 4 cm^{-1} resolution, and 32 scans for each sample spectrum were performed. The spectral values are in cm^{-1}.

Results and Discussion

Our findings show that lignin content increases with the progress of plant development both in leaves and stem (Table 1). From the first to the third stage of development, lignin content in leaves increased from 4.21 to 4.91% of DM, and in stem from 8.45 to 11.92% of DM. The content of nitrogen, carbon, hydrogen and sulphur in lignin from leaf and stem is presented in Table 1. The highest content of nitrogen and carbon were determined at the second stage of development in leaf (7.01% and 62.77% of DM, respectively). The content of carbon in stem increased from 55.68 to 60.08% of DM. The higher hydrogen content was determined in alfalfa leaf (from 8.77 to 8.25% of DM), than that found in stem (from 6.36 to 6.83% of DM). In leaf, the sulphur concentration decreased from first to third stage of development (Table 1), and in stem increased from 4.25 to 4.70% of DM.

Table 1. Content of lignin and nitrogen, carbon, hydrogen and sulphur in lignin from alfalfa leaf and stem (% of dry matter)

Plant anatomical fraction	Stage of growth	Lignin	N	C	H	S
Leaf	I stage	4.21	5.37	61.99	8.77	3.82
	II stage	4.65	7.01	62.77	8.76	3.23
	III stage	4.91	4.70	60.10	8.25	3.07
Stem	I stage	8.45	1.16	55.68	6.36	4.25
	II stage	10.21	1.14	56.54	6.44	4.30
	III stage	11.92	1.15	60.08	6.83	4.70

ATR-IR spectrometry was used as a structural, non-destructive, and simple tool to qualitatively analysis the chemical composition of lignin isolated from leaf and stem of alfalfa in different stages of plant development. The spectra of lignin isolated from leaf are presented in Figure 1-3.

Peak at 1727.8 cm^{-1} show carbonyl stretching-unconjugated ketone and carboxyl groups; 1600.7 cm^{-1}-aromatic skeletal vibrations; 1494.3 and 1455.3 cm^{-1}-CH-deformations; 1159.0-aromatic CH-in-plane deformations, guaiacyl type; 1034.3 cm^{-1}-aromatic C-H deformation, guaiacyl type and 856.8 cm^{-1}- aromatic C-H out of plane deformation (first stage of development). The ATR-IR spectrum of lignin from the second stage of development shows similar lignin structure. At the third stage of development (Figure 3), signal at 721.5 cm^{-1} represent aromatic CH out of plane deformation *(Faix, 1991)*.

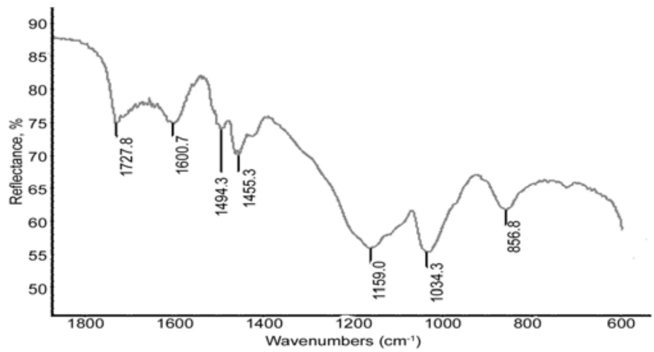

Figure 1. ATR-IR spectrum from alfalfa leaf, I stage of development

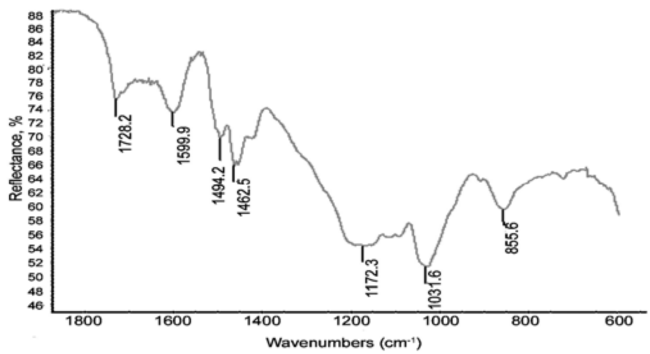

Figure 2. ATR-IR spectrum from alfalfa leaf, II stage of development

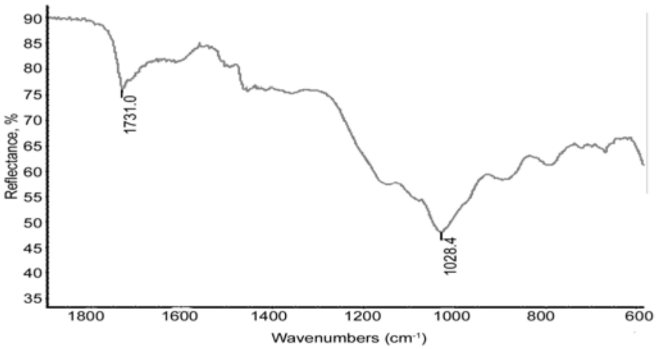

Figure 3. ATR-IR spectrum from alfalfa leaf, III stage of development

The spectra of lignin from stem at first stage of development are comparable to the spectra of lignin from leaf (Figure 4). At the second development stage (Figure 5) an intensive signal at 1462.8 cm^{-1} represent aromatic skeletal vibrations-guaiacyl-syringyl type; 1165.2 cm^{-1} show aromatic CH-in plane deformation, syringyl type; 721.8 cm^{-1}-aromatic CH out-of-plane deformations, syringyl type. At the third development stage (Figure 6) peak at 1605.5 cm^{-1} shows aromatic skeletal vibrations, syringyl type *(Faix, 1991)*.

Stems differ from leaf blades in that their tissue characteristics change greatly with age. *Terashima et al. (1993)* reported that the phenolic units (hydroxyphenyl-, guaiacyl-, and syringyl—propane) varied in the different regions and differed with maturity. The secondary wall, which is adjacent to the lumen and, therefore, most susceptible to microbial colonization, has a relatively high amount of syringyl-units. Syringyl-units are less degraded by microorganisms than are the other lignin units *(Mansson, 1983)*.

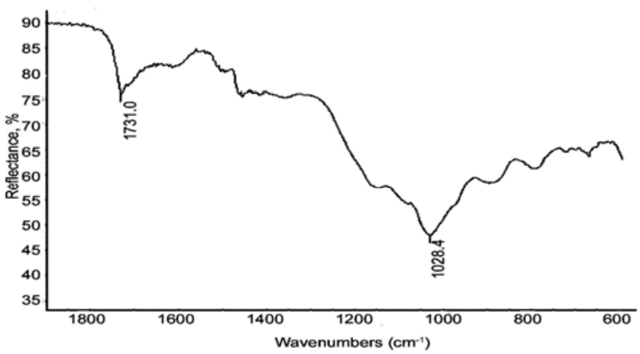

Figure 4. ATR-IR spectrum from alfalfa stem, I stage of development

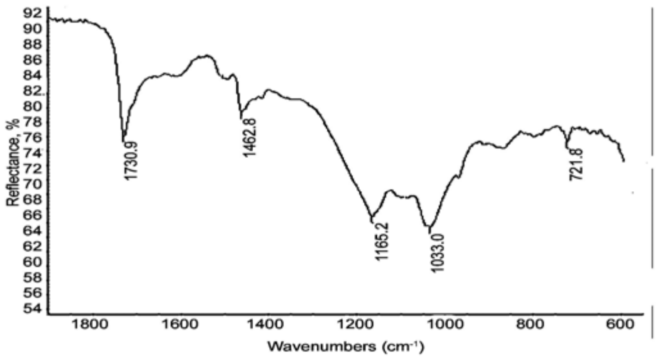

Figure 5. ATR-IR spectrum from alfalfa stem, II stage of development

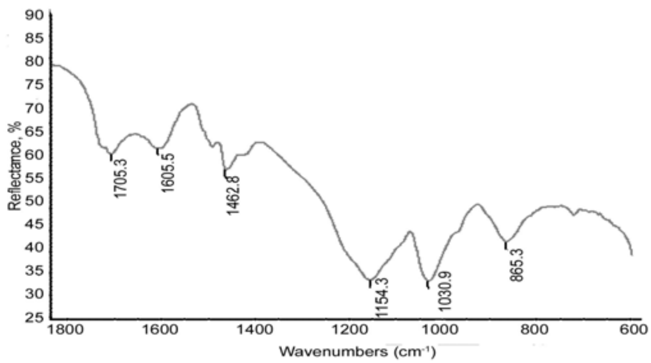

Figure 6. ATR-IR spectrum of alfalfa stem, III stage of development

Conclusion

In the present study we have applied ATR-IR spectrometry to the comparison of lignin composition from alfalfa leaf and stem with growth and development. The spectra of lignin isolated from leaf are similar. The main difference is that in the lignin from alfalfa stem, there are guaiacyl and syringyl ring breathing, whereas in the lignin from leaf there is only guaiacyl ring breathing. Comparison between the peaks from different development stages reveals appearance of new signals after 7-days intervals, which are indications of new bonds. During this period, many chemical changed occurred in the structure and the amount of lignin, which is subject of this paper.

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Promene u strukturi lignina izolovanog iz lista i stabla lucerke u različitim fazama razvića

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Rezime

Ispitivane su promene u količini i strukturi lignina izolovanog iz lista i stabla lucerke, sorta K-22 u različitim fenofazama razvića. Uzorci su košeni u drugom otkosu u sedmodnevnim intervalima – 22., 29. i 36. dana vegetacije. Količina ADL u stablu lucerke je najveća u fenofazi punog cvetanja. U ADL izolovanom iz lista sorte K-22 zabeležen je veći udeo azota. U ADL iz lista sorte K-22 utvrđena je najmanja količina vodonika u trećoj fazi razvića (8,25 %), dok je u prve dve faze razvića približna. Nije ustanovljena jasna tendencija promena udela sumpora u ADL izolovanom iz lista sorte K-22, dok se u ADL izolovanom iz stabla sorte K-22 povećava od 4,25 do 4,70 %. Sa napredovanjem faze razvića, lignin koji se sintetiše u listu lucerke ne prelazi iz gvajacil u siringil tip lignina. Nasuprot ligninu iz lista, lignin iz stabla lucerke sadrži veći udeo siringil jedinica u kasnijim fazama razvića.

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MYCOTOXINS, GMO AND BULK FEED

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Abstract: The work objective was to compare the content of mycotoxins entering the food chain from the maize silage. The paper includes three years observation. The content of mycotoxins was assessed in maize silages after four plant protection strategies against European Corn Borer (ECB), GM hybrid MON 810, commercial isoline Monumental and two insecticide protections (chemical insecticide or biological protection using wasps). Mycotoxins assessed were deoxynivalenol (DON), fumonisin (FUM), and zearalenon (ZEA). The experiments have demonstrated very low or no contamination of GMO maize by ECB and subsequent analysis of *Fusarium* mycotoxins showed a correlation with insect resistance, i.e., mycotoxin content in GMO material was lowest compared to the highest content in the control untreated maize.

Key words: maize, silage, mycotoxins

Introduction

One of the most important factors affecting the health of animals, their efficiency as producers, and the quality of livestock products is the feeding ration. Feedstuffs may contain harmful substances that negatively affect not only the health of animals, but also the safety and acceptability of their products. These harmful substances may contain contaminants that were created during the production, preservation and storage of these feedstuffs, or even during their technological processing. Often, this involves such common contaminants as fungi and their toxins. The occurrence of fungi on maize harvested for silage, which has higher stubble remaining after cutting, is partially eliminated as long as the upper part of the ear is not contaminated by European corn borer (ECB - *Ostrinia nubilalis* Hbn). Corn borer damage leads to fungal contamination which can subsequently spread to other parts of the plant. In the case of harvesting severely contaminated and older maize plants with high dry matter content, the material is usually contaminated with *Fusarium* spp., which leaves its toxins in the silage, decreasing its digestibility, reducing production efficiency, and negatively influencing animals' health. The appearance of fungi and their mycotoxins can also be expected if the silage production technique was not in accordance with proper standards. Especially problematic are slow and interrupted ensiling, contamination with soil, failure to cover the material, leakage of rainwater, insufficient sealing against air, etc. These factors lead to greater contamination with undesirable bacteria and fungi that might cause secondary fermentations and not only result in loss of nutrients but also put at risk the health and physiological functions of the animal consuming the silage.

Mycotoxins are secondary metabolic products from moulds belonging in particular to the *Aspergillus, Penicillium* and *Fusarium* genera. More than 300 secondary metabolites have been identified although only around 30 have true toxic properties which are of some concern. Toxinogenic moulds may develop under all climatic conditions on any solid or liquid supports as soon as nutritional substances and moisture (water activity Aw over 0.6) are present, hence the wide variety of contaminated foodstuff substrates. These toxins are found as natural contaminants in many feedstuffs of plant origin, especially cereals but also fruits, hazelnuts, almonds, seeds, fodder and foods consisting of, or manufactured from, these products and intended for human or animal consumption. Two groups of toxinogenic (mycotoxin producing) fungi can be distinguished. The first one consists of fungi (such as *Fusarium*) which invade their substrate and produce mycotoxins on the growing plants before harvesting: this is the category of field (pre-harvest) toxins. Aflatoxins and *Fusarium* toxins are included in this group. The other group contains fungi which produce toxins after harvesting and during crop storage and transportation. These toxins are named storage (or post-harvest) toxins and ochratoxin A belongs to this group. Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties.

Issues regarding mycotoxins in forages have been much discussed in recent years. In considering the health risks connected with livestock consumption of corn silage contaminated by mycotoxins, we can divide this topic into three parts: 1) issues associated with the growing of maize; 2) issues relating to maize harvesting and silage production; and 3) the feeding process and the possible removal of dangerous contaminants from the feedstuffs. In the Czech Republic's soil and weather conditions, the main mycotoxin producers are soil fungi of the genus *Fusarium*. In discussing this area attention has focussed on growers' interventions that may decrease the contamination of plants by these pathogens and so to create conditions for a lower content of toxic metabolites. From the entire spectrum of technological possibilities, the most important are: the cultivar and its type, dry matter content, and phytopathological and biotechnological steps toward reducing damage to plants by ECB. Among biotechnological steps, we refer mainly to the cultivation of GM maize with incorporated Bt toxin.

Material and Methods

Damage to plants by ECB allows infection by fungal pathogens, and is one of the factors that increase the possibility of contamination by mycotoxins. Experiments carried out over several years have compared protection of maize against ECB using 1) a genetically modified Bt-hybrid, 2) traditional protection using insecticides, 3) biological protection using wasps of genus *Trichogramma,* and 4) a control variant (isoline to Bt-hybrid Monumental). ELISA quantitative tests for mycotoxins analyses were used.

Results and Discussion

These experiments have demonstrated very low or no contamination of GMO maize by ECB. A 70-95 % effectiveness was achieved using insecticides. The effectiveness of biological approaches was strongly dependent upon the weather conditions, but the average effectiveness was less than that using chemical protection. Subsequent analysis of *Fusarium* mycotoxins showed a correlation with insect resistance, i.e., mycotoxin content in GMO material was lowest compared to the highest content in the control untreated maize (Table 1).

Table 1. Number of insect corridors (50 plants) (example from 2007)

	silage	corn grain	DON
control	100 % (43)	100 % (60)	100 % (540 ppb)
insecticide	4 %	8 %	0
biological control	50 %	50 %	60 %
BT hybrid	0 %	0 %	10 %

It should be noted that the mycotoxin content in GMO material was not always zero. Even if this material was not attack by ECB, the material could still have been contaminated by fungi of the genus *Fusarium*, because the genetic modification is intended as a protection against damage done by insects and it does not increase the resistance against fungal pathogens. Notwithstanding all the questions that are related to the use of genetically modified plants, cultivation of GM maize can be recommended from the viewpoint of decreasing mycotoxine contamination. Thirty four studies about *Fusarium* mycotoxin contamination in isogenic BT maize and non-Bt maize hybrids grown in Europe, USA, South America and Asia were analyzed. Thirty out of total amount of studies on Bt maize came to the conclusion that Bt maize is less contaminated with mycotoxins (FUM, DON, ZEA) than the conventional control variety in each case *(Ostry et al, 2009)*.

A list of mycotoxins of interest which are of some concern for the safety of animal feed in the European Union was published in an EU SCAN report *(EU SCAN, 2003)*. It includes Aflatoxin B1 (AFB1) and ergot sclerotia, which are subject to *Commission Regulation (EC) No 1881/2006*. Zearalenone (ZEA), deoxynivalenol (DON), ochratoxin A (OTA) and fumonisins (especially fumonisin B1, FB1), the maximum levels of which are now recommended *(Commission Recommendation 2006/576/EC)*. This list also includes T-2 and HT-2 toxins.

Bt-maize was cultivating on 8,300 ha in the Czech Republic in the year 2008 and making the country the second-largest grower of Bt-maize in the EU after Spain, with about 70,000 ha. In 2009 was a little smaller area about 7000 ha. Borer is afforded not only for maize grain production but also for silage production from maize. This protection is more important for locations with a higher occurrence of this pest in the past, and in later-maturing maize hybrids, for which the interval between the Corn Borer's invasion and the harvest is extended. The results from the subsequent experiments indicate the equivalence of the nutrient composition and feeding value between Bt maize and its near-isogenic control. The digestibility of crude fibre and nitrogen-free extracts of Bt silage determined on whether was higher than that of the control silage *(Křížová et al. 2009)*.

A separate chapter relates to the harvesting of maize, its quality, the subsequent speed and quality of its ensiling, and the quickest possible sealing of the silage against air and its covering. Recommendations, for increasing the quality of silage are provided. There can be no exception in practice if there is not to be secondary contamination of the ensiled material by "storage fungi", which may be connected with mycotoxin production. The article's authors have analysed a wide range of samples collected during the ensiling processes from individual locations of trench silos, as well as samples that have been taken from the face of the silage when loading out the silage for feeding. The results confirmed that if the ensiled material contains a greater amount of mycotoxins, then these can be found across the entire profile of the final silage. If mycotoxins are present during the period of silage fermentation they are also present at the final opening of the silo. The conclusion is drawn, that if maize cannot be cultivated without the possibility of mycotoxins being present, then the ensiling process will not decrease the amount of those substances, because these are chemical compounds with high thermal and chemical stability.

Conclusion

Mycotoxins contamination of maize products could be important negative factor decreasing feed safety for farm animals. Plant protection strategies against European Corn Borer including GMO Bt-hybrid is good way to reduce not only insect incidence, but mycotoxin content too.

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Mikotoksini, GMO i kabasta hrana

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Rezime

Cilj rada je bio da se uporede sadržaji mikotoksina koji se uključuju u lanac ishrane preko kukuruzne silaže. U rad su uključena trogodišnja zapažanja. Procena sadržaja mikotoksina u kukuruznim silažama je vršena nakon primene četiri strategije zaštite biljaka od evropskog kukuruznog plamenca (ECB), GM hibrid 810, komercijalni isolin Monumental i dve insekticidne mere (hemijski insekticidi ili biološka zaštita korišćenjem osa). Analizirani mikotoksini su bili deoksinivalenol (DON), fumonizin (FUM) i zearalenon (ZEA). U eksperimentima je pokazana veoma niska ili nikakva kontaminacija GMO kukuruza sa ECB-om i naknadna analiza *Fusarium* mikotoksina je pokazala korelaciju sa otpornošću na insekte, npr. sadržaj mikotoksina u GMO materijalu je najmanji, a najviši sadržaj je u kontrolnom netretiranom kukuruzu.

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CONSERVATION OF ALFALFA AND ORCHARD GRASS BIOMASS USING THE ENSILING METHOD

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Abstract: The modern alfalfa and orchard grass ensiling technologies implies the understanding of the plant's properties for conservation, such as the sufficient content of fermenTable sugar, buffer capacity, or their favourability for ensilage. The study of ensiling of the alfalfa and orchard grass, as pure (100%) and in mixture in equal ratio (50%:50%), was conducted. Carbohydrate additive was used in amount of 3% and 6% and inoculant. It was determined that in silo-mass of alfalfa and orchard grass the level of DM is lower in leaf than in stem. The values of buffer capacity were higher in leaf than in stem. Ensilability of both leaf and stem of alfalfa and orchard grass depends on water-soluble carbohydrates and on buffer capacity

Key words: inoculants, buffer capacity, silage, carbohydrate feed, sugars

Introduction

One of the most appropriate ways of preservation of alfalfa biomass and orchard grass is ensiling. This is gaining importance in the last ten years due to the global trend of using conserved bulky feed, particularly silage, during the whole year to maximise stability of milk production, *Đorđević and Dinić (2003).* In recent years, in our country, ensiling of alfalfa and orchard grass biomass gets considerable attention. The reason of insufficient ensiling of alfalfa and orchard grass biomass is insufficient knowledge of the technologies of ensiling of the mentioned biomasses. In order to increase the nutritional value or improve the conditions of fermentation, different types of supplements are used during the ensiling process.

The aim of this research is to determine the most appropriate relations of alfalfa and orchard grass for ensiling for the use with the different doses of carbohydrate additives and inoculants application.

Materials and Methods

The following materials for ensiling with the application of the following methods, according to the scheme from Table 1 were used. Following treatments were done:

Table 1. The plan for conservation of alfalfa and orchard grass

Research factors						
Species - A	alfalfa (a_1)			orchard grass (a_2)		
Biomass rations - B	alfalfa 100% (b_1)	orchard grass 100% (b_2)			alf. 50%+orc.gr.50% (b_3)	
Ground corn- C	c_1	c_2	c_3	c_1	c_2	c_3
Applic. of inoc. - D	d_1		d_2	d_1	d_2	

b_1-100% alf.biomass; b_2-100% orch.gr.biomass; b_3-50% alf. biomass+50% orch.bio;
c_1-0% (without additives); c_2-3% ground corn; c_3-6% ground corn;
d_1-0% (without inoculants); d_2-with inoculants;

The experiment was set up using the method of three-factor experiment in three repetitions (Table 1). Alfalfa and orchard grass were both cut in the flowering stage (70%). The floristic composition was determined and the crop was very clean (Table 2).

Table 2. Floristic composition of alfalfa and orchard grass biomass, %

Species	%	weeds and other species	total
Alfalfa	99.02	0.98	100
Orchard grass	86.55	13.15	100

The share of steam and leaf in the plant, with no weed and other species, is shown in Table 3.

Table 3. The share of steam and leaf in the plant, %

Species	development phase	
	blossoming	
	leaf	stem
Alfalfa	39.00	60.00
Orchard grass	26.89	73.10

Results and Discussion

For successful of alfalfa and orchard grass biomass, it is necessary to determine the content of water soluble carbohydrates-sugar (S), the buffer capacity (BC), the ratio of S/BC, the minimum dry matter content (DM) and the critical value of pH, *Weissbach (1967)*. If the dry matter content in the ensilaged material is lower, it is necessary to intensify the acidity, which would prevent the creation of butyric acid. The pH value which is necessary in a particular content of dry matter to obtain stable silage is called the critical pH value.

For fermentation to go in the desired direction, the ratio S/BC must be higher if the dry matter content is lower. The minimum dry matter content (Y) depends on the relationship S/BC(x) and is expressed as the equation Y (g kg^{-1}) =450-80x.

The botanical analysis of silo-mass in the flowering stage (70%) shows that the participation of alfalfa is 99.02%, and the remaining of 0.98% were other species and weed. Participation of steam in the green matter (or dry matter) is 60.00% (58.08%), and the participation of the leaves was 39.02% (41.92%) (Tables 2 and 3).

The botanical analysis silo-mass in the flowering stage (70%) shows that the participation of orchard grass is 86.86%, while the remaining 13.13% were other species and weed. Participation of steam in green matter (or dry matter) is 63.50% (61.08%), and the participation of the leaves was 23.35% (38.92%).

Alfalfa is one of the forage crops that are difficult to ensilage. Small content of S, the high value of BC, as well as very unfavourable ratio of S/BC, show the disadvantage of alfalfa biomass for ensilage (Table 4). To successfully ensilage alfalfa biomass without the butyric acid synthesis, the wilting or addition of nutrients to provide DM content of about 400 g kg^{-1} must be done (Table 4).

It has been determined that as the plant grows, the relative ratio of the leaves in relation to the stem decreases, and due to that comes the changes in sugar content and buffer capacity. This is because the leaves contain more nutrients essential for quality in relation to the stem, *Ignjatović et al. (1998)*. With age (ripening) of fodder crops improves the suitability for ensiling, i. e. the concentration of sugar increases, and the buffer capacity decreases.

The level of dry matter in silo-mass has great influence on the suitability of alfalfa for ensiling. In alfalfa, it was lower in the leaf (355 g kg^{-1}) than in the stem (418 g kg^{-1}). The buffer capacity of plants and their ability to confront the change of pH is an important factor in ensiling, *Dinić et al. (1998, 2005)*. The buffer capacity is defined as the amount of lactic acid that is required for the increase of the acidity of the silo-mass to pH 4, *Weissbach (1967)*. The buffer capacity of leaf and stem showed higher results in the leaf (110.0) than in stem (54.4) (Table 4).

The buffer capacity of leaf, in relation to the stem, is the result of greater amounts of crude protein and minerals.

To reduce the pH to a certain level the more lactic acid is necessary, in accordance with the fact that if there is more sugar, the silo-mass BC is higher. Calculation is performed from the ratio of sugar (g kg^{-1} DM) to the buffer capacity (meqv lactic acid/100gDM), whereby the use of abbreviations S/BC. If the conversion of sugar into lactic acid is complete and without loss, the ratio S/BC=1 would be sufficient to ensure necessary acidity. But, because of the process of breathing and the creation of acetic acid, alcohol and other products of the fermentation, is always a considerable amount of sugar dissolved and thus does not participate in the creation of lactic acid Therefore, the relationship S/BC should be significantly higher than 1, in order to ensure a stable pH, which is in our tests, (Table 4).

The suitability of the alfalfa leaf and stem for ensilage depends on the balance of the water soluble carbohydrates and the buffer capacity. This ratio, in our tests, in the leaf of alfalfa is 0.79 which shows that it is not suitable for ensiling. The ratio of water soluble carbohydrates and the buffer capacity in the stem of alfalfa is twice better and is 1.63. The ratio of S/PC in the alfalfa leaf was 0.736, while the ratio of S/BC in the stem of alfalfa was 0.931

Table 4. Suitability of alfalfa biomass from the first cut for ensiling g kg^{-1} DM

Parameters / Description of the samples	Dry matter g kg^{-1}	Buffer capacity, meqv lactic acid/100g dry matter	Mono saccharides	Total sugar content	S/BC ratio
Alfalfa leaf	355	110.0	78.7	86.9	0.79
Alfala stem	418	54.4	66.6	88.8	1.63
Xsl	386	64.9	85.0	99.5	1.53

Xsl-suitability of alfalfa biomass for ensiling (g kg^{-1}DM and meqv lactic acid/100gDM-average (chopped alfalfa)

Forage crops from the group of bladed grasses are more suitable for ensilage than the forage plants from the legume group. Among the forage crops, in terms of ratio S/BC, alfalfa is the last, while the ratio in the orchard grass and other bladed grasses is somewhat better. Among grasses, there are large differences between species, both in content of DM and in the ratio of S/BC. Orchard grass is very insufficient in sugar compared to the other species (Table 5).

The cutting of blade grass should be done when they from the inflorescences (in phase of earing). The cutting in the later stages leads to significant reduction in protein content, minerals, crude fibre content increases, i. e. reduction of the quality or the low nutritional value.

Table 5. Suitability of orchard grass biomass from the first cut for ensiling g kg^{-1} DM

Parameters / Description of the samples	Dry matter, g kg^{-1}	Buffer capacity, meqv lactic acid/100g dry matter	Mono saccharides	Total sugar	S/BC ratio
Orchard leaf	429	72.0	132.1	142.3	1.97
Or.grass stem	445	25.6	66.3	82.8	3.23
Xsj	424	39.6	94.9	112.7	2.83

Xsl-suitability of orchard grass biomass for ensiling (g kg^{-1}DM and meqv lactic acid/100 g DM-average (chopped orchard grass)

For each S/BC ratio, the content of SM, required for stable silage, can be indicated, and vice versa, to every value of SM content there is a need for corresponding S/BC ratio. If these values can not be reached, the success of silage should not be counted on.

The buffer capacity of the leaf, in relation to the stem, is the result of greater amount of crude protein and minerals in the mentioned phase of cutting (Table 5).

It is know that there are differences in sugar content between cultivars within species. *Dent and Aldrich (quoted McDonald-a) (1985)* have found that orchard grass cultivars that mature early had higher sugar content that those that mature late. The suitability of crops for ensiling can be accurately determined based on the relations of the sugar content and the buffer capacity, *Dinić et al.(1998)*.

Suitability for ensiling is not a constant trait, and therefore, in its assessment should be based on the specific conditions in each case.

Conclusion

It can be seen that, analysing the suitability for ensilage of leaf and stem of alfalfa in relation to orchard grass and vice versa, the buffer capacity is much higher in alfalfa, while all other results were better in orchard grass.

Konzervisanje biomase lucerke i ježevice metodom siliranja

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Rezime

Savremeni pristup tehnologije siliranja lucerke i ježevice podrazumeva poznavanje njihovih osobina za konzervisanje u pogledu dovoljnog sadržaja fermentirajućih šećera, puferskog kapaciteta, tj. pogodnosti za siliranje. U ovom radu izvršena su istraživanja siliranja lucerke i ježevice u čistom obliku (100%) i u smeši sa jednakim udelom (50%:50%) Korišćen je ugljenohidratni dodatak u količini od 3% i 6% i inokulant. Ustanovljeno je da je u silomasi lucerke i ježevice nivo SM niži u listu u odnosu na stablo. Puferni kapacitet je pokazao veće rezultate kod lista nego kod stabla. Silažnost lista i stabla lucerke i ježevice zavisi od odnosa u vodi rastvorljivih ugljenih hidrata i pufernog kapaciteta.

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IMPROVEMENT OF QUALITY OF SILAGES FROM SORGHUM X SUDAN GRASS HYBRID

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Original scientific paper

Abstract: Objective of this study was to assess the nutritive value of *Sorghum bicolor x Sorghum sudanense* hybrid GK-Csaba (HU) and to test the effectiveness of biological ensilaging preparations on fermentation process during ensilaging. The hybrid was grown in identical agro-climatic conditions, in four different sites. Plants in ensilaging ripeness contained medium to higher content of crude protein (131 – 186 g kg^{-1} dry matter), fibre (260 – 291 g kg^{-1} DM) and ash (65 – 81 g kg^{-1} DM). Content of NDF was in all samples higher than 540 g kg^{-1} dry matter. NEL represented 5.81 – 5.99 MJ.kg^{-1} dry matter. The ensilaged Sudan grass hybrid was divided into 1 control variant without treatment and 4 experimental variants treated with bacterial inoculants. The ensilaged mass contained 33 % dry matter. pH measurement on day 3 from ensilaging showed that the most rapid start of fermentation was in silages, which were treated with biological preparations in liquid form (pH 4.09, 4.16), then in silages treated with granulated inoculants (pH 4.29, 4.35), and the slowest one in the untreated silage (pH 4.49). The following course of fermentation was in line with its start. The inoculated silages had lower pH, higher content of lactic acid, lower content of volatile fatty acids, alcohol as well as ammonia nitrogen compared with non-treated silage. Content of nutrients in *Sorghum bicolor x Sorghum sudanense* hybrid depends on growing conditions and level of nutrition. Results obtained in our experiments show good ensilaging capacity of sorghum x Sudan grass hybrid. Bacterial inoculants on the basis of homo-fermentative as well as combination of homo- and hetero-fermentative bacteria of lactic fermentation speed up the start and improve the course of the fermentation process.

Key words: *Sorghum bicolor x Sorghum sudanense* hybrid, nutrition value, silage, fermentation, inoculation

Introduction

The average annual air temperature rose at the ground level of Earth's atmosphere by 0.3 to 0.6^oC, by 0.8^o C in Europe and by 0.9 to 1.1^o C in Slovakia during the last 100 years. The increase in temperature influences also other characteristics of the environment, mainly atmospheric precipitations, solar radiation, evapotranspiration, soil moisture and other.

It is necessary to compensate the increase of average temperature by 1^{o} C by means of precipitations increase by 15 % compared with the normal in order to keep steady growth of plants. As it does not occur, we see for some years already that the raise of temperature at simultaneous decrease in precipitations during the vegetation causes decrease in plant production. Losses arising from climatic changes influence significantly the economy in whole agriculture. One of the possibilities how to eliminate these losses, at least partly, is diversification of plant production and gradual introduction of drought withstanding crops into the crop rotation. Sorghums seem to be very suitable in the feedstuff production for ruminants.

Sorghums are plants of physiological type C_4, with more intensive photosynthesis and lower water evaporation caused by waxy leaves with lower number of stomas *(Avasi et al., 2001)*. They are noted for quick growth, tolerance of high temperatures and long lasting draughts. They give enough feed also in dry years. Importance of sorghums growing increases in regions, in which is the yield of maize uncertain because of soil and climatic conditions. They are mainly maize production regions with lighter soils, lower level of underground water and low atmospheric precipitations. Cold and wet soils limit their growth, therefore it is recommended not to grow them in higher situated regions *(Lang, 2001)*.

Hybrids of *Sorghum sudanense* can be mown several times and they can give three cuts during the vegetation period with total yield of fresh green matter up to 80 tons per hectare. It is possible to use the obtained feed in many ways; for grazing, green feeding, ensilaging in horizontal silos, and packed into bales. It is a problem to use them for hay because of thick stalks. Classic technology used at harvest of other bulky feeds is used also at sorghum harvest.

Aim of this work was to assess the nutritive value of hybrid *Sorghum sudanense x Sorghum bicolor,* variety GK Czaba (HU), as well as to test the effectiveness of biological ensilaging preparations on fermentation process at ensilaging.

Materials and Methods

Sorghum sudanense hybrid (variety GK-Csaba) was sown on 4 different sites in south Slovakia, at the altitude of 118 m above sea level. It was sown in the first decade of May at seeding rate 50 kg seed per hectare. Samples of stand were taken immediately before heading of plants to assess nutritive value of feed. Average annual temperature varied from 9 to 10.5^{o} C and annual precipitation depth from 530 to 650 mm in the course of this task.

The stand wilted to 33 % content of dry matter for ensilaging. At ensilaging we used one control variant (C) not treated with ensilaging additive and four experimental variants in which was the ensilaged matter treated with following preparations:

E_1 – biological preparation (*Lactobacillus plantarum* DSM 3676 and 3677, *Propionic bacterium* DSM 9576 and 9577), application rate 2 ml of preparations' activated solution per 1 kg ensilaged feed
E_2 – biological preparation (*Enterococcus faecium* M 74, *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus species*), application rate 1.1 ml solution of preparation per 1 kg ensilaged feed
E_3 – granulated biological preparation (*Lactobacillus rhamnosus* NCIMB 30121, *Enterococcus faecium* NCIMB 30122), application rate 0.5 g per 1 kg feed
E_4 – granulated biological preparation composed of strains of *Lactobacillus plantarum* DSM 4784-7, *Enterococcus faecium* DSM 4788-9), application rate 0.5 g granulated preparation per 1 kg ensilaged feed

In the course of fermentation were assessed weight losses in silages by weighing in regular 21 days intervals; dry matter losses in silages were calculated. Chemical analysis was done in samples of fresh feeds and silages; results were statistically processed and evaluated. The results were statistically evaluated by one-factorial analysis of variance, and compared by Student-t test.

Results and Discussion

Samples of stand were taken at ensiling ripeness, which was assessed for the period immediately before heading of plants. On the basis of nutrients concentration assessed in plants at individual stands (Table 1) it is possible to state that in this fodder crop is medium to higher content of crude protein (131 – 186 g kg^{-1} dry matter), fibre (260 – 291g kg^{-1} dry matter) and ash (65 – 81 g kg^{-1} dry matter) compared with other fodder crops grown normally in our country. NDF content was higher than 540 g kg^{-1} dry matter in all samples and we consider it for very high as far as nutrients requirement of high yielding dairy cows are concerned. On the contrary, fat concentration in the hybrid was low.

Table 1. Content of nutrients in hybrids *Sorghum bicolour x Sorghum sudanense*

Parameter, in g kg^{-1} dry matter	Stand			
	1	2	3	4
Dry matter	182,82	198,67	216,10	212,33
Organic matter	918,82	923,18	934,68	931,58
Crude protein	186,32	180,92	130,61	133,96
Fibre	286,14	290,81	259,65	271,10
ADF	316,84	316,76	276,11	292,11
NDF	549,69	552,98	540,79	542,09
Hemicelluloses	232,85	236,22	264,68	249,98
Nitrogen-free extract	387,70	398,57	522,63	518,55
Fat	18,66	19,88	21,80	22,15
Ash	81,18	76,82	65,32	68,42
Total sugars	54,03	58,10	156,99	137,63
Reducing sugars	33,04	37,12	106,06	120,43
ME in MJ.kg^{-1} DM	9,86	9,90	10,11	10,08
NEL in MJ.kg^{-1} DM	5,81	5,84	5,99	5,97
NEV in MJ.kg^{-1} DM	5,65	5,68	5,88	5,86
PDIN in g kg^{-1} DM	113,40	110,11	79,49	81,53
PDIE in g kg^{-1} DM	99,01	97,99	88,37	88,84

Content of nutrients in hybrid varied at individual sites. Nutritive as well as energy value in *Sorghum sudanense* hybrid was closely connected to cultivation conditions (soil, nutrition). *Wheeler and McKinlay (1998), Undersander and Lane (2001), Kaiser and Pilz (2002), Rajcakova et al (2004, 2005)* indicated instability in nutritive value. *Lang (2001)* reported 550 – 680 g NDF in kilogram of dry matter in *Sorghum sudanense* hybrids; it is more than was assessed in our experiment. Content of crude protein in our experiment was similar.

Table 2. Content of nutrients in ensilaged hybrid *Sorghum bicolor x Sorghum sudanense*

Parameter	Value (g kg^{-1} DM)
Dry matter	332,52
Organic matter	931,58
Crude protein	133,96
Crude fibre	271,10
ADF	292,11
NDF	542,09
Nitrogen-free extract	518,55
Fat	22,15
Ash	68,42
Total sugars	137,63
Reducing sugars	120,43

Table 3 gives results of fermentation in *Sorghum sudanense* hybrid ensilaged at optimum content of dry matter 33 %. Parameters of fermentation process in individual silages show positive influence of applied biological inoculants. Measurement of pH on day 3 of silages fermentation shows that the fastest onset of fermentation was in silages treated with biological preparations in liquid form, followed by silages treated with granulated preparations and the slowest onset of fermentation process was in the untreated silage. Further course of fermentation was also in line with its onset. Inoculated silages had lower pH, higher content of lactic acid, lower content of volatile fatty acids, alcohol and ammonia nitrogen compared with untreated silage.

Table 3. Parameters of fermentation process and selected nutrients in silage of hybrid *Sorghum bicolor x Sorghum sudanense* (in g kg^{-1} dry matter)

Parameter		Silage variants					Statistical of differences	
		C	E1	E2	E3	E4	p ‹ 0,05	p ‹ 0,01
pH	$\bar{x}$	3,85	3,69	3,75	3,81	3,79		E1:C,E3,E4 E3:E4
	s	0,06	0,01	0,09	0,03	0,01		
pH on day 3 of fermentation		4,49	4,09	4,16	4,35	4,29		
Acids - lactic	$\bar{x}$	50,91	68,28	67,65	72,37	69,71		C: E1,E2,E3,E4
	s	3,66	5,59	5,87	6,16	4,99		
- acetic	$\bar{x}$	4,54	2,96	3,02	4,13	4,72	C:E1	E2:C,E3,E4 E1:E4
	s	1,00	0,51	0,14	0,64	0,57		
- propionic	$\bar{x}$	0,68	0,41	0,31	0,43	0,35	C:E1	C:E2,E3,E4
	s	0,19	0,12	0,08	0,07	0,06		
- butyric + i.b	$\bar{x}$	0,12	0,50	0,37	0,32	0,25	E1:E4 C:E2,E3	C:E1
	s	0,08	0,12	0,15	0,18	0,16		
VFA total	$\bar{x}$	5,89	4,22	4,05	5,22	5,69		E4:E1,E2
	s	1,08	0,71	0,24	1,12	0,55		
Alcohol	$\bar{x}$	3,93	3,39	3,46	2,93	3,88	C:E3	
	s	0,92	0,47	0,89	0,18	1,19		
NH_3-N of total N in %	$\bar{x}$	4,02	3,36	3,40	3,96	3,62	E1:C,E3 E2: E4	E2 : C,E3
	s	0,36	0,21	0,08	0,32	0,16		
Dry matter in	$\bar{x}$	310,5	311,8	311,4	309,3	308,8		
	s	6,37	5,81	7,94	2,92	6,29		
Losses of DM in %	$\bar{x}$	8,2	6,8	7,5	7,3	8,1		
	s	1,16	1,76	1,22	0,87	1,40		
Crude protein	$\bar{x}$	129,2	131,0	132,4	130,2	130,5		
	s	6,53	2,43	2,69	5,73	1,04		
Crude fibre	$\bar{x}$	287,0	292,0	293,8	291,8	287,8		
	s	3,93	3,62	5,83	3,49	3,83		
Total sugars	$\bar{x}$	64,7	69,2	65,0	48,5	52,0	C:E2	E1: C,E2,E3,E4 E2: E3,E4
	s	3,53	1,86	2,99	2,38	2,79		
Reducing sugars	$\bar{x}$	51,1	58,0	51,3	35,1	43,2	C:E2	E1:C,E2,E3,E4 E2: E3,E4
	s	9,07	1,51	3,74	3,63	7,68		
Fat	$\bar{x}$	29,6	28,3	29,6	28,0	26,2		
	s	2,00	1,82	1,27	3,07	2,48		
Ash	$\bar{x}$	63,4	62,3	63, 8	66,0	65,3		
	s	0,72	1,67	0,45	0,94	1,44		

Conclusion

Hybrids of *Sorghum sudanense* (*Sorghum bicolour x Sorghum sudanense*) are feeds suitable for growing in the driest regions of south Slovakia because of fast growth, tolerance of high temperatures and long lasting droughts. This feed does not reach the quality of classic fodder crops; however, it brings higher yields, which enables to enrich the feeding base for cattle during dry years.

Content of nutrients in hybrids of *Sorghum sudanense* depends on cultivation conditions and level of nutrition. In our conditions, it contains medium to higher content of crude protein (13 – 18.6 %), fibre (25 – 29 %) and ash (6.5 – 12 %), high content of neutral detergent fibre (54 – 55 %) and hemicellulose (23 – 26.5 %), and low content of fat (1.9 – 2.2 %). We consider ensilaging during suitable vegetation stage to be the most convenient utilization of sorghums in nutrition of cattle. For common sorghum it is the stage of wax ripeness, for the hybrid of *Sorghum sudanense* it is the period closely before heading of plants. This fodder plant is ensilaged by two-stage harvest. Optimum content of dry matter is on the level 30-35 % after wilting of the chopped matter. Results obtained in our experiments show good ensilability of *Sorghum sudanense* hybrids. Biological inoculants based on homo-fermentative as well as on combination of homo- and hetero-fermentative bacteria of lactic fermentation, accelerate the onset and improve the course of fermentation process. As wilting of sorghum is problematic, we recommend wilting of the matter during the moist weather for necessary time only, and ensilage the feed at lower content of dry matter using chemical conserving additives. *Sorghum sudanense* silage is very well applicable in combination with lucerne and maize silages in the feed ration for cattle.

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Poboljšanje kvaliteta silaže spravljane od sirka x hibrida sudanske trave

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Rezime

Cilj rada je određivanje hranljive vrednosti *Sorghum bicolor x Sorghum sudanense* hibrid GK-Csaba (HU) i testiranje efikasnosti biološke pripreme silaže u odnosu na proces fermentacije tokom siliranja. Biljke za silažu su imale srednji do

visoki sadržaj sirovih proteina (131 – 186 g kg^{-1} suve materije), celuloze (260 – 291 g kg^{-1} SM) i pepela (65 – 81 g kg^{-1} SM). Sadržaj NDF je u svim uzorcima bio veći od 540 g kg^{-1} suve materije. NEL je bio 5.81 – 5.99 MJ kg^{-1} suve materije. Silirani hibrid sudanske trave je postavljen u kontrolnoj varijanti bez tretmana i 4 eksperimentalne varijante tretirane bakterijskim inokulantima. Silirana masa je sadržala 33% suve materije. Merenje pH, tri dana nakon siliranja, je pokazalo da je proces siliranja najbrže otpočeo u silažama koje su tretirane biološkim rastvorima u tečnom obliku (pH 4.09, 4.16), zatim u silažama tretiranim granuliranim inokulatima (pH 4.29, 4.35), a najsporije u silažama koje nisu bile tretirane (pH 4.49). Fermentacija je nastavljena u istom pravcu kao i na početku. Inokulirane silaže su imale niži pH, viši sadržaj mlečne kiseline, niži sadržaj isparljivih masnih kiselina, alkohola, kao i amonijačnog azota u odnosu na silažu koja nije tretirana.

Dobijeni rezultati pokazuju da smeša sirak x hibrid sudanske trave ima dobar kapacitet za silažu. Bakterijski inokulanti, bazirani na homofermentativnim i kombinaciji homo- i heterofermentativnim bakterijama mlečno-kiselinske fermentacije, ubrzavaju početak i poboljšavaju tok procesa fermentacije.

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PRODUCTION OF FODDER IN THE HUNTING GROUNDS FOR GAME FEEDING AND DECREASE OF DAMAGES IN AGRICULTURE AND FORESTRY

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Review paper

Abstract: One of the possibilities for hunting business in order to reduce damages in agriculture is to produce certain crops within the hunting ground for additional feeding of game animals. Most suitable for that purpose are legumes (lucerne and others), cereals (maize, ray, oats, triticale etc.), roots and tubers (beet, potato), cabbages (canola, kale, perko…) and others. The mentioned plants may be used directly as green feeds, or as pasture, but also may be conserved for winter feeding – as hay, silage and haylage, and conserved in other ways to be used when needed. The feeds should be planted on several places in the hunting ground, so that animals wander searching for them. The culture production should be in a form of a "green conveyer" so that green forages are continuously available for animals. Planted fields should have some fence so that animals are allowed to eat forages when they are in the optimal phase. With the described measures it is possible to achieve significant reduction of crop damages and increase in the number of animals and their trophy values, also the decrease in animal losses during the winter period.

Key words: game, damages, feeding, fodder, conserved feeds

Introduction

In addition to economic benefits that man obtains from game and its positive influence on the habitat, it is also important to consider negative impact of the game, both in forestry and farming and on domestic animals and wildlife itself. Damages caused by game are usually caused by lack of, poor quality and inadequate distribution of food within the hunting grounds, as well as by disturbing of game and even by habits of the game *(Popović, 2006a)*. Wildlife from the carnivore order may cause specific type of damage both in hunting grounds and on livestock and beehives. „Predatory" type of damage may also be caused by wild boars, although this species is an omnivorous even-toed ungulate and not a carnivore *(Popović et al., 2009)*.

Damages may be reduced or prevented by adequate management of the hunting grounds *(Popović, 2006b; Đorđević et al., 2007)*. Increase in natural food production within the hunting grounds and provision of additional food from other sources that is conducted during the period when damages occur are among the more important measures *(Đorđević et al., 2006a; 2008)*. These procedures may help to maintain adequate game population *(Popović et al., 2008)*, control losses *(Popović and Bogdanović, 2001)* and increase trophy value *(Popović and Bogdanović, 2002; Popović and Gačić, 2006)*. A series of protective measures for farming and horticulture crops, orchards and vineyards, forests, domestic animals and beehives, as well as fencing the hunting grounds, which allows breeding game in a controlled environment, may be undertaken *(Popović and Đorđević, 2009)*. High degree of wildlife control and protection is obtained in national parks *(Beuković et al., 2006)*.

Feeding potentials of Serbian hunting grounds

Hunting association of Serbia is managing an area of 7.891.318 ha of hunting grounds where dominant species are small game and roe deer. The percentage of forests is one of the better parameters of suitability for life of most game animals. In that respect Vojvodina is not in very good situation (only 7.07% of forests), while Zlatibor, Raška, South Serbia, Timok, Kosovo and Metohija are in better position (above 40% forests, Table 1).

Table 1. Overview of total hunting grounds (ha) in Serbia *(Hunting Association of Serbia, 2001)*

Name of the area	Total	Forests and forest lands	Meadows and pastures	Arable land	Fruit and vineyards	Other land
Bačka	890975.60	40925.00	45078.50	703647.00	12188.00	89137.10
Banat	883169.00	42001.50	109705.00	635265.00	10175.00	86022.50
Srem	378491.00	69351.00	18684.00	241533.00	10961.00	37962.00
Vojvodina	2152535.60	152277.50	173467.50	1580445.00	33324.00	213121.60
Beograd	315685.00	36328.00	24864.00	199186.00	19835.00	35472.00
Podunavlje	499469.47	118802.47	67863.00	238566.00	30751.00	43487.00
Šumadija	691826.00	185420.00	110346.00	290347.00	59180.00	46533.00
Kolubara	573844.00	142224.00	70931.00	288631.00	35514.00	36544.00
Zlatibor	724339.00	281769.00	271713.00	113863.00	14335.00	42659.00
Raška	705913.67	292860.61	169298.69	163871.30	33072.00	45811.07
Timok	724269.45	302786.80	153614.50	205987.83	16481.33	45398.99
South eastern Serbia	722297.00	238461.00	174293.00	233667.00	31623.00	44253.00
South Serbia	625986.10	241498.60	155816.00	170804.00	24739.00	33128.50
Central Serbia	5583629.69	1840150.48	1198739.19	1904923.13	265530.33	374286.56
Kosovo	602287.00	257518.00	126476.00	183515.00	3459.00	31319.00
Metohija	489976.00	207825.00	126877.00	118953.00	8532.00	27789.00
Kosovo and Metohija	1092263.00	465343.00	253353.00	302468.00	11991.00	59108.00
Serbia	8828528.29	2457770.98	1625559.69	3787836.13	310845.33	646516.16

Forests present suitable environment for game animals and provide natural feeds in the form of pastures, browse, seeds, mushrooms and small animals (for omnivores and carnivores), as well as water springs. In forest environment there is very little need for supplemental feeding, and may be considered only in cold winters with high snows *(Đorđević et al., 2008)*. The lowland type of hunting ground, which is present in Vojvodina and in parts of Central Serbia, is much less abundant in natural feeds. The reason for that is intensive agriculture, the use of chemicals, high density of human and animal populations. In such circumstances the additional feeding of game is becoming indispensable, particularly during the winter *(Đorđević et al., 2005)*.

Types and degree of damages in agriculture and forestry

Damages in agriculture may be created by game on crops (maize, lucerne, wheat, barley, potato and others) are generally less than 1%, and therefore may be considered as negligible *(Kaluzinski, 1982; Obrtel and Holišova, 1983)*. The biggest damage is created by wild boars and ruminants, while birds may be harmful only in period when maize is in early growth *(Holand, 1994; Holišova et al., 1984, 1986)*. The greatest damages on crops are observable on soya and kidney beans during the growing period and somewhat less in flowering phase. The damages on crops usually occur during the late spring and summer particularly on maize and sunflower, and the number of plants affected is not very high (Table 2).

Table 2. Degree of maize devastation by roe deer in various development phases *(Obrtel and Holišova, 1983)*

Sect	Row lenght m	Number of plants in a row	Total number of plants	A (2. July 1980)			B (23. august 1980)		
				Damaged	Undamag.	% damaged	Damaged	Undamag.	% damaged
1	150	585	3.510	397	3.113	11,31	62	3.448	1,77
2	84	328	1.968	251	1.717	12,75	280	1.688	14,23
3	140	546	3.276	238	3.038	7,26	86	3.190	2,63
4	142	554	3.324	62	3.262	1,87	47	3.277	1,41
5	152	593	3.558	92	3.466	2,59	320	3.238	8,99
6	127	495	2.970	105	2.865	3,54	285	2.685	9,60
7	266	1.037	6.222	89	6.133	1,43	314	5.908	5,05
8	150	585	3.510	12	3.498	0,34	61	3.449	1,74
9	146	569	3.414	153	3.261	4,48	29	3.385	0,85
Total	1.357	5.292	31.752	1.399	30.353	4,41	1.484	30.268	4,67

Total damage in orchards (distraction of young plants and bark cutting) may occur in periods when natural feeds are deficient, or if the number of animals becomes too high for the size of the hunting ground *(Đorđević et al., 2009b)*. That kind of damage is characteristic for hares and wild ungulates *(Malik and Karnet, 2007)*.

Damages in forestry occur on plants in various phases of their growth and development, or on forest seeds and fruits that are important in plant reproduction. The highest intensity of those damages is during the winter when there are no fresh feeds available on meadows and in forests. That kind of damage may be substantial in fenced hunting grounds, with inadequate production of natural feeds and there is no additional feeding available to animals.

One of the common damage in forestry happens due to plant browsing. It is starting with the end of autumn and lasts until the spring, usually by wild ruminants: red and roe deer *(Đorđević et al., 2009a)*. Based on the anatomy of their digestive organs wild ruminants are classified in three groups: browsers, grazers and those that use both methods of feeding *(Hofmann, 1989)*. Browsers are best adapted to rapidly fermented feeds usually with lower digestibility. Grazers slowly digest plant material, but they digest more efficiently. The third group uses many feeds and is more flexible because of that.

Stripping tree bark is mostly happening because of the inadequate feed supply, certain nutrient deficit (minerals or vitamins), or the need for unidentified substances with medicinal properties. It may occur at any time of the year. Most often it is produced by red deer. On the damaged parts of the tree the rotting or parasites may develop. The tree is weakening and slowing its growth, and the technical properties of timber are changed, or the tree may be broken on very severely damaged places (Table 3).

Table 3. Changes in chemical composition of pine and spruce bark due to damage produced by deer, g/kg *(Malik and Karnet, 2007)*

Tree species and degree of damage	Nitrogen matters	Fats	Ash	Pulp	Non-fat extract	Sugars	Ca	P
Scottish pine								
Unaffected bark	35.72	59.05	38.25	209.15	655.,43	146.57	5.19	0.64
Damaged bark	46.86	52.51	34.45	221.70	644.15	156.42	3.64	0.81
Norwegian spruce								
Unaffected bark	34.35	47.01	43.14	246.99	630.69	140.85	9.07	0.60
Damaged bark	31.40	50.75	47.80	217.80	652.15	142.44	7.34	0.65

Green fodder production in hunting grounds

The dominant plant species on our meadows are grasses and legumes of variable nutritional quality, also weeds and some harmful and poisonous plants. With the appropriate agrotechnical measures, like irrigation, plowing, cutting and fertilizing, the botanical composition may be changed in some areas of hunting grounds, and improved the quality and quantity of natural feeds *(Đorđević et al.,* *2006b)*. Natural pastures should be mowed two times per year, and plowed and fertilized once a year with 100 kg of mineral fertilizer per hectare *(Novaković, 1999)*. Those methods decrease the possibility for weeds to grow and produce their seeds, which is changing the floristic composition and nutritive value of the green mass.

It is also important to plant fruit and other trees that produce feeds for animals in the hunting grounds. Such trees are oak, beech, chestnut, plum, apple, pear, mulberry and similar *(Nečas, 1972)*.

Aside from the improvement of natural pasture, it is possible to create new meadows in some areas. Usually they are made as mixtures of perennial grasses which have different life duration, morphological composition and nutritive value (table 4). Due to variability, such mixtures are suitable for various terrains and ecological conditions. The composition of those mixtures depends on many factors, especially on chemical composition of the soil *(Ocokoljić et al., 1983)*.

Table 4. Areas for deer feeding, ha/animal *(Novaković, 1999)*

Type	Fenced hunting ground	Open hunting ground
Natural pasture	0.10	0.05
Artificial pasture	0.04	0.02
Arable land	0.05	0.03

It's one of the possibilities to produce green forages in hunting grounds. Most suitable for that purpose are legumes (lucerne and others), cereals (maize, ray, oats, triticale etc.), roots and tubers (beet, potato), cabbages (canola, kale, perko…) and others *(Đorđević and Dinić, 2007)*. Winter cereals seeded at the end of summer, with sufficient rain, can produce abundant green fodder during the winter. Excellent culture for such production can be kale. It is very resistant on low temperatures (to -15°C) so the animals may use it as green feed during the whole winter if the snow is not very deep. It has average content of 2.4% crude protein and 1.7-2.4% crude fiber, and it is rich in calcium and all vitamins. Especially suitable for hunting grounds is topinambur (*Heliantus tuberosus* L.) It can be used for green mass and tuber production, and its crops can be used for animal to hide, since the plant may be up to 3 m high. Red and roe deer like to eat its plant, and the dig the soil to collect the roots. It is helpful if the soil is shallowly ploughed. The topinambur tubers are eaten by wild boars with great appetite. Once established topinambur plantation may last for year (and decades). Its tubers have 22% dry matter, 1% crude fiber, 0.2% fat, 2% crude proteins, of which 30-40% is in form of amides *(Đorđević et al., 1996)*.

The mentioned plants may be used directly as green feeds, or as pasture, but also may be conserved for winter feeding – as hay, silage and haylage (Table 5), and kept in other ways to be used when needed *(Đorđević et al., 2007b)*. The feeds should be planted on several places in the hunting ground, so that animals wander searching for them. The culture production should be in a form of a "green conveyer" so that green forages are continuously available for animals. Planted fields should have some fence so that animals are allowed to eat forages when they are in the optimal phase.

Table 5. Chemical composition of silages prepared for winter supplemental feeding of roe deer, g kg^{-1} DM *(Đorđević et al., 2007)*

Factors	Dry matter g kg^{-1}	Crude protein	Crude fiber	Crude lipid	NFE	Ash
Starting material						
Whole maize plant	360.46	72. 45	169.04	35.08	674.90	48.53
Alfalfa	384.00	198.56	249.09	31.33	400.65	120.37
Oak leaves	513.21	102.09	226.90	38.27	595.48	37.56
Maple leaves	521.88	113.91	214.51	51.28	537.00	83.30
Silages						
Whole maize plant	357.11a	72.29a	185.35a	91.10a	606.09b	45.17b
Whole maize plant + oak leaves (10%)	354.84a	70.96a	245.96b	100.35a	540.12a	42.61a
Whole maize plant + maple leaves (10%)	368.05b	74.08a	199.32a	96.62a	587.19b	42.79a
∅	360.00	72.44	210.21	96.02	577.80	43.52
Alfalfa	378.60a	186.15a	253.40a	122.50a	331.57a	106.38b
Alfalfa + oak leaves (10%)	374.69a	177.48a	259.90a	109.41a	352.73a	100.48a
Alfalfa + maple leaves (10%)	380.32a	177.20a	273.47ab	113.96a	328.90a	106.47
∅	377.87	180.28	262.26	115.29	337.73	104.44b

Conclusion

It is possible to improve quality and quantity of natural feeds in the hunting grounds with various management measures, to produce feeds on meadows and arable lands and organize supplemental feeding of game animals with such feeds. Topinambur is certainly one of the best fodder species for hunting grounds, both for green mass and tubers, while at the same time it provides shelter for the animals. As a result, it is possible to reduce damages produced by game on agricultural crops and damages on game itself. However, the only effective solution is to fence the hunting grounds and organize completely controlled management.

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Rezime

Jedna od važnih mogućnosti za lovnu privredu u cilju smanjenja šteta u poljoprivredi jeste gajenje određenih krmnih kultura u samom lovištu u cilju dodatne ishrane divljači. Za ovu svrhu najpogodnije su krmne leguminoze (lucerka, grahorica...), žita (kukuruz, raž, ovas, tritikale...), korenasto-krtolaste biljke (čičoka, repa, krompir,...), kupusnjače (repica, stočni kelj, perko...) i dr. Nabrojane biljne vrste životinje mogu koristiti direktno, u zelenom stanju i za ispašu, dok se viškovi mogu čuvati za ishranu divljači u zimskom periodu, i to u vidu sena, silaže i senaže, utrapljenih plodova i koncentrovane zrnaste hrane. Zasejane površine treba formirati na više međusobno udaljenih mesta u lovištu, kako bi se time izvršila i prirodna disperzija životinja u potrazi za hranom. Setva kultura treba da bude organizovana po modelu zelenog konvejera, i time omogući kontinuirano pristizanje zelene hrane u toku vegetacije. Polja sa zasejanim kulturama treba po mogućstvu ograditi, a divljači dopustiti ulazak tek kada biljke stignu u optimalnu fazu za korišćenje. Navedenim merama može se postići značajno smanjenje šteta na poljoprivrednim kulturama, povećanje brojnosti i trofejne vrednosti divljači, smanjenje zimskih gubitaka divljači i dr.

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DEGRADABILITY AND FERMENTATION QUALITY IN PEA-LUCERNE SILAGE

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Original scientific paper

Abstract: The subject of our work was to study the effect of a biological preparation on the fermentation process in pea-lucerne (90% *Pisum sativum*, 10% *Medicago sativa*) silage and effective degradation of organic matter and crude protein. The pea was cut in the vegetation stage of milk ripeness. The biological preparation consisted of *Lactobacillus plantarum* DSM 3676, 3677 and *Propionic bacterium* DSM 9576, 9577. We applied 2 ml into 1 kg ensilaged feed. Non-treated silage had higher content of acetic and butyric acids (21.66 and 1.66 g.kg^{-1} dry matter. respectively), and NH_3-N of total N (10.07%), but not statistically significant**.** Improved fermentation had a positive effect in the treated silage upon the nutrient level. The content of crude protein varied from 164.07 to 166.12 g kg^{-1} dry matter. The effective degradability of crude protein was higher in non-treated silage (85.4) than in treated silage (84.1). The potentially degradable fraction of crude protein decreased in the treated silage. Application of biological preparation had positive effect on the quality of the fermentation process nutrient composition and degradation of crude protein in pea-lucerne silage.

Key words: pea-lucerne silage, fermentation process, organic matter degradation and crude protein

Introduction

New pea varieties denominated as „semi-leafless“are leafless, tendrillar. They do not cover the lower part of stand; therefore, they are suitable as cover crop for a number of fodder crops, mostly for lucerne. Pea-lucerne silages produced of this mixture are a good source of energy as well as crude protein for dairy cows of lower production or in the period of rising lactation phase, when the daily milk production does not exceed 25 kg milk. High proportion of soluble fraction of crude protein in pea (more than 50% out of total N) cause that their effective degradability is also high on the level about 78% *(Mustafa et al., 2000, 2002)*. Application of lactic fermentation bacteria contributes also to improvement of total quality of ensilaged pea-lucerne mixtures. Such inoculated silages contain at the beginning of fermentation process more homo-fermentative bacteria of lactic fermentation, with higher biological activity *(Kung, 2009)*. Increased fermentation speed in such treated feeds restrains proteolysis and deamination of proteins in the ensilaged matter. Decrease in proteolysis level improves utilization of crude protein from silage.

The subject of our work was to study the effect of a biological preparation on the fermentation process in pea-lucerne (*Pisum sativum, Medicago sativa*) silage, and effective degradation of organic matter and crude protein.

Materials and Methods

Stand of pea-lucerne mixture (90% *Pisum sativum*, 10% *Medicago sativa*) was cut at the phase of milk ripeness of pea. The matter was chopped after 6 hours wilting, homogenized and filled into laboratory fermentors (volume 1.7 l). The experiment consisted of one control variant with ensilaged feed without additives and one experimental variant treated with the additive of activated life culture bacteria of lactic fermentation (*Lactobacillus plantarum* DSM 3676, 3677 and *Propionic bacterium* DSM 9576, 9577), 2 ml into 1 kg ensilaged feed was applied.

The filled fermentors were placed in dark room at 22^0 C. After 105 days, the experiment was terminated and the following wilting parameters were determined in samples: nutrient content, DM losses calculated in% of original DM contents, pH of silage extract (electrometrically), lactic acid and volatile fatty acid (VFA) levels by gas chromatography, alcohol and ammonia levels by microdiffusion method according to Conway. Index of proteolysis was calculated from the content of ammonia expressed as% NH_3-N out of total N. Analytical procedures were described in actual norm (MA SK, 2004). Energy and PDI concentrations in the silages were calculated as mentioned by *Petrikovic and Sommer et al. (2002).*

The *in sacco* experiments were performed in two cows of Holstein breed with large rumen cannulas, during the dry period (average live weight 600 kg). Animals were fed maintenance ration composed of lucerne hay, maize silage and cereal coarse meal (mixture of wheat and barley in proportion 1 : 1). Forage feeds created 70% dry matter in the ration. Samples of silages were incubated 6, 9, 16, 24, 48 and 72 hours (6 bags of each feed, animal and incubation) in fresh state, chopped to the size approx. 1.5 cm. After incubation in rumen were the bags rinsed under running water, washed in washing machine in a cycle 3 x 5 minutes, then dried at a temperature 55°C, ground to the size 1 mm and content of crude protein and ash was assessed. At the same time was performed also zero incubation in which were assessed losses by washing in a cycle 3 x 5 minutes. Effective degradability (Edg) was then calculated according to the equation *(Orskov and McDonald, 1979)* in the programme Neway (Edg = a + ((b × c)/(k + c))). Where k is the rumen outflow rate assumed to be 5% / h and a, b, and c are as described above.

Results were statistically processed by the method of variance analysis and differences between the experimental groups compared by Student t-test.

Results and Discussion

Content of nutrients in ensilaged stand of pea-lucerne mixture is in Table 1. The stand was characterized by low dry matter, slightly lower content of crude protein, higher content of ADF and NDF and low content of starch. *Mustafa et al. (2002)* reported lower content of ADF and NDF (25.7 to 29.3, 34.1 to 44.6 g kg^{-1} dry matter) and higher content of crude protein (171 – 204 g kg^{-1} dry matter) in stands of various pea varieties compared with our results.

Table 1. Pea-lucerne mixture – fresh matter

DM	OM	CP	ADF	NDF	Total sugar	Starch	Fat	ME	NEL	PDI
in g	in g kg^{-1} DM							MJ kg^{-1} DM		G kg^{-1} DM
235.8	880.62	167.8	356.6	455.1	51.3	64.9	19.5	9,73	5.82	89.9

Composition of nutrients and parameters of fermentation process in pea-lucerne silage are in Table 2.

Table 2.Nutrient composition and fermentation parameters in pea-lucerne silage in g kg^{-1} DM

Parameter n = 6	Non-treated		Biological preparation		Statistical significance of differences	
	x	s	x	s	P < 0.05	P < 0.01
DM in g.kg^{-1} FM	234.49	1.29	234.75	0.44		
Losses DM in%	0.92	0.27	0.84	0.17		
OM	883.83	2.02	887.71	5.72		
Crude protein	164.07	2.16	166.12	4.32		
ADF	366.54	2.95	373.36	7.15		
NDF	419.86	5.38	428.91	3.08	**	
Total sugar	13.34	0.12	12.53	2.08		
Starch	39.18	1.83	32.68	0.06		**
Fat	27.87	0.34	25.30	0.66		
ME /MJ/	9.61	0.02	9.63	0.07		
NEL /MJ/	5.68	0.01	5.69	0.03		
PDI	81.70	1.02	82.85	0.84		
pH	3.97	0.06	3.92	0.02		
Acids						
- lactic	105.19	6.53	102.15	5.53		
- acetic	21.66	2.96	19.53	2.82		
- butyric + isobutyric	1.66	0.49	1.61	0.54		
Total organic acids	132.84	6.70	126.23	6.36		
Alcohol	2.18	0.31	2.35	0.09		
NH_3-N of total N in%	10.07	1.82	9.18	0.52		

The effect of inoculation with life culture bacteria of lactic fermentation did not show itself significantly in the fermentation process of ensilaged pea-lucerne mixture.

Concentration of lactic acid in silages was balanced; it was slightly lower in the treated silage (105.19 vs 102.15 g kg^{-1} dry matter). In line with it was also the same level of pH. Content of butyric acid 1.66 vs 1.61 g kg^{-1} dry matter shows balanced course of fermentation process. The additive of lactic fermentation bacteria showed itself only slightly in decreased content of acetic acid (19.53 vs 21.66 g kg^{-1} dry matter) and NH_3-N out of total N (9.18 vs 10.07%). In comparison with our work, *Borreani et al. (2009)* founded statistically significantly higher influence of lactic fermentation bacteria on fermentation process in ensilaging of pea stand.

We suppose that the higher effect of inoculation was caused by higher content of dry matter (302 g kg^{-1} dry matter) in conserved feed. Low content of dry matter in our experiment caused intensive course of fermentation process in treated as well as in untreated silage; therefore were differences in amount of fermentation products low.

Course of fermentation process manifested itself also in nutrient composition of pea-Lucerne silage. Losses of dry matter were balanced. Content of crude protein and fibre complex in treated silage was slightly higher. Even here was found no statistical significance of differences. Higher content of NDF (419.86 vs 428.91) and lower content of starch (39.19 vs 32.68 g kg^{-1} dry matter) in treated silage were exceptions.

Parameters of degradation and effective degradability of organic matter and crude protein are in table 3. Inoculation of pea-lucerne mixture with content of 23% influenced effective degradability of OM and CP to a minimum. The lowest effective degradability of OM and CP was noticed in treated silage (OM 70.9 vs 69.6, CP 85.4 vs 84.1). Detected differences were not statistically significant. Similar results reported also *Hart et al. (2003) and Sinclair et al. (2009*) in their works.

Table 3. Parameters of organic matter and crude protein degradation in pea-Lucerne silage

Parameter	Non-treated	Biological preparation	Non-treated	Biological preparation
	OM		CP	
a (%)	50.4	48.8	68.7	65.8
b (%)	31.7	30.6	22.8	20.4
c ($\%.hr^{-1}$)	0.074	0.081	0,074	0.081
a + b	82.1	79.4	91.5	86.2
Edg[1]	70.9	69.6	85.4	84.1

*at Edg calculation lag time 1,9 h was taken into consideration,
a – Immediately soluble fraction, b – Degradable part ot the insoluble fraction, c – Fractional rate,
a + b – Potential degradable fraction, Edg – Effective degradability
[1]Calculated assuming a ruminal outflow rate of 0.05 $fraction.hr^{-1}$

Conclusion

During ensilaging of pea-lucerne mixture (90% *Pisum sativum*, 10% *Medicago sativa*) we found out that intensive fermentation process took place in untreated as well as treated silage under the influence of low level of dry matter in conserved feed. The influence of life culture bacteria of lactic fermentation in silage was low because of the mentioned reason. Level of pH and concentration of lactic acid were balanced. We observed only slight decrease in content of acetic acid (19.53 vs 21.66 g kg^{-1} dry matter) and NH_3-N out of total N (9.18 vs 10.07%) in the treated silage. The effect of proteolysis decrease in silage with life bacteria additive became evident also in higher content of crude protein (166.12 g kg^{-1} dry matter). Effective degradability of organic matter and crude protein decreased under the influence of additive only minimally (OM 70.9 vs 69.6, CP 85.4 vs 84.1).

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Degradacija i kvalitet fermentacije u silaži od graška i lucerke

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Rezime

Tema rada je proučavanje efekta biološke pripreme biomase na proces fermentacije silaže spravljene od graška i lucerke (90% *Pisum sativum*, 10% *Medicago sativa*) kao i na efektivnu degradaciju organske materije i sirovih proteina. Grašak je košen u vegetacionoj fazi mlečne zrelosti. Biološka priprema je uključivala primenu *Lactobacillus plantarum* DSM 3676, 3677 i *Propionic bacterium* DSM 9576, 9577, u količini od 2 ml na 1 kg siliranog hraniva. Silaže koje nisu tretirane su imale veći sadržaj sirćetne i buterne kiseline (21.66 i 1.66 g kg^{-1} suve materije) kao i NH_3-N od ukupnog N (10.07%). Međutim, ovi podaci nisu pokazali statističku značajnost. Poboljšana fermentacija je imala pozitivne efekte na nivo hranljivosti u tretiranoj silaži. Sadržaj sirovih proteina je varirao od 164.07 do 166.12 g kg^{-1} suve materije. Efektivna degradibilnost sirovih proteina je bila veća u silaži koja nije tretirana (85.4) nego u tretiranoj silaži (84.1). Potencijalno razgradljiva frakcija sirovih proteina je smanjena u tretiranoj silaži.

Primena bioloških preparata je imala pozitivne efekte na kvalitet procesa fermentacije, sastav hranljivih materija i degradaciju sirovih proteina u silaži graška i lucerke.

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THE USE OF A SILAGE INOCULANT IN SILAGES OF GRAINS OF FIELD BEAN (*Vicia faba*)

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Original scientific paper

Abstract: A silage trial under laboratory conditions with mini silos was conducted with field bean grains (*Vicia faba*). A biological silage additive (Biomin® BioStabil Plus, *L. plantarum, L. brevis* y *E. faecium*, 1 x 10^5 or 1 x 10^6 cfug^{-1} silage) and molasses (without or with 2% of the fresh matter) were used as additives in order to improve the silage quality and enhance the aerobic stability. The influence of the additives on the vicine and convincine content was also studied. The silages were open after 0, 2, 7, 44 and 90 days. DM content as well as the fermentation profile, the pH value, the aerobic stability *(Honig, 1990)* and the content of vicine and convincine was determined. The inclusion of the silage inoculant had a positive effect on the acidification and the fermentation profile, as well as on the aerobic stability compared with the control treatment without additives. The content of vicine and convincine after 90 days of ensiling was reduced from 30 to 80%. The silage quality and the aerobic stability were not improved essentially by the use of molasses.

Key words: *Vicia faba*, inoculant, silage, quality, tannins

Introduction

The purpose of this trial was to test the efficiency of the bacterial silage inoculant (Biomin® BioStabil Plus, a blend of *L. plantarum, L. brevis* and *E. faecium*) in different concentrations with or without molasses on the quality of *Vicia faba* grains, the aerobic stability and the vicine and convincine content.

Material and Methods

Dried grains of *Vicia faba* were used for the experiment and moistened to adjust their moisture content to 70% by adding water. The material was ensiled using the treatments as shown in Table 1, in buckets with a capacity of 5 liters.

Table 1. Treatments for the trial

Treatment	Additive	Concentration of the biological inoculants (cfu g^{-1} silage)
BS6	Biomin® BioStabil Plus	1 x 10^6
BS6+2	Biomin® BioStabil Plus + 2% molasses	1 x 10^6
BS5	Biomin® BioStabil Plus	1 x 10^5
BS5+2	Biomin® BioStabil Plus + 2% molasses	1 x 10^5
C	Control	-

The additives were sprayed on the material and well homogenized. The material was put in the buckets and well compacted and sealed for reaching anaerobic conditions. The mini silos were opened at the days 0, 2, 7, 44 and 90. Each treatment had 3 replicates. The parameters under study were DM, pH, fermentation products (HPLC), the content of the vicine and convincine, as well as the aerobic stability during a week after the opening of the mini silos *(Honig, 1990)*, evaluating the difference between the room and the silage temperature. Differences over 2 °C were considered as a sign of instability.

Results and Discussion

The control treatment without additives had the highest dry matter losses (3.0 – 4.5%, Figure 1).

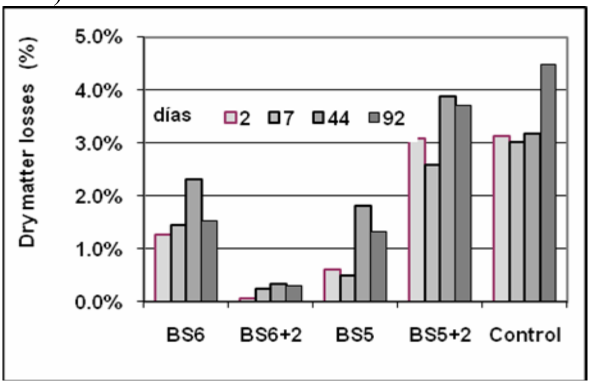

Figure 1. Dry matter losses in silages of *Vicia faba* grains

The dry matter losses were lowest in the treatment BS6+2 (<0.5%) followed by BS6 and BS5 with losses between 0.5 and 2.5% respectively. The acidification in the control treatment was relatively low and insufficient for an adequate preservation (pH of approx. 6, Figure 2). The treatments in which the inoculation was of 1 x 10^6 $cfug^{-1}$ silage, the acidification process was faster and deeper.

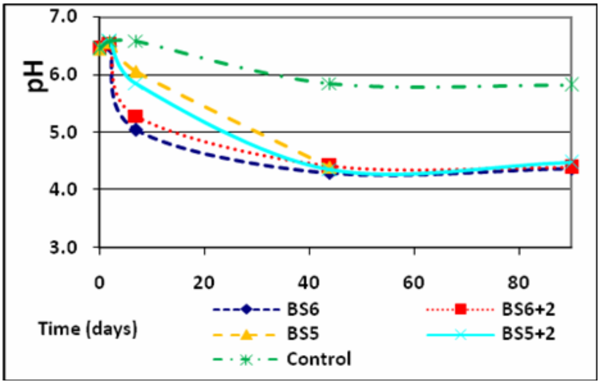

Figure 2. pH values in silages of *Vicia faba* grains

The lactic acid production is shown in Figure 3.

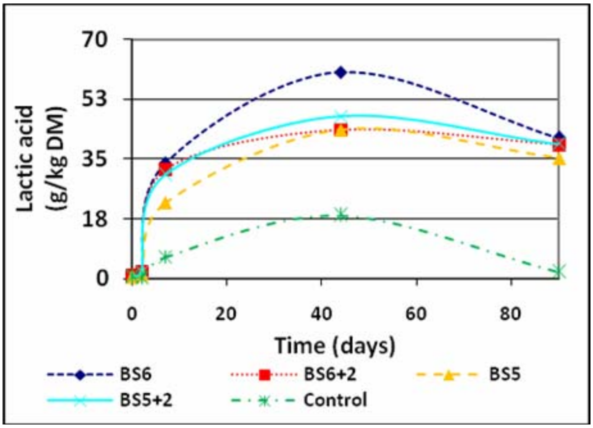

Figure 3. Lactic acid production in silages of *Vicia faba* grains

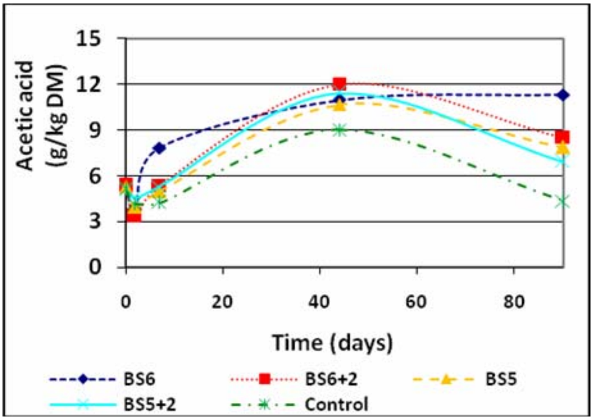

Figure 4. Acetic acid production in silages of *Vicia faba* grains

The best results were obtained when inoculants were used. The production of lactic acid in the control treatment was relatively low compared to inoculated silages. The differences in acetic acid production were not so markedly high, however its content is also lower in the control treatment (Figure 4).

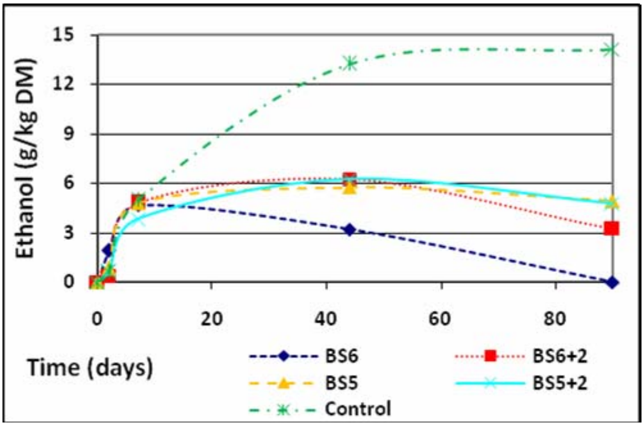

Figure 5. Ethanol content in silages of *Vicia faba* grains

Aerobic stability was lower only in the control treatment (7.7 days), compared to the non treated silages of *Vicia faba* grains presenting a high ethanol production (15 gkg^{-1} DM, Figure 5). The other treatments had less than 6 g of ethanol/ kg DM at 90 days. No butyric acid was found in any of the silages treated (or not) with the rest of the treatments (stable up to 8 days). The vicine and convincine contents in *Vicia faba* grains in the raw material and in silages are shown in the graphics 6 a) and 6 b).

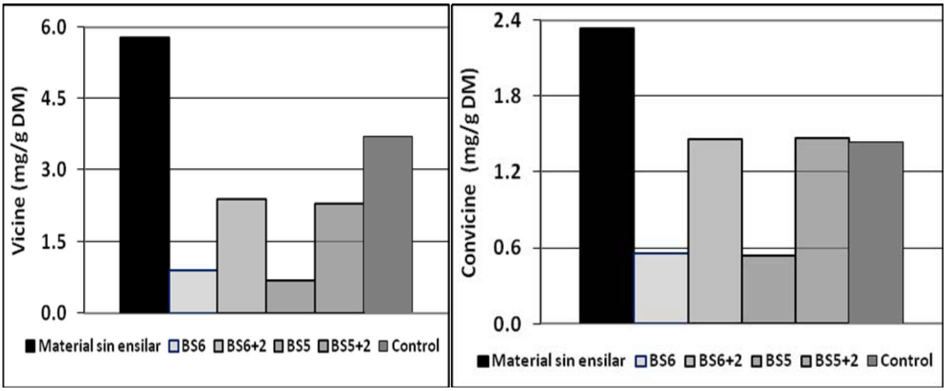

Figure 6 a) and b): Vicine (a, left) and convincine (b, right) content in *Vicia faba* grains before and after (90 days) ensiling

The vicine content was reduced in the control treatment in about 40% after 90 days of ensiling (from 5.8 to 3.7 mg g^{-1} DM). However, in treatments BS6+2 and BS5+2, the vicine content reduction was of about 60% and in treatments BS5 and BS6 86%. The same tendency was found in the convincine content: 2.3 mg/ g DM in the raw material and 60% lower in BS5+2 and BS6+2, and only 30% in BS5 and BS6.

Muuduli et al. (1982) found that 1% of vicine in the diet of laying hens can reduce the egg weight and the fertility for about 60% compared with diets which did not contain *Vicia faba*.

Conclusion

The inoculation of the silages had a positive effect on the silage quality and the aerobic stability of silages of *Vicia faba* grain compared with the control treatment.

The vicine and convincine content was reduced to a lower level in the treated silages than in the non treated one.

The use of molasses as silage additive for this type of silage did not bring an additional improvement of the analyzed parameters.

Korišćenje silažnih inokulanata u silažama zrna stočnog boba (*Vicia faba*)

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Rezime

Vršeno je ispitivanje silaže stočnog boba (*Vicia faba),* u laboratorijskim uslovima korišćenjem mini silosa. Dodavani su biološki silažni aditivi (Biomin® BioStabil Plus, *L. plantarum, L. brevis* y *E. faecium*, 1 x 10^5 ili 1 x 10^6 cfu g^{-1} silaže) i melasa (bez ili 2% od sveže materije), u cilju poboljšanja kvaliteta silaže i povećanja aerobne stabilnosti. Silaže su otvarane nakon 0, 2, 7, 44 i 90 dana. Utvrđen je sadržaj SM, kao i fermentacioni profil, pH vrednost, aerobna stabilnost *(Honig, 1990)* i sadržaj vicina i konvicina. Uključivanje silažnih inokulanata je imalo pozitivne efekte na zakišeljavanje i na fermentacioni profil, kao i na aerobnu stabilnost u odnosu na kontrolne tretmane bez aditiva. Sadržaj vicina i konvicina nakon 90 dana siliranja je bio smanjen sa 30 na 80%. Korišćenje melase nije poboljšalo kvalitet silaže ni aerobnu stabilnost.

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THE INFLUENCE OF DIFFERENT TYPE OF MOWERS ON ALFA-ALFA DRYING SPEED

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Original scientific paper

Abstract: This paper covers the results obtained for three types of mowers for alfalfa mowing and drying processes: self-propelled mower and hay crusher Fortschritt E 302; oscillatory mower with classic cutting mechanism and rotary mower with drums BRK 1650. The drying process was done on natural manner, with no application of machines for mowed mass manipulation. Alfalfa was dried on the field in swaths formed after mowing by appropriate mower, i.e. there was neither hay ted nor hay loosing. The results of the comparative analysis for three types of mowers have indicated a significant difference in mass drying speed between the self-propelled mower -hay crusher and other two types of the mowers. When the self-propelled mower -hay crusher was used, the drying process resulting in optimal humidity of 20% lasted 28 hours. When the oscillatory mower with classic cutting mechanism was used, the drying process lasted for 47 hours, whereas it took 55 hours for drying process when the rotary mower was used.

Key words: alfalfa, self-propelled mowers, hay drying,

Introduction

The mowing is the first technical operation performed in the scope of quality cattle food preparation technology. It needs to be done within optimal agro-technical time framework in order to decrease potential negative impacts of the external factors (rain, storm). The selection of the appropriate mowing apparatus significantly impacts the quality of cattle food preparation.

In order to produce good quality of bulky cattle food, in this case alfalfa, containing materials of higher nutrition values (proteins, minerals), the time period for green mass drying process up to 20% of its optimal humidity needs to be shortened so that it can be stocked. This will also result in loss decrease expressed in percentages of the fallen leaves containing nutritious materials. The swath needs to be formed in such a way to enable equal drying of the green mass on its whole width. The above given facts clearly indicate that the selection of appropriate mowing mechanism, with appropriate working performances, definitely impacts the speed of green mass drying and decreases partial operations for storage hay preparation.

While analyzing the impact of roll pressure on the alfalfa drying speed, *Tanevski (1990)* found out that the humidity of the mass at 11th hour of the second day was 19.20%. During exploitation testing of the self-propelled mower-hay crusher Fortschritt E302, the same author indicated that the average speed is about 5.35 kmh^{-1}.

According to *Wener (1987)* the recommended working speed of the oscillatory mower with classic cutting mechanism is ranging from 5 to 8 kmh^{-1}, whereas it is from 8 to 15 kmh^{-1} when the rotary mower with drums is used. *Koprivica and Stanimirović (1996)* indicate that the humidity of the crushed mass made by the self-propelled mower-hay crusher decreased from 74.48% to 18.87% within 30 hours.

The aim of the research is to use the comparative analysis of different technical mower performances in order to obtain the optimal parametric values related to the mass drying aspect, in scope of the given criteria for analyzed machine application.

Materials and Methods

The research covered three types of mowers: self-propelled mower - hay crusher; oscillatory mower with classic cutting mechanism and rotary mower with two drums. The testing was performed during alfalfa mowing period when the self-propelled mower - hay crusher was used on the parcel with average yield of 3.5 t ha^{-1} (without irrigation). The testing of other two types of mowers was performed on the parcels with average yield of 3.3 t ha^{-1} (without irrigation). It is noteworthy that the weather factors (drought) caused such a low yield, as well as the fact that the alfalfa was in blooming phase when mowed.

The green mass yield was determined based on alfalfa mass measurements taken from a length meter with swath width, calculated per hectare. The alfalfa humidity was determined by lab method, i.e. determination of dry material alfalfa content per hectare. The drying speed was determined based on humidity of mowed mass sample taken three times a day, during daylight, over entire alfalfa drying period.

Results and Discussion

The results have indicated a significant difference in mass drying speed between the self-propelled mower -hay crusher and other two types of the mowers.

The drying process of the mass mowed by the self-propelled mower -hay crusher, reaching 20% of humidity was accomplished on the second day till 13:00 o'clock. If we take into consideration the fact that mowing was done around 9:00 o'clock on the previous day, then the drying process was accomplished within 28 hours. The average pre-mowing alfalfa humidity was 70.17%. Immediately after mowing, the average humidity was 45.66%. The second day after mowing, the average humidity was 33.85 at 9:00 o'clock. When the next humidity test was done around 13:00 hours, the average humidity was 20.40%. The alfalfa drying process was accomplished with this percentage (Table 1).

Table 1. Drying process speed of the mass mowed by the self-propelled mower -hay crusher

Test	Pre-mowing humidity (%)	Post mowing humidity (%)	Mass humidity on the second day			Mass humidity on the third day		
			At 9.00 hrs	At 13.00 hrs	At 17.00 hrs	At 9.00 hrs	At 13.00 hrs	At 17.00 hrs
1.	70.21	45.25	33.68	20.21	-	-	-	-
2.	70.15	44.63	33.32	20.17	-	-	-	-
3.	70.13	47.41	34.72	21.06	-	-	-	-
4.	70.20	45.34	33.67	20.18	-	-	-	-
Average	70.17	45.66	33.85	20.40				

The drying process of the mass mowed by the oscillatory mower with classic cutting mechanism was accomplished on the third day till 9.00 hours. (Table 2). The mowing was done around 10.00 o'clock, so the drying process was finished within 47 hours, on the third day after mowing.

Table 2. Drying process speed of the mass mowed by the oscillatory mower with classic cutting mechanism

Test	Pre-mowing humidity (%)	Post mowing humidity (%)	Mass humidity on the second day			Mass humidity on the third day		
			At 9.00 hrs	At 13.00 hrs	At 17.00 hrs	At 9.00 hrs	At 13.00 hrs	At 17.00 hrs
1.	68.76	52.81	37.61	30.53	23.26	20.73	-	-
2.	68.90	49.84	35.60	30.25	23.11	20.45	-	-
3.	68.04	47.76	34.43	29.63	22.47	19.68	-	-
Average	68.83	50.17	35.88	30.14	22.95	20.29	-	-

The average alfalfa humidity was 6.83%. Immediately after mowing, the average humidity was 50.17%. The first tests made at 9.00 o'clock of the second day after mowing showed that the average humidity was 35.88, then 30.14% at

13.00 hours, whereas the average humidity was 22.95% at 17:00 hrs. The drying process was done on the third day after mowing at 9:00 o'clock, when the average humidity of the mass mowed by the oscillatory mower with classic cutting was 20.29%.

The drying process of the mass mowed by the rotary mower with drums was also accomplished on the third day around 17:00 hours. The drying process was done within 55 hours (Table 3). The average pre-mowing alfalfa humidity was 67.18%. The post-mowing average humidity was 50.17%, which is identical value to the values obtained by oscillatory mower with classic cutting. The first humidity test of the mass mowed with rotary mower was made on the second day around 9.00 o'clock (average humidity 42.52%), then at 13.00 hrs (average humidity 36.17) and at 17.00 hrs (average humidity 32.18%). The drying process was finished on the third day after mowing at 17.00 hrs when the average humidity of mass mowed by the rotary mower with two drums was 20.02%.

Table 3. Drying process speed of the mass mowed by rotary mower with two drums

Test	Pre-mowing humidity (%)	Post mowing humidity (%)	Mass humidity on the second day			Mass humidity on the third day		
			At 9.00 hrs	At 13.00 hrs	At 17.00 hrs	At 9.00 hrs	At 13.00 hrs	At 17.00 hrs
1.	67.32	51.23	43.24	36.48	32.31	29.94	24.23	20.17
2.	67.35	49.67	42.17	36.12	32.17	29.56	24.16	20.05
3.	66.87	49.62	42.16	35.92	32.07	29.60	23.92	19.85
Average	67.18	50.17	42.52	36.17	32.18	29.70	24.10	20.02

In the experiments, mass drying parameters, the F tests (Table 4) were highly significant, except for post-mowing humidity, when F test was significant. Pre-mowing humidity was higher when self-propelled mower -hay crusher was used, followed by oscillatory and finally rotary mowers. Differences were highly significant. Post mowing humidity was significantly higher in case of oscillatory and finally rotary mowers in comparison to the self-propelled mower -hay crusher, whereas there were no differences amongst them.

Table 4. Statistic analyses of the mass drying speed

Parameters	F-test	Lsd 0.05	Lsd 0.01
Pre-mowing humidity (%)	94.790 **	0.347	0.498
Post-mowing humidity (%)	8.965*	1.990	2.858
Humidity at 9.00 o'clock of the second day	113.313**	0.942	1.354
Humidity at 13.00 hrs of the second day	1344.152**	0.492	0.707
Humidity at 17.00 hrs of the second day	1343.089**	0.561	0.880
Humidity at 9.00 o'clock of the third day	783.627**	0.749	1.174

The last two values are related only to the oscillatory mower with classic cutting mechanism and rotary mower with drums.

The humidity at 9.00 o’clock of the second day was the highest with the rotary mower, followed by oscillatory, whereas it was the lowest with the self-propelled mower -hay crusher. All differences were highly significant.

The humidity at 13.00 hrs of the second day was the highest with rotary mower, followed by oscillatory, whereas it was the lowest with the self-propelled mower -hay crusher. All differences were highly significant.

The humidity at 17.00 hrs of the second day was the highest with the rotary mower, and the lowest with the oscillatory mower. All differences were highly significant.

The humidity at 9.00 o’clock of the third day was the highest with rotary mower, and the lowest with the oscillatory mower. All differences were highly significant.

Conclusion

Comparison of the obtained results lead to conclusion that the self-propelled mower -hay crusher showed the best results. The significant difference in mass drying speed between self-propelled mower -hay crusher and other two types of mowers was noted. The mass drying process was shorted for 19 hours in comparison to the oscillatory mower with classic cutting apparatus, whereas it was shorter for 27 hours in comparison to the rotary mower with two drums. The self-propelled mower -hay crusher obtained such results due to the fact that it has a crushing device which performs partial pressing, i.e. crushing of the green mass, or to be more precise, crushing of the alfalfa stem. That provides possibility for stem and leaf to be dried within the same time period. In given conditions, the rotary mower with drums obtained poorer results.

Based on the above given facts, it may be concluded that the application of the self-propelled mower -hay crusher significantly shortens on-field green mass drying process, which consequently gives better quality of bulky cattle food.

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Uporedna analiza brzine sušenja lucerke pokošene različitim tipovima kosačica

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Rezime

Prikazani su rezultati tri tipa kosačica pri košenju i sušenju lucerke: samohodne kosačice gnječilice Fortschritt E 302, oscilatorne kosačice sa klasičnim režućim aparatom i rotacione kosačice sa bubnjevima BRK1650. Samo sušenje mase vršeno je prirodno, bez upotrebe odgovarajućih mašina za manipulaciju sa pokošenom masom. Lucerka je sušena na parceli u otkosu koji je formiran nakon košenja odgovarajućim tipom kosačice, odnosno nije bilo prevrtanja, rastresanja sena. Rezultati komparativnih ispitivanja sva tri tipa kosačica, su pokazala da postoji značajna razlika u brzini sušenja mase između samohodne kosačice gnječilice, u poređenju sa ostala dva tipa ispitivanih kosačica. Kod samohodne kosačice gnječilice proces sušenja do optimalne vlažnosti od 20%, trajao je 28 sati. Kod oscilatorne kosačice sa klasičnim režućim aparatom proces je trajao 47 sati, a kod rotacione kosačice sa bubnjevima 55 sati.

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MONITORING OF VEGETATION CHANGES ON DYKES AFTER SOWING SELECTED SPECIES

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Original scientific paper

Abstract: Within the frame of the Eureka project: "E! 3824 INWASCOMP From Industrial Waste To Commercial Products" the possibilities of built embankments re-vegetation by use of the material called „EnviMIX" are solved. Selected industrial waste (slag, ashes, fly-ashes etc) are used as components of the material „EnviMIX". Since 2008 ten selected plant species (*Scirpus sylvaticus, Poa pratensis* agg, *Trifolium repens, Festuca arundinacea, Dactylis glomerata, Poa trivialis, Prunella vulgaris, Carex hirta, Cerastium holosteoides, Achillea millefolium* agg.) plus control have been tested in the pot and pilot plan experiments with the use of "EnviMIX". Two localities (the embankment of a brook and the dyke of a pond) were selected for pilot trials using "EnviMIX" in the form of vegetation tiles. The mixture of 10 above mentioned species plus 8 more species was used for re-vegetation on these two localities. Vegetation changes are monitored twice a year. Preliminary results and perspectives of „EnviMIX" use are presented in this paper.

Keywords: vegetation changes, dykes, embankment, industrial waste, pot trials

Introduction

The project „INWASCOMP"(Eureka E!3824) is aimed at studying the possibilities of waterside embankment construction in the Czech Republic with the use of EnviMIX product. It is a special material constructed with the use of selected industrial waste (slag, ashes, fly ashes etc.) and grinded limestone. EnviMIX is an ecological alternative to waste disposal of industrial waste; its low eco-toxicity was verified by various tests. This project supposes elimination of negative influences connected with erasure of stabilizing elements in countryside (especially soil erosion, high surface runoff, floods). EnviMIX product should help to restore, strengthen and enhance the damaged dykes both along rivers and ponds, which are very similar from the point of view of vegetation cover and patterns. Alluvia of the rivers are territories influenced by man for a long time. These territories were first deforested and were used as agricultural land. After deforestation of highlands and mountain areas alluvia were covered by loamy sediments because of enhancement of erosion. After deforestation of the countryside flow rates of rivers are more fluctuated and after long lasting rains floods are very frequent. In last years the most important factor is the man, who influences river alluvia by direct interventions *(Ložek, 1973)*. Riparian zones represent unusually wide spectrum of species and environmental processes. Ecological diversity is connected with regular regime of floods, changes of climatic conditions and the river corridor within the river stream. Innovations in management of the riparian zone are effective thanks to solving many ecological problems, concerning land use and the quality of environment. These zones play important roles in aquaculture and landscape planning and also in the restoration of water ecosystems *(Naiman and Décamps, 1997)*. Such disturbed habitats are suitable biotopes for non-native plant species. After disturbances caused by floods, aquaculture interferences, etc., new niches are loosen. These niches are occupied also by non-native plant species. Many species are not limited in the new environment by diseases and pests. That is why these species could spread intensively *(Jehlík, 1998)*. Seeds, sometimes also vegetative parts of these plants, are quickly distributed by water along rivers. On the places occupied by these species diversity of native plant species is lower. Less competitive species are ejected from the ecosystem. Principles of ecological restoration could be used widely. *Simmons et al. (2007)* recommend studying and using native plant species originating on the places of restoration. From the point of view of our project it is restoration ecology. It is a scientific discipline studying ecology of renewal places disturbed by man. Increasing biodiversity, enlargement of the water retentive capacity, protection against erosion, establishment of new ecosystems are the main tasks of the discipline. Restoration ecology as an important scientific discipline was mentioned by *Hobbs and Harris (2001)*. Spontaneous succession is used very often, because it is a very cheap variant of restoration of plant coverage on the locality. Its importance is within our project on the places surrounded by semi natural or natural vegetation. *Prach (2003)* discusses different aspects of spontaneous succession and its future potential. The concrete comparison of 16 localities in the Czech Republic was published by *Prach and Pyšek (2001)*. Re-cultivation is better to be used on the places that are not integrated into natural vegetation or if they are too large. First of all it is necessary to make technical re-cultivation (in our case with use of ENVIMIX) and prepare the locality for biological re-cultivation. Then it is necessary to sow a suitable mixture of plants or plant trees to re-cultivate the area.

Materials and Methods

Verification of the EnviMIX material in the form of granules (EnviMIX I) and special shaped waterside concrete blocks (EnviMIX III) has been performed since 2008. The pot and pilot plant experiments were established to study suitability of the material for re-cultivation processes and restoration ecology.

Pot trials. Growth of selected plant species in pots filled with a mixture of soil and EnviMIX I has been studied. An optimal variant of EnviMIX and soil mixture was proposed on the base of pH value. EnviMIX has pH value 12.8 (high alcalic). The mixture of soil (Z_1), garden substrate and EnviMIX I in the ratio 2:2:0,5 with pH in KCl 7.3 was selected as optimal. The value indicates a slightly alcalic soil. The content of elements was fully convenient: P – 94 $mgkg^{-1}$ (high content), K – 402 $mgkg^{-1}$ (very high content), Mg – 456 $mgkg^{-1}$ (very high content), N – 0.168% (medial content), Humus – 2.52% (medial content). Pots of size 30 x 30 x 30 cm were filled with the mixture. Three repetitions in the dry and in the wet variant plus the control pots (for chemical analyses) were established. These pots were placed in a cold greenhouse. Irrigation in the wet variant represented two times higher level of irrigation than in the dry variant. The intensity of irrigation was also influenced by the climatic conditions in the greenhouse; during vegetation season (15.4.-15.10.) the dose of water was two times higher (two litres four times a week per one pot) than during winter season (one litre of water four times a week per one pot). Fifty seeds of each selected species (*Prunella vulgaris, Trifolium repens, Festuca arundinacea, Poa pratensis* agg.*, Poa trivialis, Carex hirta, Cerastium holosteoides, Achillea millefolium* agg.*, Scirpus sylvaticus, Dactylis glomerata*) were sown. The evaluation of actual coverage of plants was performed on 8.6.2009 and 8.10.2009.

Pilot plan trials. In the year 2008 two experimental localities were selected. The first locality is a breed fishpond of the country estate "Podsatzka-Lichtenstein", nearby the town Velké Meziříčí (60 km W from Brno, altitude 460 m a. s. l., mesophyticum) and the second is a shore of the brook "Troubský potok" in the cadastral area of Troubsko (10 km SW from Brno, altitude 260 m a. s. l., thermophyticum). Permanent plots for vegetation relevés recording were established in spring 2008 (5 on the first locality and 2 on the second locality). Pilot plan trials with the use of EnviMIX I and EnviMIX III were established in spring 2009. On both localities plots of 2 x 10 meters were traced. After sod removal on both dykes the special shaped waterside concrete blocks (EnviMIX III) were laid down. On one third of each plot material EnviMIX I in the form of granules was applied. Afterwards, a mixture of herb seeds was sown, see Table 1. The selection of suitable herb species was based on previous botanical monitoring on the breed fishpond dyke in the years 2007 and 2008. Sowing rate 2g/m^2 was chosen.

Table 1. Composition of the mixture which was used for testing on selected localities. The weight of seeds represents the amount of seeds of each species added into the mixture

Species	Weight of seeds (g)	Species	Weight of seeds (g)
Carex hirta	8	*Glechoma headracea*	4
Carex pallescens	6	*Prunella vulgaris*	10
Juncus effuses	4	*Cerastium holosteoides*	10
Poa palustris	50	*Lysimachia nummularia*	2
Poa trivialis	10	*Veronica chamaedrys*	2
Scirpus sylvaticus	4	*Dactylis glomerata*	40
Lathyrus pratensis	6	*Festuca arundinacea*	40
Achillea millefolium agg.	10	*Poa pratensis* agg.	40
Alchemia vulgaris agg.	4	Total	250

On both localities phyto-sociological relevés on permanent plots of 16 m^2 were recorded. The recording date was 12.6.2009, immediately before haymaking. On both dykes, where the special shaped waterside concrete blocks (EnviMIX III) were laid down, relevés were recorded later in the autumn (5.10.2009). Phyto-sociological relevés were recorded according to a standard methodology in seven points of Braun-Blanquet scale. Individual points of the scale indicate the following values of coverage of individual species: r: 1%, +: 2%, 1: 3 and 4%, 2: 5-24%, 3: 25-49%, 4: 50-74%, 5: 75-100%. Statistical evaluation of herb layer coverage in pots was performed by Anova testing in the software Statistica for Windows (StatSoft CZ, Ltd. 2007). Phyto-sociological relevés were recorded into TURBOVEG database and were analysed in the programme JUICE by method of numerical classification TWINSPAN. The nomenclature of plant names in the paper was unified according to *Kubát et al. (2002)*.

Results and Discussion

Results and discussion are divided in two separate parts: pot trials and pilot plan trials.

Pot trials. From the obtained results presented in Figure 1 and 2 it can be concluded that there were substantial differences found not only between the coverage in dry and wet variant, but also among tested species. In the dry variant the best performance was observed in *Festuca arundinacea* and *Dactylis glomerata*. In the wet variant the best performance was observed in *Festuca* *arundinacea* and *Dactylis glomerata*, in autumnal term also *Prunella vulgaris* and *Trifolium repens*.

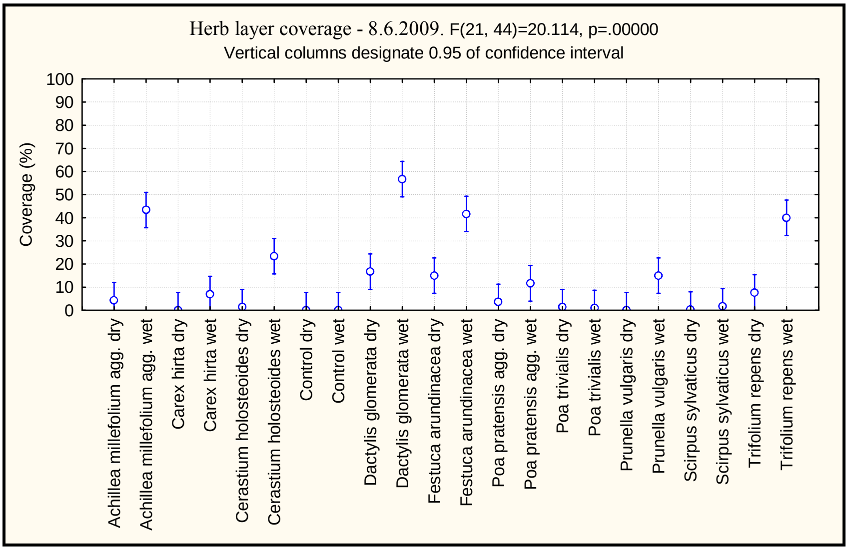

Figure 1. Results of Anova testing - herb layer coverage in 10 tested plant species – 8.6.2009

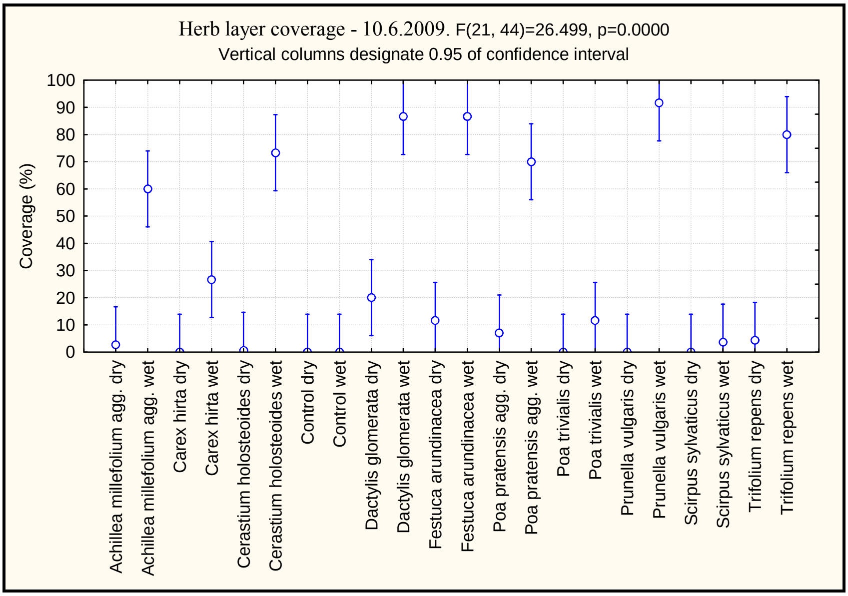

Figure 2. Results of Anova testing - herb layer coverage in 10 tested plant species - 8.10.2009

At the end of the year 2009 (25.11.) biomass of the plants planted in pots was harvested and weighted; the results are presented in Table 2. The highest yields were observed in *Festuca arundinacea*, *Cerastium holosteoides* and *Dactylis glomerata*. These species had also the highest coverage in pots.

Table 2. The yields of fresh biomass – pots, 25.11.2009

		Wet variant				Dry variant		
Species	1	2	3	Average	1	2	3	Average
Prunella vulgaris	0.10	0.06	0.05	0.07	0.00	0.00	0.00	0.04
Trifolium repens	0.08	0.08	0.05	0.07	0.01	0.00	0.00	0.04
Festuca arudinacea	0.09	0.08	0.11	0.09	0.01	0.01	0.00	0.05
Poa pratensis	0.07	0.05	0.05	0.06	0.01	0.01	0.00	0.03
Poa trivialis	0.05	0.03	0.00	0.03	0.00	0.00	0.00	0.02
Carex hirta	0.07	0.00	0.00	0.02	0.00	0.00	0.00	0.01
Cerastium holosteoides	0.16	0.07	0.11	0.11	0.00	0.00	0.00	0.06
Achillea milefolium agg.	0.04	0.04	0.04	0.04	0.00	0.01	0.00	0.03
Scirpius sylvaticus	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dactylis glomerata	0.06	0.08	0.07	0.07	0.02	0.01	0.01	0.05
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Pilot plan trials. The numbers of plant species in relevés differed substantially. The lowest number of recorded species was 15 (relevés No. 17 and 18 – the first locality, Troubsko). The highest numbers were 38 (relevé No. 13) and 42 (relevé No. 12). Both relevés 13 and 12 were recorded on the first locality – Velké Meziříčí. The average number of species per relevé was 26. Species composition of the embankment vegetation is presented in the Table 3.Analysis of phyto-sociological relevés by the method of numerical classification TWINSPAN divided them into four clearly defined groups (separated in the Table 3 by vertical lines). In the first group there are relevés recorded on permanent plots on the brook "Troubský potok" in cadastral area of Troubsko. Typical species are *Carex hirta, Urtica dioica, Equisetum arvense*, *Elymus repens, Convolvulus arvensis, Calystegia sepium* and *Potentilla reptans*. These species could be considered ruderal species of dry and warm disturbed areas in the Czech Republic. They are accompanied by some typical meadow species like *Dactylis glomerata, Festuca rubra* agg., *Arrhenatherum elatius* and *Geranium pratense*. In the second group there are relevés recorded on the plots where waterside concrete blocks were installed. The relevés contain species like *Carex hirta, Urtica dioica, Achillea millefolium* agg., *Trifolium repens, Taraxacum* sect. *Ruderalia, Equisetum arvense, Elymus repens, Convolvulus arvensis, Calystegia sepium, Potentilla reptans, Festuca arundinacea, Amaranthus retroflexus, Lolium multiflorum, Artemisia vulgaris*. Both sown and from the soil seed bank germinated species are present. Ruderal species prevail in this group. In the third group there are relevés from the current vegetation on the dyke of the breed fishpond. In these relevés there is occurrence of species like *Carex hirta, Urtica dioica, Achillea millefolium* agg., *Trifolium repens, Taraxacum* sect. *Ruderalia, Calamagrostis epigejos, Rumex obtusifolius, Equisetum arvense, Poa palustris, Tussilago farfara, Poa pratensis* agg., *Ranunculus repens, Cirsium arvense, Cerastium holosteoides, Galium aparine, Campanula patula, Glechoma hederacea, Alnus glutinosa* juv., *Poa trivialis, Veronica chamaedrys* agg., *Impatiens parviflora, Carex leporina, Festuca gigantea, Mentha arvensis* etc. It is a common mesophilous meadow vegetation of the alliance *Arrhenatherion*, completed by some wetland species. Presence of the species is given by the location of the experimental plots on the dyke of the pond, reaching the shores of the brook flowing under this dyke. In the fourth group there occur species like *Achillea millefolium* agg., *Trifolium repens, Taraxacum* sect. *Ruderalia, Calamagrostis epigejos, Rumex obtusifolius, Cerastium holosteoides, Plantago major, Stellaria media, Juncus articulatus, Poa annua, Lemna minor, Persicaria lapathifolia, Juncus bufonius, Rorippa palustris, Epilobium* sp., *Festuca pratensis* etc. These are mostly species of disturbed wet habitats, like exposed fishpond bottoms, fluvial deposits, etc. More results will be available after more years of evaluation of permanent plots on both experimental localities.

Table 3. Phytosociological relevés

Relevé number	**11** **10**	**111** **879**	**11111** **53462**	**222** **201**
Carex hirta	11	.++	.11+1	..r
Urtica dioica	11	.r+	.112+	r..
Achillea millefolium agg.	..	rrr	.1+.+	+22
Trifolium repens	..	rr.	2+1+2	.22
Taraxacum sect. *Ruderalia*	..	r.+	+++++	+r.
Calamagrostis epigejos	..	...	23212	+11
Rumex obtusifolius	..	...	+r+2+	+++
Equisetum arvense	22	+++	.+..+	...
Poa palustris	..	...	22222	.++
Tussilago farfara	..	...	+++r+	.++
Elymus repens	33	222	1	...
Convolvulus arvensis	11	++2	.+...	...
Dactylis glomerata	++	33.	..+.+	...
Festuca rubra agg.	++	...	.1..1	.11
Poa pratensis	+.	...	22..2	.22
Ranunculus repens	..	...	1.111	+.+
Cirsium arvense	..	...	++.r+	r+.
Cerastium holosteoides	..	...	.++.+	+++
Calystegia sepium	+1	222	.....	...
Potentilla reptans	++	+++	.....	...
Galium aparine	..	+..	++++.	...
Campanula patula	..	...	+11+1	...
Glechoma hederacea	..	...	+1.1+	+..
Alnus glutinosa juv.	..	...	.11.+	+++
Poa trivialis	..	...	11.3+	...
Veronica chamaedrys agg.	..	...	1+.11	...
Impatiens parviflora	..	...	+++1.	...
Carex leporina	..	...	++++.	...
Festuca gigantea	..	...	+.+1+	...
Mentha arvensis	..	...	+.+++	...
Lathyrus pratensis	..	...	.+..+	.+r
Plantago major	..	...	.r...	+++
Stellaria media	..	...	...+.	+++
Juncus articulatus	..	...	....+	2++
Arrhenatherum elatius	33	..+	.....	...
Geranium pratense	+1	..r	.....	...
Aegopodium podagraria	+.	...	+.+..	...
Lolium perenne	.+	...	.....	.++
Prunella vulgaris	..	r..	.....	.++
Alchemilla vulgaris agg.	..	...	+.+.+	...
Stachys sylvatica	..	...	+.+.r	...
Salix caprea juv.	..	...	r...r	r..
Myosoton aquaticum	..	...	.+++.	...
Juncus effusus	..	...	...+.	.++
Poa annua	..	...	.....	+++
Lemna minor	..	...	.....	+++
Persicaria lapathifolia	..	...	.....	+++
Festuca arundinacea	..	++.	.....	...
Amaranthus retroflexus	..	++.	.....	...
Lolium multiflorum	..	++.	.....	...
Artemisia vulgaris	..	r.r	.....	...
Stellaria alsine	..	...	+..+.	...
Lychnis flos-cuculi	..	...	+...+	...
Impatiens noli-tangere	..	...	r..+.	...
Lycopus europaeus	..	...	r....	..r
Tanacetum vulgare	..	...	.2..r	...
Juncus conglomeratus	..	...	.++..	...
Veronica arvensis	..	...	.++..	...
Persicaria mitis	..	...	.r.r.	...
Myosotis palustris agg.	..	...	..++.	...
Deschampsia cespitosa	..	...	...++	...
Angelica sylvestris	..	...	...++	...
Scirpus sylvaticus	..	...	....r	.+.
Juncus bufonius	..	...	.....	1+.
Rorippa palustris	..	...	.....	+.+
Epilobium sp. juv.	..	...	.....	+.+
Festuca pratensis	..	...	.....	.22

Relevés recorded on the locality brook "Troubský potok" in cadastral area of Troubsko are marked by red colour. Relevés recorded on the dyke of breed fishpond of the country estate "Podsatzka-Lichtenstein", nearby the town Velké Meziříčí, are marked by black colour.

Species presented only in one relevé (No of relevé : coverage value):
Lathyrus tuberosus 11: +; *Galium album* 11: +; *Mentha longifolia* 11: r; *Symphytum officinale* 10: +; *Festuca pratensis* 10: +; *Heracleum sphondylium* 10: +; *Lactuca serriola* 10: r; *Atriplex sagittata* 17: +; *Capsella bursa-pastoris* 17: r; *Bidens frondosa* 19: r; *Digitaria sanguinalis* 19: r; *Arenaria serpyllifolia* 19: r; *Echinochloa crus-galli* 19: r; *Cardamine amara* 15: +; *Epilobium tetragonum* 13: +; *Fragaria vesca* 13: +; *Fraxinus excelsior* juv. 13: +; *Epilobium montanum* 13: +; *Torilis japonica* 13: +; *Scrophularia nodosa* 13: +; *Picea abies* juv. 13: r; *Betula pendula* juv. 13: r; *Poa pratensis* 14: 2; *Moehringia trinervia* 14: +; *Vicia cracca* 14: +; *Carex pallescens* 14: +; *Cirsium palustre* 14: r; *Anthriscus sylvestris* 16: +; *Alopecurus pratensis* 12: +; *Stachys palustris* 12: +; *Cirsium vulgare* 12: +; *Carex muricata* agg. 12: +; *Securigera varia* 12: +; *Erigeron annuus* 12: +; *Geum urbanum* 12: r; *Acer pseudoplatanus* juv. 12: r; *Salix fragilis* juv. 22: +; *Glyceria fluitans* 22: +; *Chenopodium polyspermum* 22: +; *Conyza canadensis* 20: +; *Salix* sp. juv. 21: +.
Notice: juv. means juvenile (young) individuals.

Conclusion

The results of testing in the year 2009 clearly indicated that selected plant species had much better performance in the wet variant (variant with double level of irrigation). Plants growing in the wet variant were in much better condition, were higher and reached higher coverage levels. It can be concluded that EnviMIX I is not negatively influencing the growth and development of the tested plants. On the contrary, the limiting factor for the growth and development was water and irrigation. The pilot plan trials outlined the possibilities of the use of both selected species seed mixture and also spontaneous succession. Both variants seem to be suitable, but spontaneous succession is a much cheaper alternative. No invasive species were observed during the development of plant communities.

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Praćenje promena vegetacije na nasipima nakon setve odabranih vrsta biljaka

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Rezime

U okviru Eureka projekta "E! 3824 INWASCOMP Od industrijskog otpada do komercijalnog proizvoda", rešene su mogućnosti revegetacije izgrađenih nasipa korišćenjem materijala zvanog „EnviMIX“. Odabrani industrijski otpad (šljaka, pepeo, čađ i dr.) su iskorišćeni kao komponente materijala “EnviMIX“. Od 2008. godine deset odabranih biljnih vrsta (*Scirpus sylvaticus, Poa pratensis* agg, *Trifolium repens, Festuca arundinacea, Dactylis glomerata, Poa trivialis, Prunella vulgaris, Carex hirta, Cerastium holosteoides, Achillea millefolium* agg.) i jedna kontrolna su testirane u sudovima u pilot plan eksperimentu uz korišćenje “EnviMIX”-a. Dva lokaliteta (nasip na potoku i nasip na bari) su izabrana za početna ispitivanja korišćenja “EnviMIX” kao podloga za vegetaciju. Smeša 10 navedenih vrsta i još 8 drugih vrsta su korišćene za revegetaciju ova dva lokaliteta. Vegetacione promene su praćene dva puta godišnje. U ovom radu su predstavljeni rezultati i perspektive korišćenja „EnviMIX-a“.

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PHYTOREMEDITATION AS AN ECOLOGIC MEASURE FOR CLEANING UP OF SOILS CONTAMINATED WITH HEAVY METALS (Pb, Cd and Zn) IN THE VELES REGION

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Abstract: One of the measures for revitalization of the contaminated soils with heavy metals is phytoremediation. It consists of cultivation of plants capable of conducting phytoextraction of the heavy metals from the soil solution and hyperaccumulation into the surface biomass**.** The Veles region is one of the typical parameters where proved contamination of soils and other media of life environment with toxic metals: lead, cadmium, zinc and other, as a consequence of the emission of these elements from the lead and zinc smeltery located in Veles. Based on these acknowledgments in the Veles region, an experiment has been set up with four plants in four repetitions: rape – *Brassica napuss sp.oleifera D.C.,* white clover – *Trifolium repens L.,* alfalfa - *Medicago sativa L,* and corn – *Zea mays L*. Before the set up of the experiment, soil samples have been taken for laboratory analyses. The comparison between the examined crops during the first year of vegetation in relation to the heavy metal extraction power has indicated that the white clover has the greatest lead, cadmium and zinc extraction power. The oilseed rape has the weakest lead and zinc accumulation power, while corn has the weakest cadmium accumulation power. The total coefficient of bioaccumulation in the two-year research of alfalfa and corn has indicated that alfalfa is a stronger bioaccumulator of lead, and corn is a stronger bioaccumulator of cadmium and zinc.

Key words: phytoremediation, soil, heavy metals, Pb, Cd, Zn, bioaccumulators

Introduction

The contamination of the life environment is a subject of broad scientific examination of the contemporary world. Concerning the decontamination of the contaminated soils it is practice to cultivate plants able for hyperaccumulation of the heavy metals that contaminate the soil. This measure in the literature is called phytoremediation (*Sekulić, 2003*).

Plants belonging to the group of hyperaccumulators of heavy metals are characteristic by their high coefficient of bioaccumulation of heavy metals (*Ebbs et.al 1997, Ebbs and Kochian 1997, Huang et.al 1997, Hajiboland 2005, McGrath and Zhao 2003*). With their cultivation on the contaminated soils with heavy metals, it is possible that their presence in the soil will be reduced for a few years.
In the R. Macedonia, there are 16 hot spots (Environmental statistic, (2007), where the soils are contaminated with heavy metals.

Within the "Local Agenda 21 for the Municipality of Veles" concerning results have been presented in relation to the contaminated soils with heavy metals. The high contamination of the soils with Pb and Zn provides conditions for acceptance and accumulation of these elements in the above-ground parts of the plants which are cultivated in the Veles region.

So far, in R. Macedonia there are no examinations for decontamination of the contaminated soils with heavy metals whereby tested the cultivated plants, and from which it will be determined the most suitable one for phytoremediation. This will initiate a solution for one of the biggest problems of the people in Veles, and which are related to the healthy life and productive environment.

Materials and methods

The experiment is set up in 2008 on the productive areas of PE Derven in Veles, according to the method of randomized block system in four repetitions, with four variances. The variances are the following: rape – *Brassica napus ssp. Oleifera D.C.,* white clover - *Trifolium repens L.,* alfalfa - *Medicago sativa L.,* corn – *Zea mays* L.

Before the set up of the experiment, there have been taken average soil samples from 0-20cm and 20-40 cm for determination of the total and soluble forms of heavy metals (lead, cadmium and zinc) in the soil. Heavy metals were extracted from soil by digestion method with the Aqua Regia, and EDTA. (*Đamić, 1996*) and from the plant material by digestion method with a nitric (HNO_3), (*Jones, 1990*). Concentrations of heavy metals were determined by inductively coupled Plasma optical emission spectrometry using a Varian.

During the vegetation, the following parameters have been followed: fen-phased development of plant, plant height, number of plants per m^2 (vegetative composition) yield of green mass and chemical analyses of the vegetable material. The examinations include generally accepted methods for filed and laboratory examination of soils and the vegetative material (*Mitrikeski and Mitkova, 2006, Dzamić R., 1996*), and the obtained data is presented in tables.

Results and Discussion

The experiment was made on the right side of the bank of the Vardar River, formed with the sedimentation of the suspended alluvium from the river water. The chemical properties and the mechanical composition of the soil apply to the average trials of the experiment (Tables 1 and 2).

Table 1. Texture of the soil

Soil separates in %						
Depth (cm)	Coarse sand 0.2-2 mm	Fine sand 0.02-0.2 mm	Total sand 0.02-2 mm	Silt 0.002-0.02 mm	Clay <0.002 mm	Silt + clay <0.02 mm
0 - 20	25.00	57.70	82.70	10.30	7.00	17.30
20 - 40	21.20	52.50	73.70	12.90	13.40	26.30
average	23.10	55.10	78.20	11.60	10.20	21.80

In soil separates, the fine sand fraction (55.10%) dominates and together with the coarse sand fraction amount to 78.20 %, the silt fraction is third (11.60%) and the clay friction is last (10.20%). The soils are characterized by favourable mechanical composition which is confirmed with the classification of the fluvisols in texture classes (fine sand loam) according to *Scheffer and Schachtschabel*, (*Mitrikeski and Mitkova, 2006*).

The results of the research of the chemical properties of the alluvial soil (table 2) show that the examined soil has little humus contents (contains 1.37 % of humus), medium contents of carbonate, (classification according to *Penkov 1996*), contains 5.95 % of $CaCO_3$ which influences the reaction of the soil solution (7.67 % low alkaline).

Table 2. Chemical properties of the soil

Depth (cm)	Humus %	N %	pH		$CaCO_3$ %	Available nutrients in mg/100g soil	
			H_2O	NKCl		P_2O_5	K_2O
0 - 20	1.61	0.0966	7.60	6.95	5.20	3.96	12.11
20 - 40	1.14	0.0684	7.75	7.00	6.70	1.75	10.41
average	1.37	0.0825	7.67	6.98	5.95	2.85	11.26

The neutral and low alkaline reaction of the soil solution as well as the organic and mineral colloids, limit the heavy metal migration, including their easily available forms. In the Republic of Macedonia we still refer to the EU standards on maximally permitted concentration of heavy metals in the soil. The results on the total contents of heavy metals in the soil have indicated that all three elements are present over the maximally permitted concentrations, which means that the soils are not safe for healthy food production. This condition of soil contamination is suitable for implementation of the phytoremediation measure which is not very efficient when it comes to greater contents of heavy metals in the soil.

Table 3 shows the total and soluble forms of heavy metals in soil. The soluble forms of zinc amount to 46% of the total, cadmium 62% and zinc 21% of the total forms of these heavy metals in the soil.

Table 3. Content of heavy metals of the soil

Depth (cm)	Total (mg kg^{-1})			Soluble (mg kg^{-1})		
	Pb	Cd	Zn	Pb	Cd	Zn
0 - 20	175.96	5.43	298.17	74.20	3.28	62.83
20 - 40	177.63	5.73	314.50	89.00	3.64	65.71
average	176.79	5.58	306.33	81.60	3.46	64.27

The results from the two-year research shown in Table 4 refer only to alfalfa and corn. In the first year (2008), oilseed rape, considering its biology, was planted in autumn, contrary to the other species which were planted in spring. The white clover significantly retreated from the surface which leads to its elimination from the research for that year. In the second year (2009) the results showed that all four crops are suitable for phytoremediation.

Table 4. Extraction of heavy metals from the soil concentration into plant

Soluble forms of heavy metals (mg kg^{-1})			
	Pb	Cd	Zn
average	81.60	3.46	64.27
mg kg^{-1} in the dry plant material			
	Pb	Cd	Zn
lucerne - 2008	2.25	0.66	38.34
lucerne - 2009	8.68	0.03	12.03
lucerne - total	10.93	0.69	50.37
corn - 2008	1.88	0.31	39.13
corn - 2009	6.75	1.00	106.33
corn - total	8.63	1.31	145.46
white clover - 2009	5.58	1.23	73.34
cole - *Brassica oleracea* - 2009	1.25	0.53	16.41

The two-year research period showed that alfalfa has a total lead extraction power of 10.93 mg kg^{-1}, cadmium extraction power of 0.69 mg kg^{-1} and zinc extraction power of 50.37 mg kg^{-1}. Accumulation of lead (2.25 in the first year and 8.68 mg kg^{-1}) has increased by several times in the second year in comparison to the cadmium and zinc accumulation. Compared on an annual basis, it was noted that in the second year alfalfa had a far stronger lead extraction power compared to its cadmium extraction power (0.66 in the first year and 0.03 $mgkg^{-1}$) and zinc extraction power (38.34 in the first year and 12.03 $mgkg^{-1}$) in the same year.

The two-year research period showed that corn has a total lead extraction power of 8.63 mg kg^{-1}, cadmium extraction power of 1.31 mg kg^{-1} and zinc extraction power of 145.46 mg kg^{-1}. Unlike from the lead and cadmium which are toxic, zinc as trace element is necessary for plant development. Maize show sensitivity of lack of this element and has a greater needs *(Vukadinović et. al, 1998)*. Its accumulation of lead (from 1.88 in the first year to 6.67 mg kg^{-1}), cadmium (from 0.31 in the first year to 1.00 mg kg^{-1}) and zinc (from 39.13 in the first year to 106.33 mg kg^{-1}) has increased by several times in the second year. Compared on an annual basis, it was noted that corn had a far stronger extraction power with the three tested heavy metals in the second year.

In the two-year research of alfalfa and corn, it was shown that alfalfa has greater lead extraction power, while corn has greater cadmium and zinc extraction power. The one-year period of research showed that the white clover has the greatest extraction power with zinc (73.34 mg kg^{-1}) in comparison to the extraction power of lead (5.58 mg kg^{-1}) and cadmium (1.23 mg kg^{-1}).

The one-year period of research showed that the oilseed rape has the greatest extraction power with zinc (16.41mg kg^{-1}) in comparison to the extraction power of lead (1.25 mg kg^{-1}) and cadmium (0.53 mg kg^{-1}).

The comparison between the examined crops during the first year of vegetation in relation to the heavy metal extraction power has indicated that the white clover has the greatest lead, cadmium and zinc extraction power. The oilseed rape has the weakest lead and zinc accumulation power, while corn has the weakest cadmium accumulation power.

Table 5. Power of the extraction of the heavy metals

Coefficient of bioaccumulation			
	Pb	Cd	Zn
lucerne - 2008	0.03	0.19	0.60
lucerne - 2009	0.10	0.01	0.18
lucerne - total	0.13	0.20	0.78
corn - 2008	0.02	0.08	0.60
corn - 2009	0.08	0.30	1.66
corn - total	0.10	0.38	2.26
white clover	0.07	0.35	1.14
cole - Brassica oleracea	0.02	0.15	0.25

The results shown in Table 5 indicate that the ratio between the metal concentration in the plant and the metal concentration in the soluble forms of the soil solution (accumulation coefficient) has a big span of variation. This situation depends from the type of the metal and its concentration, varieties and possibility for translocation of metals from the root to the upper part of the plants.

The total coefficient of bioaccumulation in the two-year research of alfalfa and corn has indicated that alfalfa is a stronger bioaccumulator of lead, and corn is a stronger bioaccumulator of cadmium and zinc.

The comparison between the examined crops during the first year of vegetation in relation to the heavy metal bioaccumulation coefficient has indicated that the white clover is the best lead, cadmium and zinc bioaccumulator. In the first year of vegetation, corn is the weakest lead and cadmium bioaccumulator, while corn and alfalfa are the weakest zinc bioaccumulators of the tested crops.

Conclusion

The two-year research of alfalfa and corn has indicated that alfalfa has greater lead extraction power, while corn has greater cadmium and zinc extraction power. The comparison between the examined crops during the first year of vegetation in relation to the heavy metal extraction power has indicated that the white clover has the greatest lead, cadmium and zinc extraction power. The oilseed rape has the weakest lead and zinc accumulation power, while corn has the weakest cadmium accumulation power. The total coefficient of bioaccumulation in the two-year research of alfalfa and corn has indicated that alfalfa is a stronger bioaccumulator of lead, and corn is a stronger bioaccumulator of cadmium and zinc. The comparison between the examined crops during the first year of vegetation in relation to the heavy metal bioaccumulation coefficient has indicated that the white clover is the best lead, cadmium and zinc bioaccumulator. In the first year of vegetation, corn is the weakest lead and cadmium bioaccumulator, while corn and alfalfa are the weakest zinc bioaccumulators of the tested crops. This is the first scientific knowledge on the phytoremediation of soils with heavy metal contamination in our country.

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Fitoremedijacija kao ekološka mera čišćenja zagađenih površina teškim metalima (Pb, Cd i Zn) u reonu Velesa

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Rezime

Fitoremedijacija kontaminiranih površina toksičnim metalima je vrlo skup, dug i složen proces. Ona se sostoji od gajenja kultura koje imaju sposobnost fitoekstrakcije teških metala iz zemljišnog rastvora i hiperakumulacije u njihovom zelenom delu iznad površine zemljišta. Region grada Velesa je jedan od tipičnih primera gde je dokazano zagađivanje zemljista i drugih medijuma životne sredine toksičnim metalima kao sto su olovo, kadmium, cink i drugi, kao posledica njihove emisije topionice za olovo i cink lociranoj u Velesu. U veleškom regionu je postavljen ogled sa četiri kultura u četiri ponavljanja: repica – *Brassic napus ssp. oleifera D.C.,* bela detelina – *Trifolium repens L.,* lucerka - *Medicago sativa L.,* i kukuruz - *Zea mays L*. Rezultati o ukupnom sadržaju teških metala u zemljištu su pokazali da su sva tri elementa zastupljena iznad maksimalno dozvoljenih koncentracija. Istraživanja su pokazala da najveću moć izvlačenja olova, kadmiuma i cinka ima bela detelina. Najmanju moć akumulacije olova i cinka ima uljana repica a kadmiuma kukuruz. Istraživanja su pokazala da u prvoj vegetacionoj godini najveći koeficijent bioakumulacije svih ispitivanih teških metala ima bela detelina. Najmanji koeficijent bioakumulacije olova i kadmiuma ima kukuruz, a cinka kukuruz i lucerka. Ovo su prva naučna saznanja o fitoremedijaciji kontaminiranih zemljišta teškim metalima u našoj zemlji.

Ključne reči: fitoremedijacija, zemljište, teški metali, Pb, Cd, Zn, bioakumulatori

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A NEW TECHNOLOGICAL PROCESS FOR PRODUCING BIO-ETHANOL BY CONTINUOUS BIOCHEMICAL CONVERSION OF SWEET SORGHUM JUICE

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Abstract The Institute of Field and Vegetable Crops in Novi Sad has developed the sweet sorghum variety Novosadski Šećerac and the sweet sorghum hybrid NS Šećerac. In a two-year trial carried out under different growing conditions in four locations, Novosadski Šećerac produced a fresh weight yield of 37.1-72.0 t ha^{-1}, while the hybrid NS Šećerac produced 44.6-87.2 t ha^{-1}. The sugar content of the sweet sorghum juice, determined by refraction and chemical analysis, was 18.8% in the variety Novosadski Šećerac and 20.0% in the hybrid NS Šećerac. A new technological procedure has been developed at the laboratory and scale-up levels whereby bio-ethanol and CO_2 are produced through continuous biochemical conversion of sweet sorghum juice. The major stages of the new procedure have been analyzed and technologically optimized, such as the extraction and purification of the juice, continuous biochemical conversion of sucrose, continuous bacterial fermentation of glucose, and adsorptive concentration of ethanol and CO_2.

Key words: sweet sorghum, sugar content, bio-ethanol, biochemical conversion.

Introduction

Due to a drastic rise in crude oil prices and the global effects of environmental pollution caused by combustion gases, great efforts have been under way lately to find new raw materials that can be used for the production of renewable, environmentally friendly fuels. The present demand for gasoline in Serbia has been estimated at 1.500.000 t year^{-1}, while the demand for diesel fuels in the country is put at 1.800.000 t year^{-1}. Owing to its high photosynthetic efficiency, high yields of fresh weight per unit area, and high levels of sugar and lignocellulosic biomass *(Billa et al., 1997; Brayan, 1990)*, sweet sorghum

(*Sorghum biocolor* (L.) Moench) is a highly suitable raw material for the manufacture of the renewable, environmentally friendly fuel bio-ethanol.

Different sorghum species are expanding rapidly to all the continents and currently rank fourth among cereals in acreage planted (80 million hectares). The world's largest producers of sorghum are India, China and the U.S. The area sown to the crop in Serbia is negligible.

The Institute of Field and Vegetable Crops in Novi Sad has developed several cultivars of sweet sorghum, the best-known among which are the variety Novosadski Šećerac *(Lazić and Lazić., 1973)* and the hybrid NS Šećerac *(Pataki et al., 2007)*. As part of the project Production and Use of Ethyl Alcohols as Energy Sources, *Adnađević (2005)* developed a new technology for the manufacture of bio-ethanol using continuous biochemical conversion of juice obtained from the sweet sorghum variety SUCROSORGO 405.RTM (Northum King Comp.USA)

The present paper examines the possibility of obtaining bio-ethanol through continuous biochemical conversion of sweet sorghum juice contained in the hybrid NS Šećerac.

Materials and Methods

Sweet sorghum production. Sweet sorghum is most often planted at a spacing of 70 cm between the rows and 12-15 cm within the rows. It is a thermophilic plant species that is planted when soil temperature rises above 10 ^{0}C. The production technology, crop management, and protection against pests, weeds, and diseases for sweet sorghum are the most similar to the practices used in maize. An experiment was set up in 2005 and 2006 in the Novi sad, Kruševc, Pančevo and Zaječar, with the variety Novosadski Šećerac and the hybrid NS Šećerac. The size of the experimental unit was 10 m^2 and there were four replicates. The crop was harvested at waxy ripeness of the grain and the yields were determined by measuring all of the above-ground biomass and extrapolating the results to tons per hectare.

Production of sweet sorghum juice. Right after harvesting, the sorghum was taken to the laboratory to be tested. The panicle and the grains were removed and the stalk was stripped of all the leaves. The stalk was cut into 1 cm long pieces, which were then tested for moisture and mineral contents. The juice was pressed out of the stalk pieces and total and reduced sugar levels and the pH were determined.

Methods of analysis. The levels of total and reduced sugars in the juice were determined by the HPLC method *(Saha and Bothast., 1999)*, while the pH of the juice was measured on a pH meter. The moisture content was determined gravimetrically by drying to constant mass. The levels of mineral substances were also determined through gravimetric analysis by burning the samples at T=500 ^{0}C. The ethanol content was determined by gas chromatography *(Voogd et al., 1991)*.

Results and Discussion

Table 1 shows a comparative review of fresh weight yield (FWY), grain yield (GY), yield of bare stalk (YBS), juice yield (JY), total sugar content (TSC), reduced sugar content (RSC), and mineral content (MC) of the variety Novosadski Šećerac (NŠ) and the hybrid NS Šećerac (HNS).

Table 1. Yield and components of yield of the sweet sorghum variety Novosadski Šećerac and NS Šećerac

Genotype	FWY $t\ ha^{-1}$	GY $t\ ha^{-1}$	YBS $t\ ha^{-1}$	JY $t\ ha^{-1}$	TSC %	RSC %	pH	Moisture %	MC %
HNS	87.2	-	62.4	47.4	20	4.1	4.8	70.0	2.0
NŠ	72.0	4.5	44.0	30.0	18.8	2.1	4.85	70.6	2.2

Compared to Novosadski Šećerac, the hybrid NS Šećerac has higher fresh weight and juice yields and higher total and reduced sugar contents and has the capacity to produce 5.0 t ha-[1] of bio-ethanol.

Figure 1. A process flow diagram of bio-ethanol production

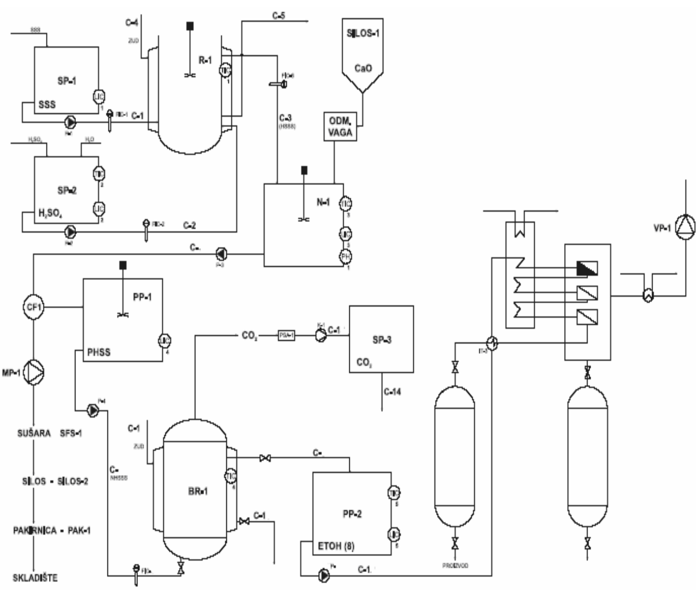

Figure 1. depicts the process of producing bio-ethanol by continuous biochemical conversion of sweet sorghum juice. R-reactors, N-neutralizer, BR-bioreactor, PVP-pervaporation unit, PSA-dryer unit, IT- temperature control unit, SP-storage container, PP-receptacle, P- pumps, C-pipeline

The production of ethanol from sweet sorghum juice is based on four interconnected processes:
- obtainment and mechanical purification of sweet sorghum juice (SSJ),
- continuous acid-catalyzed hydrolysis of sucrose and starch (CACHS),
- continuous fermentation of mono-saccharides to ethanol (CFME) and
- pervaporation of a concentrated ethanol solution.

Table 2 shows the optimal values of the major parameters in the process of continuous acid-catalyzed hydrolysis of sucrose and starch.

Table 2. Optimal parameter values in acid-catalyzed hydrolysis of sucrose and starch

Parameter	Value
Sugar concentration in sweet sorghum juice	17%
Temperature	40°C
Contact time	1 h
Neutralizing agent	$Ca(OH)_2$ powder

Table 3 shows optimal parameter values for continuous fermentation of monosaccharides to ethanol.

Table 3. Optimal parameter values in continuous fermentation of mono saccharides to ethanol

Parameter	Value
Concentration of mono saccharides in neutralized hydrolyzed sweet sorghum juice	10%
Temperature	35°C
Contact time	1h
Percentage of bioreactor capacity filled	80%

The optimal parameter values for pervaporation are shown in Table 4.

Table 4. Optimal parameter values for pervaporation

Parameter	Value
Temperature	40°C
Vacuum	0.01 bar
Degree of recirculation	3
Ethanol concentration	6.7%

An evaluation has been made of the economic viability of constructing a facility with a capacity to produce 20,000 tons of bio-ethanol per year (Table 5).

The results shown in Table 5 clearly show that the manufacture of bio-ethanol from sweet sorghum juice is a highly advanced and profitable investment.

Table 5. Techno-economic viability of constructing a 20000-ton-capacity plant for bio-ethanol production

Criteria	Parameter
1. Eliminative	
1.1 Investment return time	10 months
1.2 Value created	33.120.000 €
1.3 Internal profitability rate	23%
2. Descriptive	
2.1 Accumulativeness	4.98
2.2 Reproductive capability	5.07
2.3 Sensitivity	Low
3. Functional	positive

Conclusion

The juice of the sweet sorghum hybrid NS Šećerac is a highly suitable bio-renewable raw material for the production of bio-ethanol.

Bio-ethanol production by continuous biochemical conversion of sweet sorghum juice is an original new technology for obtaining bio-ethanol that is highly profitable. The investment return time is 10 months and the new value created is worth 33.000.000 €.

Novi tehnološki proces proizvodnje bioetanola kontinualnom biohemijskom konverzijom soka slatkog sirka

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Rezime

U Institutu za ratarstvo i povrtarstvo stvorena je sorta sirka šećerca Novosadski šećerac i hibrid NS šećerac. U dvogodišnjem ispitivanju u četiri agroekološki različita lokaliteta sorta Novosadski šećerac postigao je prinos zelene mase od 37,1 t ha^{-1} do 72,0 t ha^{-1}, dok je hibrid NS šećerac od 44,6 t ha^{-1} do 87,2 t ha^{-1}. Sadržaj šećera u soku utvrđen metodom refrakcije i hemijskom analizom, kod sorte Novosadski šećerac je iznosio 18,8%, a kod hibrida NS šećerac 20,0%. Razvijen je na laboratorijskom i scale-up nivou, novi tehnološki postupak kontinualne proizvodnje bioetanola i CO_2 biohemijskom konverzijom soka slatkog sirka. Analizirane su i tehnološki optimizirane osnovne faze novog procesa: izdvajanje i prečišćavanje soka, kontinualna biohemijska konverzija saharoze,

kontinualna bakterijska fermentacija glukoze i adsorpciono koncentrovanje etanola i CO_2.

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POSSIBILITIES OF HERBICIDE CONTROL OF ECONOMICALLY SIGNIFICANT WEEDS IN FODDER CROPS

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Review paper

Abstract: Basic harmful effect of weed species in fodder crops is reflected through competition for water, nutrition and sunlight. Economically most significant weed species in lucerne and clover belong to life form of geophytes, of which in our country the most distributed are *Cirsium arvense, Sorghum halepense* and *Convolvulus arvensis*. Of quarantine weed species the most frequently occurring are parasitic flowering plants from genus *Cuscuta,* as well as adventive species from family Asteraceae, i.e. *Ambrosia artemisiifolia, Xanthium strumarium,* and *Erigeron canadensis*. Some weed species characteristically occurring in fodder crops belong to poisonous species such as *Datura stramonium, Gratiola officinalis, Ranunculus repens, Saponaria officinalis, Euphorbia cyparissias, Aristolochia clematitis* and *Sinapis arvensis*). With the aim of chemical control, treatments can be applied after shooting of crop and weeds up to third trefoil leaves phase, then in the phase of 1-3 trefoil leaves of lucerne, or after first mowing, when *Cuscuta* spp. is also controlled with some of the following herbicides that have license for marketing in our country: bentazon, clethodim, propizamid, diquat-dichloride, metribuzin, 2-4 DB and thifensulfuron-methyl.

Key words: weeds, fodder crops, control, herbicides

Introduction

Weeds reduce yield of fodder crops by concurrence for water, nutrition and sunlight, at the same time reducing seed quality. If weed species become dominant until the first mowing of lucerne, clover or birds foot trefoil (*Lotus corniculatus* L.), they have significant impact to yield, cause problems during harvest, and indirectly cause frequency of diseases and pest occurrence. With the aim of achieving as high yields as possible, intensive production has been introduced, for which it is necessary to establish contemporary measures of crop cultivation that involve also chemical weed control. Weeds in fodder represent one of the crucial factors in limitation of yields, and their removal is one of the important conditions for profitable production. For achieving of this, knowledge of weed biology, on herbicides and their action mechanisms, persistence, selectivity and other properties are needed.

Due to specific way of cropping in suitable agro-ecological conditions there exist also convenient conditions for occurrence, development and formation of weed community. Weed community of fodder crops, in its structure and seasonal dynamic significantly differ from those in annual crops. Multi-year exploitation of forage crops, results in forming of stable weed community in which dominant become perennial weeds from the group of geophytes, whose control is still not fully resolved in a satisfactory manner (*Strbac et al., 1996*). However as perennial, fodder plants are frequently grown in crop rotation with other crops, for they leave arable land, relatively clean from weeds.

Some weed species can be: **poisonous** – those that domestic livestock grazing to avoid throughout grazing, but can eat them if found in hay or silage; – **hosts** to insects and parasites, agents of lucerne plant diseases; and to posses unpleasant smells and thus lower quality of animal products, or they can have leaves with thorns and damage skin of the mouth and digestive tract of domestic animals (*Čuturilo and Nikolić, 1986; Konstantinović et al., 2006*). Weed competition is especially expressed in the first phases of the crop development, for weeds then shoot and develop faster, occupying living space. Number and weed species that can occur in the mass and spread very fast to the area under fodder plants is not high. Beside annual weeds species, mass occurrence of perennial species is very important, especially during multi-year exploitation and before plough down of fodder crops and before sowing of the following crop in crop rotation. To the small number of weeds that are of utmost economic importance belong primarily the following types of perennial species: Johnson grass (*Sorghum halepense*), Creeping Thistle (*Cirsium arvense*), Field Bindweed (*Convolvulus arvensis*) and Dodder (*Cuscuta* sp.) as typical quarantine lucerne parasite. On the territory of EX SR of Yugoslavia in lucerne crop about 162 weed species were determined, of which even 84 can be considered harmful (*Čuturilo and Nikolić, 1986*). Certainly, this number in Serbia and Montenegro is significantly lower. In neighboring Bulgaria, by *Kolev (1963),* in lucerne were found 215 species of weeds, of which 92 are considered economically harmful (*Strbac et al., 1996*).

Economically the most harmful weeds of fodder crops

Last years, relatively narrow crop rotation, shallow tillage, inadequate pre-sowing preparation and use of the same or identical herbicide groups enabled spread and domination of adventive weed species such as *Ambrosia artemisiifolia, Iva xanthifolia, Xanthium strumarium* (*Konstantinović et al., 2005*). If they adapt to the conditions on the arable fields in unchanged conditions of weed control, these species may cause greater problems than native weed ones. As all invasive species, they are also of utmost biological potential, high competitive abilities, adaptive and aggressive. They result from significant ecological changes accompanied by globalization and human activities, and consequences are mainly significant losses in economy, biological diversity and in the function of inhabited ecosystems.

Weedy ruderal species *Ambrosia artemisiifolia* L., of fast end intensive spread represents great problem. It is widespread in Austria, Belgium, Luxembourg, Czechoslovakia, France, Germany, Hungary, Italy, Serbia, Portugal, Poland, Romania, USSR (southwest region). It belongs to the adventive floristic element (Adv.), and in 1800, it was introduced from North and Central America to Europe. While spreading it began to adapt to the open vegetation on waste terrains, primarily of ruderal or weedy types. It is now invading natural vegetation of semi-closed type on degraded pastures. It weeds all crops and it weeds almost all crops and plantations, spreads quickly and becomes cosmopolitan. Since the time when it was introduced into Europe, it had a long period of rest, and it has started spread rapidly in the first half of the twentieth century and it continues its expansion for which it requires appropriate environmental factors. In our country, it was found in a greater number of sites (*Konstantinović et al., 2004*). It was found in Srem and Backa regions near the Danube in Petrovaradin, Sremski Karlovci and Novi Sad. It has also continued its spread in some parts of Serbia, especially in the region of Nis and in the valley of the river Morava. It is a thermophylic species that shoots throughout the summer, and one plant can make 150 000 seeds and its control is extremely difficult and expensive.

The species *Iva xanthifolia,* newly introduced weed species in our country; predominantly weed of ruderal sites, in last years has also been occurred as great problem in crops, especially in row ones and it spreads intensively. The danger of this weed is reflected primarily in its extremely robust appearance (height over 2m), strong competition influence on the crop (uniform emergence) and huge seed production. Also, from the aspect of human health this species is very dangerous, because it is very strong allergen like the species *Ambrosia artemisiifolia. Iva xanthifolia* Nutt. is spread in Austria, Czechoslovakia, France, Germany, Hungary, Poland, Romania, USSR (central, south-western, south-eastern region). It is naturalized in the central-eastern and south-eastern Europe and France and elsewhere it is a random occurrence. In 1966 in the literature of our area it was described for the first time. In the relatively limited number of localities in Backa, Banat and Srem, for a long time it occurred just as the weed of ruderal sites. Last few years studies indicate a very aggressive expansion of the weed, so it is present in almost the whole territory of Srem, southern and central Backa, Banat and almost in the entire area of Stig. In places with favourable conditions for its development, it absolutely dominates and creates a clean community because due to its properties it virtually has no competitors in other weed species (*Marisavljević et al., 2006*).

Xanthium strumarium, Clotbur is frequent and harmful weed. It is one of the most frequent and the most dangerous weeds in row crops, less frequently in orchards and vineyards. It is wide spread weedy-ruderal species that is frequently occurring on lawns, uncultivated land, land boundaries, pastures and ruderal habitats, along roads and other neglected areas. Although nowadays being widely spread, almost cosmopolitan species, it originates from America. It belongs to the adventive floristic element (Adv.) and widespread in Albania, Austria, Belgium, Luxembourg, Bulgaria, Corsica, Crete, Czechoslovakia, France, Germany, Greece, Switzerland, Hungary, Italy, Yugoslavia, Luxembourg, Poland, Romania, USSR (all regions except the northern, which means the Baltic, central, south-east-south-western region and Krym), Sardinia, Sicily, Turkey (*Konstantinović et al., 2002*).

The highest damages in lucerne are caused by quarantine flowering plant– *Cuscuta europea* L, that significantly reduces yield and quality of fodder crops, prevents flowering and seed maturing of seed-growing lucerne (*Konstantinović, 1999; Konstantinović and Meseldžija, 2009*). Beside this species, in our country lucerne and red clover are also significantly endangered by species *Cuscuta trifolii* Link. and *C. epythimum* Murr. (*Mijatović and Stojanović, 1968*). Parasitic flowering plants from the genus *Cuscuta*, are annual species that flower from July to October and produce very tiny, bright seeds. They are usually found in humid places and the water for irrigation (*Smith et al., 2003*) often transfers seeds to the agricultural areas. In average, one plant can produce up to 16000 seeds, whose vitality may vary between 10 to 20 years, with postponed germination (*Rohse, 1998*). Dodder does not over winter only as seed that germinates from may to September, but also in a vegetative manner, usually in the root neck, and in early spring it continues to develop and propagate (*Stojanović, 1963; Lanini and Kogan, 2005*). Seed quality is very important for lucerne and other legumes that have tiny seeds, and in production of lucerne seed, Dodder is quarantine weed (*Popović and Stjepanović, 1995*).

Weed control in fodder crops

Control of economically harmful weed species is very difficult but the damage they cause today point to the seriousness of this problem and require access to recording of the presence and spread of these weeds, and finding of optimal ways of their suppression. The most effective use of integrated measures, which include also preventive measures (cultural practice) and control of the already infected crops (application of mechanical measures such as mowing or burning of infected oasis, and chemical control measures). Beside measures of cultural practice, such as primarily crop rotation, followed by all measures that enable good soil preparation, timely and quality sowing as well as later cropping, herbicide weed control represents one of the basic protection measures. As perennial cultures, fodder Crops remains many years on the same land, which makes problem of weed control harder and more delicate. Eventual inadequate herbicide choice could have negative impact to evolution of weedy florae, more than it is the case with annual cultures (*Konstantinović, 1999*). All weed control measures that can be applied, such as choice of the sowing land, crop rotation, soil cultivation, pure seed material, cultivation, irrigation, herbicide application and mowing must be applied integrally with the aim of effective weed control (*Dowson, 2004*). In lucerne, clover or birds foot trefoil (*Lotus corniculatus* L.) crops, composition of weed florae in the first year of growing differs in its composition from one after the second, third or fourth year, which must be taken into consideration during herbicide choice (*Kojić et al., 1972; Andreasen et al., 2002*). During establishment of the crop, during spring, present are winter and early spring weed species. In the second year of growing of fodder crops dominant become perennial weed species and some species of summer weeds, and in the last year of exploitation the most distributed become perennial and some annual weeds, which is especially expressed if control measures are not applied (*Štrbac et al., 1996; Andreasen et al., 2002*). Considering expressed susceptibility of fodder crops toward a row of herbicides, the great number of them is applied in the phase before sowing or after sowing and before shooting of the crop, in young crops, i.e. in the phase of vegetation rest in older crops (*Mitić, 2004*).

The applied weed control preventive measures include prevention of the weed occurrence, elimination of some survived weeds as sources of infestation, identification of weed species and their mapping, detection of weed occurrence during year, identification and elimination of new weed species before their expansion, use of sanitary measures for prevention of spreading of some weed species from neighbouring non-agricultural areas, cleaning of tools and machines before entering into the crop, cleaning of the pure sowing material, monitoring of the applied canal irrigation water (*Dowson, 2004*). Reduction of Dodder infestation is done preventively, by making lucerne seed free of Dodder seed in plants for production and marketing of lucerne seed, with the application of electromagnetic separators, or curative, by application of herbicides aimed primarily to treatment of local Dodder (*Konstantinović, 1999*). In weed seeds that are hard to separate from lucerne seed presence of the following weed species was established: *Cuscuta sp., Rumex sp., Plantago lanceolata, Amaranthus retroflexus., Myosotis arvensis., Polygonum lapathifolium., Setaria sp.* and *Sorghum halepense*, and during seed processing on magnetic separator is usually lost about 20% of fodder plants seeds (*Gajić and Stjepanović, 1995*). During fodder plant seed cleaning from seeds of weed species, beside loses of great quantity of the crop seeds, seeds of quarantine weed species from the genus *Cuscuta*, are often hard to be completely removed, which disables sell of such seed material (*Dawson, 2004*).

If there are indications that the crop will be intensively attacked by Dodder at the beginning of vegetation, preventive treatment is performed simultaneously with sowing or immediately after it. Intervention against parasitic weeds from the genus *Cuscuta* in the year of the lucerne crop establishment enables complete eradication of this parasitic flowering plant. In the last 150 years, Dodder seeds are predominantly distributed in antropohorn manner, more by human activity than naturally (*Dowson, 2004*). If control is began very late, when the crop is already hardly infested by Dodder, it should be in the same year treated twice, before starting of vegetation and after the first mowing (*Konstantinović, 1999, 2008*). There are several herbicides that can be successfully applied in fodder crops, certainly timely and in accordance with other measures of cultural practice (*Dowson, 2004).* Chemical weed control can be performed in three periods, i.e. before sowing, in the period of the crop establishment and in already established crop.

Table 1. Herbicides that can be applied with the aim of weed control in lucerne crops (*Anonymus, 2009*)

Herbicides (a.i.)	Controlled weeds	Rate	Time of use	Number of treatment in a year
Reglone Forte, Dikvat (diquat dibromide)	*Cuscuta sp.*	5 l/ha	treatment of focuses	1
Kerb 50-WP (propizamid)	*Cuscuta sp.*	3-4 kg/ha	after the first mowing	1
Select Super, Nikas (chletodim)	annual and perennial grass weeds	0,8-2 l/ha	after shooting of the crop	1
Sencor WP-70, Mistral (metribuzin)	annual broad-leaf weeds	0.75-1.2 kg/ha	-	1
Harmony 75WG (thifensulfuron-methyl)	annual broad-leaf weeds	15-20 g/ha	lucerne in the second year of older – early spring before start of vegetation	1
Basagran, Galbenon, Deltazon 48-SL, Bentazon SL-48, Bevezon, Bentasav (bentazon)	annual and perennial broad-leaf weeds (*Taraxacum officinale*)	3 l/ha	Crop height of 10-15 cm, and weeds with 2-6 leaves	1
Butoxone-DB (2.4DB-Na salt)	annual and perennial broad-leaf weeds (*Cirsium arvense*)	1.5-3.0 l/ha	after shooting up to the third triplet	1

Perennial weeds are more difficult for control in fodder crops, in relation to broad-leaved ones, due to which preventive measures must be fully applied with the aim of reduction or elimination of perennial weed species seeds before sowing of the crop. Use of crop rotation in great extent reduces use of cultural practice or chemical measures. In small grain cereals numerous herbicides can be applied at which lucerne, clover or birds foot trefoil (*Lotus corniculatus* L.) crops are not tolerant, but that are highly efficient in control of perennial weed species *Cirsium arvense.,* and *Convolvulus arvensis*. Non-selective herbicides such as glyphosate or gluphosinate ammonium can be applied against perennial broad-leaved and narrow-leaved weeds such as *Sorghum halepense* in early autumn, after harvest of any annual crop, but attention must be paid upon time between their application and sowing of fodder crops. With the aim of control of late spring broad-leaved weeds, as well as perennial species such as *Sorghum halepense*, treatment can be done in the phase of the 1-3 triplet, or after the first mowing when *Cuscuta sp.* is also controlled by herbicides based upon active ingredients: bentazon, propyzamide, clethodim and diquat-dibromide (*Anonymus, 2009; Dowson, 2004; Mesbah and Miller, 2005; Konstantinovic, 2008*). Split application bentazon with imazamox or imazethapyr for control of perennial species *Cirsium arvense* in lucerne crops for seed production (*Mesbah and Miller, 2005*). Herbicide trifluralin is also highly efficiently applied with the aim of Dodder control if used between 15 January and 30 April; before shooting of this weed, it is highly efficient (*Dowson et al., 1984*). Treatments that depend upon weediness and weather conditions are performed during early spring in the phase of fodder plants rest by herbicide metribuzin, which is aimed for elimination of annual broad-leaved weeds, and it is applied in a rate of 0,75-1,5 kg/ha.

Three years lasting efficiency studies of herbicides hlorimuron, chletodim, flumetsulam, 2.4-DB, glyphosate and haloxyfop applied after the first or second mowing of lucerne 6-10 cm in height, and thier phytotoxicity to the crop, confirmed high efficiency of chletodim and haloxyfop to *Cynodon dactylon, Echinochloa crus-galli* and *Setaria geniculata*, which results in increased yield of lucerne. Hlorimuron, flumetsulam and glyphosate were efficient in control of *Urtica urens*, *Stellaria media, Chenopodium album*, *Xanthium spinosum* and *Veronica persica*, but they have caused great damages of crops and reduced yield (*Arregui et al., 2001*).

Use of herbicides that are inhibitors of acetolactat synthase (ALS), can cause undesired effects, such as resistance of weeds to herbicides belonging to the group of this action mechanism, such as imazethapyr and imazamox in very short period, even after two applications (*Smith et al., 2003*). Because of this it is necessary to pay attention not only to efficiency but also to the rotation herbicide with different action mechanisms.

Conclusion

In perennial crops of lucerne, clover or birds foot trefoil (*Lotus corniculatus* L.), occurr seasonal changes in weed comunity compositon. During the first year present are different species of typical field weeds, and with ageing of the crop occurre incresase in presence of ruderal and meadow weed species. Of econimic importants are perennial species such as: *Cirsium arvense* (L)Scop., *Sorghum halepense* (L) Pers., *Convolvulus arvensis* L., quarantine species from the genus *Cuscuta* and adventive species from the Family *Asteraceae*, i.e. *Ambrosia artemisiifolia, Iva xanthifolia, Xanthium strumarium* and *Erigeron canadensis*. With the aim of weed control integral measures that include choice of sowing field, crop rotation, soil tillage, sowing of the pure seed material, cultivation, irrigation, herbicide use and mowing must be applied. Chemical weed control in lucerne, clover or birds foot trefoil crops can be performed before sowing, in the period of crop establishment and in already established crop. Herbicides that are successfully applied in control of perennial weeds and parasitic flowering plants are bentazon, chletodim, diquat-dibromide, metribuzin, 2.4-DB, and thifensulfuron-methyl.

Mogućnosti suzbijanja ekonomski značajnih korova krmnog bilja primenom herbicida

B. Konstantinović, M. Meseldžija, B. Konstantinović

Rezime

Osnovno štetno delovanje korovskih vrsta u usevu krmnog bilja odražava se kroz konkurentnost za vodu, hranljive materije i svetlost. Korovi su uglavnom male hranljive vrednosti, neprijatnog mirisa i ukusa a utiču i na pogoršanje kvaliteta semena krmnog bilja. Ekonomski najznačajnije korovske vrste lucerke i deteline pripadaju životnoj formi geofita, od kojih su u našoj zemlji najzastupljenije: *Cirsium arvense, Sorghum halepense, Convolvulus arvensis.* Od karantinskih korovskih vrsta najčešće su parazitne cvetnice iz roda *Cuscuta,* kao i adventivne vrste iz fam. Asteraceae (*Ambrosia artemisiifolia, Iva xanthifolia, Xanthium strumarium, Erigeron canadensis*). Pojedine korovske vrste karakteristične za krmno bilje spadaju u otrovne vrste (*Datura stramonium, Gratiola officinalis, Ranunculus repens, Saponaria officinalis, Euphorbia cyparissias, Aristolochia clematitis* i *Sinapis arvensis*). Sve agrotehničke mere koje omogućavaju dobru pripremu zemljišta, pravovremeno i kvalitetno sejanje, kao i kasniju negu useva, predstavljaju osnovne mere suzbijanja ekonomski štetnih

korova. U cilju hemijskog suzbijanja, tretiranje se može obaviti posle nicanja useva i korova pa do treće troliske, u fazi 1-3 troliske lucerke, ili posle prvog otkosa kada se suzbija i vilina kosica, sa nekim od sledećih herbicida koji imaju dozvolu za primenu u našoj zemlji: bentazon, kletodim, propizamid, dikvat-dihlorid, metribuzin, 2,4 DB-natrijumova so, tifensulfuron-metil.

Ključne reči: korovi, krmno bilje, suzbijanje, herbicidi

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NITROGEN FIXATION OF *SINORHIZOBIUM MELILOTI*-ALFALFA SYMBIOSIS: A FIVE-YEAR FIELD TRIAL

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Abstract: In the present study the ability of alfalfa to fix nitrogen with some *Sinorhizobium meliloti* strains under field conditions was estimated during five-year trial. Nitrogen fixing efficiency was determined according to shoot dry matter, crude protein yield and the amount of total and fixed nitrogen in alfalfa. During all years single strains (234 and 236) had a significant positive effect on shoot dry matter yield compared to yields in the control treatment and treatment with mixed strains. Among the inoculums applied, strains 234 was the most effective strain in 4th year as well as in the 3rd year of utilization with the SDM yield higher from 34 to 60% in respect to the control plants. In addition, crude protein content in almost all cuttings of the last two years of alfalfa utilization was higher in treatment with strain 234 than in treatment 236. Total N content and fixed N of second cutting were in correlation with shoot dry matter. Process of N fixation through fixed N participated in total N content 32-44% in the treatments with 234 and 236. The results indicate that alfalfa inoculation with effective strains is an alternative approach to improving the long-term productivity of alfalfa with the aim of sustainable agriculture developing.

Key words: alfalfa, *Sinorhizobium meliloti*, nitrogen fixation, sustainable agriculture

Introduction

In the world, alfalfa (*Medicago sativa* L.) is important forage crop because of its high-quality of shoot dry matter and wide area of growing. It is the most commonly used forage legume for hay production. Alfalfa preferentially uses N from the soil and from applied sources such as manure, in spite of the possibility to fulfil most of its nitrogen requirement with N from the process of symbiotic N fixation. In an effort to enable increase in outputs of high-quality and, even more importantly, wholesome fodder, research has partly been focused on increasing the natural ability of alfalfa plants to uptake N_2 through inoculation with highly effective symbiotic strains in the form of microbiological N fertilizer. The interaction alfalfa /*Sinorhizobium meliloti* is one of the most effective interaction between plant and N fixing bacteria (rhizobia) with average amounts of fixed N from 140 to 210 kg ha^{-1} per year. For species such as alfalfa, in addition to inhibitory effect to N fixation, mineral fertilization decreases the useful life of the crop and the quality of protein in the dry matter *(Raun et al., 1999; Oliveira et al., 2004)*. Due to that effective management of nitrogen supply in crop production with intensively applying N_2 fixation is important to improve organic farming and sustainable agriculture. Alfalfa is successfully cultivated on chernozem, meaning that it achieves an effective nitrogen fixing process. Although alfalfa is a perennial, stands usually persist at list six years.

The effects of some highly effective *S. meliloti* strains on yield and quality of alfalfa biomass as well as effectiveness of biological N fixation were examined in five-year field trial with the aim of focusing on the need to apply alfalfa inoculation with nitrogen microbiological fertilizer on chernozem soil in alfalfa production.

Materials and method

The commercial alfalfa cultivar K-28 of the Institute for Forage Crops was used as a host plant in a five-year field trial (2005-2009). Several highly effective strains of *S. meliloti* (224, 234 and 236), originating from a collection of the Institute of Soil Science, were used as inocula. Alfalfa was hand-sown at high density in plots sized 0.2x5m ($1m^2$) in an experimental field of the Institute for Animal Husbandry, Belgrade-Zemun, in 2005. N mineral fertilizers were not used before sowing and during five years of field trial. The plants were inoculated with pure culture suspensions of the investigated strains grown for 72 h on YMB (10^9 cells ml^{-1}). The trial included 4 inoculation treatments (3 with single strains and 1 with their mixture) and an untreated control (Θ) in a random block system with 5 replicates. Fresh plant weight (kg $plot^{-1}$) and dry weight (t ha^{-1}) were measured at the beginning of flowering after each cutting (fore cutting per year) in the forth and fifth year of utilisation. Based on the percentage of N measured by chemical analyses (CNS Elemental Analyzer vario EL III, 2005) in the dry matter of plants of all treatments in second cutting for five years of utilization, the content of dry proteins, total and fixed N content (TNC) were calculated. The results were statistically processed by LSD test.

Results and discussion

The experiment was conducted on a weakly calcareous chernozem. One year before sowing, the chemical characteristics of soil (Table 1) showed that the contents of humus and total nitrogen in the top arable horizon were within normal limits, while phosphorus and potassium contents were more than well supplied. Low NO_3^- nitrogen content, expected for unfertilized soils, confirmed that the plots had not been fertilized in the initial year. Such content of nitrate (NO_3-N) is favourable for the establishment of a symbiotic association between alfalfa and *S. meliloti* (*Raun et al., 1999; Delić et al., 2007)*.

Table 1. Chemical properties of soil in 2004

Depth (cm)	Acidity (pH)		Org. mat.(%)	Humus (%)	$CaCO_3$ (%)	N mg $1000g^{-1}$			P_2O_5 mg $100g^{-1}$	K_2O mg $100g^{-1}$
	H_2O	KCl				Total	NH_4^+	NO_3^-		
0-20	7.40	7.19	7.09	4.35	0.33	1975.0	0.00	10.5	66.32	40.0

In the five-year field trial the effect of nitrogen fixing *S. meliloti* on alfalfa quantity and quality has been examined. Shoot dry matter (SDM) yield and crude protein content of alfalfa plants inoculated with *S. meliloti* strains in the forth (2008) and fifth year (2009) of alfalfa utilization were presented in Table 2. These parameters in the first three years of utilization have been analyzed in our previous paper *(Delić et al., 2007)*. In 4th and 5th year of utilization all treatments showed high SDM yield per year with the highest yield in a first cutting (Table 2). In all treatments it was noted decrease of SDM from the first to the last cutting in 2008 as well as in 2009 year which is in agreement with the results by *Eldredge et al. (2002)*. Single strains had a significant positive effect on SDM yield compared to yields in control treatment and treatment with mixed strains.

In 4th year significantly the highest SDM yield had plants of the first cutting inoculated with strain 234 as well as in 3rd year of the same cutting reported in previous paper *(Delić et al., 2007)*. In all cuttings of 4th year strain 234 achieved the highest yield (7.52, 6.30, 4.39 and 1.57 t ha^{-1}) which was higher than the control plants (for 42, 35, 60 and 34%, respectively). Among the inoculums applied, strains 234 was the most effective strain in 4th year so as in the 3rd year *(Delić et al., 2007)*. It was significantly effective than strain 236. Despite the fact that in 5th year of utilization strain 236 achieved the highest yields, there were no significant differences between plants inoculated with strains 234 and 236 with exception in forth cutting. In first, second, third and forth cutting strain 236 achieved yield higher for 19, 16, 23 and 24%, respectively than the control plants.

In 4th year of utilization, effectiveness of strain 224 was similar to strain 236 or 234 because there were no significant differences depending on cuttings.

Table 2. Shoot dry matter yield (SDM) and crude protein content (CP) in 4th and 5th year of utilization of alfalfa inoculated with strains of *Sinorhizobium meliloti* (2008-2009).

Treatment*	2008								2009							
	Cuttings (C)								Cuttings (C)							
	C_1		C_2		C_3		C_4		C_1		C_2		C_3		C_4	
	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}	SDM t ha^{-1}	CP g kg^{-1}
236	6.28	210.8	5.44	206.8	3.37	204.4	1.42	178.9	6.48	228.4	5.31	214.1	3.86	210.4	1.89	210.6
234	7.52	228.1	6.30	230.0	4.39	213.1	1.57	196.3	6.12	221.2	5.03	222.8	3.51	228.7	1.29	205.6
224	6.66	226.3	6.30	227.8	3.03	219.7	1.53	186.3	4.86	191.6	4.10	196.6	2.78	198.9	0.91	202.5
Mix	5.41	205.4	4.99	195.9	2.76	220.6	1.06	178.8	4.90	216.6	3.78	191.2	2.74	195.4	1.05	166.2
Θ	5.26	190.5	4.68	172.5	2.74	206.2	1.15	176.0	4.18	236.6	3.37	233.1	2.12	220.6	1.09	206.2
Lsd 0.05	0.49		0.44		0.37		0.21		0.37		0.41		0.25		0.19	

*Ø -untreated control; *Sinorhizobium* strains

Table 3. Total nitrogen content (TNC) and fixed N (Fix N) in alfalfa inoculated with strains of *Sinorhizobium meliloti* during five years of investigation (2005-2009) in second cutting.

Treatment	2005		2006		2007		2008		2009		X		LSD 0.05	
	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC kg ha^{-1}	Fix N kg ha^{-1}	TNC	Fix N
236	53.73	23.68	188.96	43.29	285.00	102.72	180.23	51.06	194.08	72.90	180.4	58.73	50.02	13.2
234	40.01	9.96	168.91	23.24	291.60	109.32	231.84	102.67	178.10	56.92	182.09	60.42	37.31	10.5
224	38.52	8.47	153.39	7.72	221.51	39.23	229.64	100.47	138.72	17.54	156.35	34.69	44.19	9.07
Mix	35.50	5.45	150.33	4.66	174.17	0	156.59	27.43	130.98	9.80	129.51	9.47	34.10	4.7
Xy	41.94	11.71	165.40	19.73	243.07	62.81	199.57	70.41	160.47	39.29	/	/	/	/
Θ	30.05	0	145.67	0	182.28	0	129.17	0	121.18	0	121.67	/	39.29	/
Lsd 0.05	5.12	1.30	12.50	2.05	20.70	9.72	25.40	8.43	19.05	5.92				

*Ø -untreated control; *Sinorhizobium* strains; Xy-aerage value per year ; X-verage value per treatment

However, in 5th year strain 224 showed significantly lower effectiveness in respect to these two strains. Influence of all strain mixture on SDM was not significant in most of the cuttings of the last two years of utilization. Of the inoculums applied, significant effect on shoot dry yield in the last two years of utilization had strains 236 and 234. Results in this paper were in agreement with results of the first three years of this five-year trial *(Delić et al., 2007)* indicating good effectiveness of strains 234 and 236. These results confirmed the indication from our previous findings *(Delić et al., 2007)* about good adaptability of cultivar K-28 alfalfa to environmental conditions. It is especially important to underline extremely bed weather conditions in 2009 *(www.hidmet.sr.gov.yu)*. According to the results of some authors, alfalfa yield decreased with time *(Li and Huang, 2008)*.

It is desirable to keep a crop for several years because of profitability and cost-efficiency of production (*Katić et al., 2007)*. Taking into account these facts, in our trial high yield of shoot dry mass in 4th and 5th year gives possibilities to grow alfalfa forage successfully for several years.

Quality of SDM through crude protein content was evaluated. It is known that alfalfa is high-quality fodder as its dry matter contains around 20% crude proteins. In this trial plants inoculated with single strains had high crude protein content in all cuttings (200-230 kg ha^{-1}) indicating significant quality of SDM with exception of lower crude protein content in 4th cutting of 4th year of utilization (179-196 kg ha^{-1}) (Table 2). The content of crude proteins varied depending on the year of utilisation and cutting as well as the strain. Crude protein content in almost all cuttings of the last two years of alfalfa utilization was higher in treatment with strain 234 than in treatment 236. This fact indicated influence of strain on crude protein content.

BNF of alfalfa was determined by total nitrogen content (TNC) and fixed N in second cutting in all 5 years of utilization (Table 3). TNC and fixed N were in correlation with SDM. Similar results have been noted by *West and Wedin, 1985*. They pointed out that SDM yield was the major determinant of the amount of N fixation. During all years of investigation the lowest values of TNC were marked in control treatment and treatment with mixed strains while the highest were in inoculated treatments with strain 234 and 236. In all treatments TNC of alfalfa was significantly the lowest in the year of sowing in comparison with TNC in years of utilization. In the year of sowing alfalfa has slow growing and that is why lower yields than in years of full utilization *(Radović et al., 2007)*. In inoculated treatments with single strains (236, 234 and 224) the highest values of TNC was recorded in 3rd year (strain 234 and 236 achieved 291.60 kg ha^{-1} and 285.00 kg ha^{-1}, respectively) and without significant differences in 4th year of utilization (strain 234 and 224 achieved 231.84 kg ha^{-1} and 229.64 kg ha^{-1}, respectively) while significantly lower in 2nd and 5th year. However, in inoculated treatment with mixed strains there were no significant differences between the values of this parameter in all years of utilization (2^{nd} -5^{th} year) as a possible consequence of higher competitiveness of low effective strain 224 in mixture. Similarly, in control treatment there were no significant differences between TNC in 2^{nd}, 3^{rd} and 4^{th} year of utilization in comparison with significantly lower value of this parameter in 5^{th} year of utilization. Sanders and Watson *(2002)* underlined that clover yield, the concentration of total and fixed N in the clover varies with management and climate making it difficult to predict N fixation.

Fixed N was in correlation with TNC. Strains 234 and 236 achieved the highest values of fixed N in second cutting of 3^{rd} year, 109.32 kg ha^{-1} and 102.72 kg ha^{-1}, respectively while in 4^{th} year that was strain 234 and 224 with the similar values of 102.67 kg ha^{-1} and 100.47 kg ha^{-1}, respectively. In the 4^{th} year of utilization strain 234 fixed 293 kg N ha^{-1} $year^{-1}$ which was the highest amount of fixed N per year in five-year trial. However, the best effectiveness in BNF has been noted in second year of utilization by *Freyer et al. (2005)*. It is known that symbiotic association with the bacterium *Sinorhizobium meliloti* fixes 100-400 kg N ha^{-1}, *Peoples et al. (1995)* depending on the soil type and land use regime, *Delić-Vukmir et al. (1994)*. Also nitrogen fixation depended on alfalfa variety *(Freyer et al., 2005; Appuny et al., 2008; Delić et al., 2009)*. In this trial the values of fixed N matched type of soils that have good physical and chemical characteristics such as chernozem. It contains a lot of humus that is constant, significant organic reserve of nitrogen, but because of its slowly mineralization, supplying plant with N mineral fertilizers or microbiological N fertilizer is usually recommended.

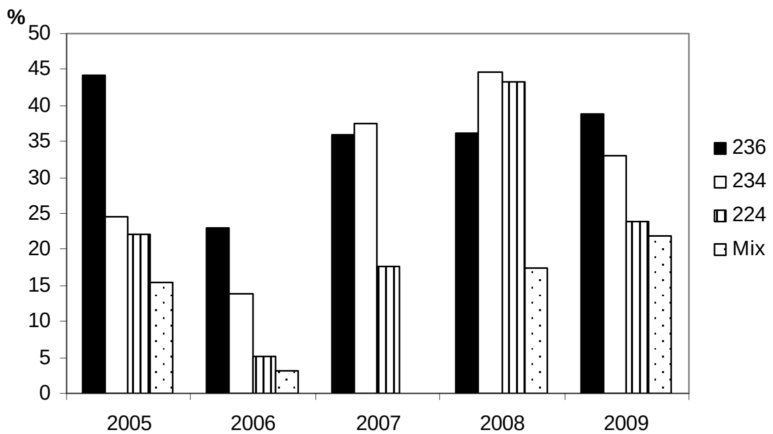

Figure 1. Percentage of fixed N in total N content (100%) in treatments with strains of *Sinorhizobium meliloti* during five years of investigation (2005-2009)

Participation of fixed N in TNC (100%) in all inoculated treatment was expressed as percentage of fixed N (Figure 1). The most effective strains in N fixation were strains 236 and 234. They fixed the most N in the last three years of utilization on average 35% (32-44%). Percentage of fixed N varied depending of treatment and year of alfalfa utilization. According these results rizobial strains and year of utilization had influence on fixed N. The highest percentage of fixed N were marked in 3rd, 4th and 5th year of utilization with maximal value of 44% (4th year) in treatment with 234 strain but the least in 2nd year (3-23%). In the year of sowing the percentage of fixed N was 15- 44%. In this trial mineral N fertilizer was not applied so that mineralization of humus and BNF by microbiological N fertilizer presented the main source of N for plant. These two sources were uninterrupted process as the source of N available for plant nourishment. A synergistic effect could occur between mineral N and N_2 fixation, leading to an increase of plant growth. This synergism may be related to the N_2 fixation efficiency of the rhizobial strain, to the concentration of mineral N *(Mendonça and Schiavinat, 2005)*. According to our results process of N fixation participated in total N content up to 45 % what was almost a half of TNC in the treatments with 234 and 236. There is a need for further investigation to improve BNF efficiency of alfalfa with applying microbiological N fertilizer in organic farming.

Conclusion

The present study showed the ability of alfalfa to produce significant yield of shoot dry mass, total and fixed nitrogen in 4th and 5th year of utilization when inoculated with effective strains which gives possibilities to grow alfalfa forage successfully for several years in organic farming without adding of mineral N.

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Simbiozna azotofiksacija lucerke u petogodišnjem poljskom ogledu

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Rezime

U radu je ispitana azotofiksaciona sposobnost lucerke sa određenim sojevima *Sinorhizobium meliloti* u petogodišnjem poljskom ogledu. Azotofiksaciona efikasnost je procenjena na osnovu prinosa suve nadzemne mase i prinosa sirovih proteina kao i sadržaja ukupnog i fiksiranog azota. Značajan uticaj na prinos suve materije i prinos proteina imali su sojevi 236 i 234 u odnosu na kontrolu bez azota i inokulaciju mešavinom sojeva u svim godinama iskorišćavanja. Tretmani sa sojevima 234 i 236 su povećali prinos suve nadzemne mase za 35-60% odnosno 19-24% u odnosu na kontrolu. Sadržaja ukupnog i fiksiranog azota drugog otkosa je bio u korelaciji sa prinosom suve nadzemne mase sa najvećim vrednostima u tri poslednje godine iskorišćavanja. Sadržaja fiksiranog azota je činio u proseku 35% ukupnog azota u tretmanima sa 234 i 236. Najveća vrednost fiksiranog N po godini ostvarena je u tretmanu sa sojem 234 u četvrtoj godini iskorišćavanja lucerke i iznosila je 293 kg N ha^{-1} god^{-1}. Rezultati ukazuju na mogućnost unapređenja prinosa lucerke primenom efikasnih sojeva *S.meliloti* u formi N mikrobiološkog đubriva tokom više godina iskorišćavanja lucerke u organskoj proizvodnji.

Ključne reči: lucerka, *Sinorhizobium meliloti*, inokulacija, azotofiksacija

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THE INFLUENCE OF DIFFERENT INOCULANTS ON THE ALFALFA GROWN ON ACID SOIL

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Original scientific paper

Abstract: The effect of *Sinorhizobium*, *Actinomycetes* and *Azotobacter* (individually and in combinations) on the alfalfa canopy parameters: height, number of sprouts and plants weight was studied. Also, the weight of the root and the number of nodules were determined. These traits were studied in three cuts. The analyses were carried out in the beginning of flowering stage. Better results for almost all studied parameters were recorded in the trials where *Sinorhizobium* was used compared to the other inoculants. Polyvalent inoculums which contained *Sinorhizobium* acted more efficient in relation to the combination of *Azotobacter* and *Actinomycetes*. Plants in the variant without inoculation had lower values for the studied parameters compared to the alfalfa plants in the treatments where inoculation was applied.

Key words: alfalfa, inoculation, *Sinorhizobium*, *Azotobacter*, *Actinomycetes*

Introduction

Alfalfa (*Medicago sativa* L.) is a plant species characterized, in addition to the potential for high yield and quality of biomass, by intensive biological nitrogen fixation process. Alfalfa can, in symbiosis with nitrogen fixating bacteria, fix up to 43-80% of required nitrogen *(Wivstad et al., 1987)*. For the successful alfalfa cultivation, the microbial inoculants are more and more applied for the better utilization of potential for the development of plant and micro organism N fixation efficiency (*Delić et al., 2007,J*arak *et al., 2005*). Among the most important environmental factors that influence the activity and the number of root nodule bacteria of alfalfa is the pH value of the soil *(Soto et al., 2004*). *Lowendorf and Alexander (1983)* state that limiting pH value for the strains of *Sinorhizobium meliloti* may differ and ranges from 5.3 to 6. To ensure successful alfalfa production on the soils with lower pH, the inoculation with the *S. melliloti* strains, which show considerable activity in such soils, can be applied before the sowing.

The rhizosphere micro organisms are of great importance, because the products of their metabolism can boost the growth and development of plants *(Govedarica and Jarak, 1995).* Also, they synthesize enzymes and suppress the activities of phytopathogens *(Jemcev et al., 2004)*. Some of these micro organisms live on the roots or near it (*Azotobacter, Actinomycetes* etc.), while *Rhizobium* enters in the root tissue. *Rhizobium*, in addition to providing its macro symbionts with nitrogen, also synthesizes vitamins, riboflavin, thiamine and polysaccharides *(Ramos et al., 1987).* Besides the utilization of the symbiotic nitrogen fixators, the increase of nitrogen balance can be achieved by using free nitrogen fixators, primarily *Azotobactera* that synthesizes gibberellin, auxin, pirodoxin and nicotinic acid. *Actinomycetes* produce antibiotics and vitamins, which act favourably on the physiological processes of plants *(Berg et al., 2001).* Knowledge of the role of certain groups of micro organisms and microbiological processes in soil can be managed in the desired direction (*Milošević et al., 2003)*. Successful introduction of strains of micro organisms in the soil depends on the number, activity or competition of indigenous populations in the soil, the activity of the host plants, soil properties and other factors *(Brockwell et al., 1995).*

The aim of this study was to examine the influence of *Sinorhizobium*, *Actinomycetes* and *Azotobacter* (especially in combinations) on the development of alfalfa canopy and roots.

Materials and methods

The experiment was set up in the semi-controlled conditions in the Institute of Forage Crops, Krusevac. For the purposes of research, the soil with increased acidity pH 4.77 (KCl) was used. Chemical composition of the soil was determined by standard methods in the chemical laboratory of the Institute of Forage Crops in Krusevac (Tab. 1). Alfalfa cultivar K-28 selected by the Institute of forage crops, Krusevac, was used as the host plant.

Table 1. The chemical composition of the soil

Parameter	Total nitrogen, %	P_2O_5 mg/100g	K_2O mg/100g	Humus, %	pH/KCl	pH/H_2O
	0.204	10.20	51.0	4.30	4.77	5.83

Inoculant variations were the following:
1. *Sinorhizobium;* 2. *Azotobacter*; 3. *Actinomycetes* ; 4. *Sinorhizobium* + *Azotobacter;* 5. *Sinorhizobium* + *Actinomycetes*; 6. *Azotobacter* + *Actinomycetes*; 7. *Sinorhizobium* + *Azotobacter* + *Actinomycetes*; 8. Control - no inoculation.

Inoculation was performed with monovalent (contain only one type of micro organisms (1, 2 and 3)) and polyvalent inoculums that contain two or more species of micro organisms (4, 5, 6 and 7). The strains of *Sinorhizobim meliloti* (the number of bacteria was 10.8 $x10^{11}$ per ml), *Azotobacter chrococcum* ($28x10^{11}$ per ml) and *Actinomycetes* ($7.6x10^{10}$ per ml) were used. These micro organisms derived from the collections of the Department of Microbiology of the Faculty of Agriculture in Novi Sad. The *Sinorhizobium* cultures were grown on YM substrate by Vincent, *Azotobacter* were grown on the liquid substrate by Fjodorov, and the *Actinomycetes* were grown on the substrate by Krasiljnikov *(Jarak and Djurić, 2006).* Alfalfa seed was sterilized with 0.2% solution of $HgCl_2$ and 70% ethanol, rinsed several times with sterile tap water and then immersed in the corresponding inoculums. After that, seed from each inoculum variant were planted in the pots filled with soil. At the bottom of the pots there was gravel for draining. There were 30 plants set in six pots for each treatment. In each pot, 10 ml of the appropriate inoculum was added.

The analysis of plant and root was done in the flowering stage in three cuts. The following traits were studied: plant height (cm), the number of stem per plant, green mass per plant (g), mass of roots (g) and number of the nodules per plant. The statistical analysis was done by ANOVA and the differences between genotypes were tested by the LSD test.

Results and discussion

The application of the inoculation before the sowing had positive effect on all studied parameters. The best results in the inoculation with individual strains of micro organisms have been achieved in the treatments with *Sinorhizobium*. Also, *Sinorhizobium* was effective in the combination with other microorganisms. The maximum plant height was recorded in the treatments with *Sinorhizobium* as inoculum in all three cuts (Tab. 2).

The plants in treatments with inoculation were significantly higher than the control in almost all variants. In the research of *Jarak et al. (2007)* the greatest values for plant height and mass of alfalfa was achieved by using *Sinorhizobium* and polyvalent inoculums containing mixture of *Sinorhizobium*, *Azotobacter* and *Actinomycetes*. Application of inoculation also gave positive effects on the length of red clover plants (*Miličić and Jarak, 2008).* Considering the number of stems, the best results were determined in treatments inoculated with individual cultures of micro organisms or a combination of all three species of bacteria. The best effect for this trait was determined in the treatments with polyvalent inoculums in the third cut. The inoculation of seeds with monovalent and polyvalent inoculums (all three micro organism cultures) gave good results for the green mass per plant and weight of roots (Tab. 2 and 3).

Table 2. The influence of inoculation on the parameters of alfalfa canopy

Parameters		Plant height (cm)			Number of stems per plant			Green mass per plant (g)		
Variants		Cut			Cut			Cut		
		1	2	3	1	2	3	1	2	3
1		47.47	46.80	32.85	3.82	5.00	5.22	6.60	5.29	2.96
2		44.49	42.50	29.52	3.78	4.70	4.90	5.88	4.45	2.72
3		46.11	42.00	33.88	3.40	5.10	5.16	3.80	4.57	3.05
4		44.32	43.60	32.86	3.38	4.00	4.49	4.72	5.16	2.71
5		47.49	44.70	35.81	3.47	3.90	4.98	4.92	5.23	2.88
6		46.82	40.50	34.16	3.11	3.90	4.60	4.40	4.84	2.38
7		44.60	42.64	32.49	3.76	4.60	5.71	5.13	5.00.	3.4
8		40.98	37.60	30.32	2.86	4.06	4.01	4.07	4.23	2.46
LSD	0.05	2.290	2.836	2.164	0.209	0.145	0.191	0.205	0.154	0.164
	0.01	3.155	3.908	2.982	0.288	0.200	0.263	0.283	0.212	0.226

Table 3. The influence of inoculation on the root weight and on the number of nodules in alfalfa

Parameters		Root weight per plant (g)			Number of nodules per plant		
Variants		Cut			Cut		
		1	2	3	1	2	3
1		6.56	9.49	12.80	139.30	106,30	193.86
2		4.46	7.09	8.10	65.60	76,20	71.50
3		5.06	8.45	9.64	84.99	102.13	85.50
4		4.08	8.99	8.69	95,6	151.30	92.19
5		4.95	8.71	9.33	104.10	130.80	86.33
6		4.31	6.23	7.23	86.00	75.50	38.50
7		6.59	6.93	8.82	135.30	119.40	95.90
8		4.20	5.52	6.65	78.63	52.38	48.50
LSD	0.05	0.202	0.133	0.200	21.707	10.539	4.99
	0.01	0.281	0.184	0.276	29.907	14.521	6.88

In almost all cases, these values differ significantly from the control. *Stevović et al. (2007)* achieved significantly increased dry matter yield of alfalfa by inoculating the seeds with pH resistant *S. meliloti* strains in acid soils. Also, *Jarak et al. (2004)* achieved larger number of symbiotic bacteria and higher yields of green forage of alfalfa by applying the inoculation of seeds. Similar results were reported by *Mulić et al. (1983)* in the research of the effect *S. meliloti* as inoculant in the production of alfalfa in acid soil. Considering the number of nodules, *Sinorhizobium* proved more effective than the other two inoculants, either as single culture or in combination with *Azotobacter* and *Actinomycetes* (Tab. 3). The nodulation in alfalfa roots is an indication of the successful symbiosis between plants and *Sinorhizobium*. Using inoculation with *S. meliloti, Rice et al. (1995)*

have recorded the increased nodulation and larger weight of alfalfa plants. This is especially important because the application of microbial inoculants may reduce the use of expensive nitrogen fertilizers, speeds up the decomposition of pesticides and increases the biological activity of soil *(Djukić et al., 2006).*

Micro organisms used for the inoculation of alfalfa seeds can, also, be found in the soil, though their number may vary. However, the indigenous strains were not always effective, so the inoculation with selected micro organism strains should be used for the better results in the alfalfa production.

Conclusion

The obtained results showed that the application of appropriate micro organisms and their combination positively affected alfalfa yield components. In order to make the cultivation of alfalfa and other plant species more successful, the microbial inoculants should be applied, allowing better utilization of nitrogen fixation and plant nutrition in certain conditions. This way, with great energy and economic savings, the cultivation “per plant” area of this forage plant can be spread on the terrains with low soil production capacity.

Uticaj različitih inokulanata na razvoj lucerke gajene na kiselom zemljištu

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Rezime

Ispitivan je uticaj rizobiuma, aktinomiceta i azotobaktera (pojedinačno i u kombinacijama) na parametre nadzemnog dela lucerke: visinu, broj izdanaka i masu biljaka. Takođe, određena je masa korena i broj nodula po biljci. Ove osobine su praćene u tri otkosa. Analize su vršene u fazi početka cvetanja biljaka. Primenom rizobiuma kod skoro svih ispitivanih parametara zabeleženi su bolji rezulatati u odnosu na uticaj drugih inolulanata. Polivalentni inokulumi koji su sadržali rizobium delovali su efikasnije u odnosu na kombinaciju azotobaktera i aktinomiceta. Biljke u tretmanu bez inokulacije odlikovale su se nižim vrednostima za ispitivane parametre u odnosu na biljke lucerke u tretmanima u kojima je primenjena inokulacija.

Ključne reči: lucerka, inokulacija, *Sinorhizobium*, azotobakter, aktinomicete

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OCCURENCE OF ROUNDUP READY SOYBEAN IN SERBIAN FEED PRODUCTS

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Original scientific paper

Abstract: The detection of genetically modified (GMO) food and feed is becoming legal necessity. This study aimed at monitoring feed products derived from Roundup Ready soybean in the Serbian market. The GMO screening was based on the detection of 35S promoter and *nos* terminator sequences. The presence of genetically modified soybean was found in 36 out of the 78 analyzed samples (46%). The positive samples were subjected to event specific detection of Roundup Ready soybean with quantitative Real-time PCR. In 18% of samples, from different feed categories, the GMO content were over 0.9%. The results demonstrate for the first time the presence of GM soybean in Serbian feed products. The presence of GM material in these samples was not indicated on their labels. These results clearly demonstrate the need for a monitoring program of feed products by the Serbian regulatory authorities.

Keywords: feed, GMO labelling, monitoring, soybean

Introduction

The world's leading soybean producers are the United States (33%), Brazil (27%), Argentina (21%), and China (7%). Each year, the European Union imports approximately 20 million tones of soybean meal from countries where cultivation and commercialization of genetically modified (GMO) plants are allowed.

The genetically modified soybeans are now widely grown in the world. According to the Agbios database there are tree approved transgenic soybeans lines: A2704-12 (ACS-GMRR5-3), MON-R4R32-6 (GTS 40-3-2) and MON-89788-1 (MON89788) for the food and feed production in European Union. The Roundup Ready soybean (GTS 40-3-2), modified to express the protein CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), which confers resistance to the herbicide glyphosate, the active ingredient in Roundup Ready *(Padgette et al., 1995)* is only approved line for the food and feed products in Serbia *(Official magazine SCG 2/2003)*. According to the EU legislations (*EC* 1829*/2003* and *EC*

1830/2003), a threshold labelling at 0.9% for the adventitious presence of approved GM material is demanded. The same requirements regarding the labelling are applied for both food and animal feed in Serbia.

The most frequently used approach to detect the presence of GMO material in different food an feed matrices is the PCR based screening for the CaMV 35S promoter and the nos terminator (T-nos) DNA sequences from Agrobacterium tumefaciens *(Reiting et al., 2007)*. PCR-based methods, both qualitative and quantitative, which can also be used for the more highly processed soybean based food products *(Lipp et al., 2001)* and feedstuffs *(Novelli et al., 2003; Kwiaten et al., 2007)*. Due to relatively high protein content of 44% to 50%, consistent availability and competitive price soybean meal dominates as a protein source in the EU feed sector. It is accounting for 53% of the total protein supplement used, with only 3% of the soybean meal derived from EU supply sources *(Brookes, 2000)*. The animal feed industry uses 77% of the soybean meal produced primarily as an amino acid and protein source in diets *(Kerley and Gary, 2003)*.

The Roundup Ready soybean (RR) was already found at the Serbian fields, as well as in food samples *(Nikolić et al., 2009 a, b)*, but there were no information's about feed samples and it is not know how feed manufactures companies have implemented GMO rules on labelling. The objectives of the study were to obtain information's about use of Roundup Ready soybean in feed products, distribution of GM soybean, and implementation of GMO labelling requirements.

Materials and Methods

The study was based on 78 unlabeled feed samples provided by the Inspection of Ministry of Agriculture, Forestry and Water Management of Serbia. The samples contained soybeans were collected from approved and registered feed manufacturers from entire territory of Serbia. The samples submitted for analysis were grouped into 4 different categories (Table 1). The Certified Reference Material were used as dried soybean powder (GTS 40-3-2) with 2%, 1%, 0.5%, 0.1% and 0% GM soybean, developed by the Institute for Reference Materials and Measurements (IRMM, Belgium).

DNA Extraction and Purification. A CTAB method *(Querci et al., 2004)* was followed for the extraction and purification of DNA from all samples. Physical quality of the extracted DNA was checked by electrophoresis in a 1% agarose gel, and purity was confirmed by OD 260/280 nm comparison. DNA concentrations in extracted samples were also confirmed by UV spectroscopy at 260 nm.

PCR method. The species specific PCR assay was accomplished on soybean using target sequence for lectin gene to confirm the quality and amplificability of the extracted DNA from the samples. For qualitative PCR amplification 25 µl of PCR mixture containing 2.5 µl of reaction buffer (Fermentas, Lithuania); MgCl 1.5 mM, 0.2 mM dNTP; 0.7 µM primers for 35S and 0.8 µM primers for T nos; 1 unit Taq native polymerase (Fermentas) and approx. 100 ng DNA were used._Amplifications were carried out in a Mastercycler ep gradient S termocycler (Eppendorf, Germany) under the following programs: denaturation at 95 °C for 2 min followed by 40 cycles of 95 °C for 25 sec, 56°C for 30 s, and 68 °C for 45 sec final extension at 68 °C for 10 min. The sequences of oligonucleotide primers used in this study for 35S promoter are reported by *Lipp et al. (1999)* for nos terminator by *Lipp et al. (2001)* and for lectin gene by *Tengel et al. (2001)*.Quantification of the Roundup Ready soybean in positive samples was done by Real-Time PCR according to the procedure published in *EN ISO 21570*.

Amplified products detection PCR products were determined using electrophoresis on 2% agarose gel containing ethidium bromide (0.5 g/mL). A Fast Ruler DNA Ladder Low Range (Fermentas) was used as marker. The visualization was performed in UV transilluminator and the images were captured with DOC PRINT system (Vilber Lourman, USA).

Results and Discussion

The presence of soybean DNA in feed samples and its amplifiability was checked using soybean specific primers for lectin gene through visualization of an amplicon of 318 bp. The lectin gene was detected in all samples (Figure 1).

(bp)
1500
200
50

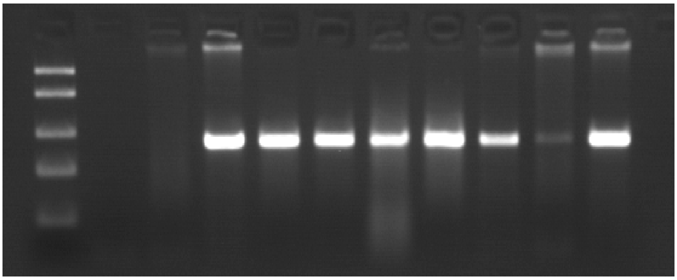

Figure 1. Lectin PCR of feed samples. Line 1: DNA ladder; Lane 2: blank (no DNA) control, Line 3: negative control, Line 4: soybean, Line 5– 11 feed samples

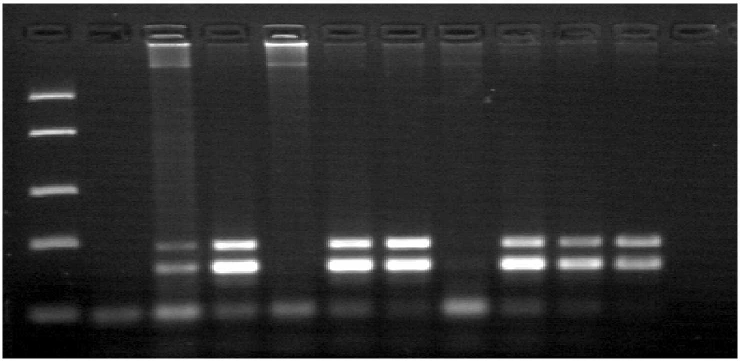

Figure 2. Electrophoresis profile of duplex PCR products of 35S promoter and nos terminator genes. Line 1: DNA ladder; Lane 2: negative (no DNA) control, Line 3: 0.1% soybean; Line 4: 1% GMO soybean, Line 5 – 11 feed samples (5, 8 GMO negative; 6, 7, 9 -11 GMO positive)

It was shown that the quality of isolated DNA using CTAB method was sufficient to make PCR analysis possible, what was in agreement with *Garay et al. (2009)* and *Jasbeer et al. (2009)*. The CTAB extraction methods which have been traditionally used for food samples may be successfully employed for feeds samples as well. The presence of GMO content have been demonstrated in 36 samples out of 78 analyzed samples. The fragments of 195 bp and 118 bp for P35S and T-nos were detected in GM positive controls and in GMO positive samples Figure 2 (lines 3, 4, 6, 7, 9-11). The results of the qualitative and quantitative PCR analysis were summarized in Table 1 and Table 2.

Table 1. Results of GMO analysis of feed samples by qualitative PCR

Samples	Number of samples	Number of positive samples for 35S and T nos	Number of positive samples in %
feed for poultry	28	14	50
feed for pigs and piglets	28	12	43
feed for calves and cattle	9	5	55
soybean meal	13	5	38
total	78	36	46

Table 2. The GMO content in positive feed samples estimated by Real Time PCR

Samples	Number of samples			
	<0.1%	0.1-0.9%	0.9-2%	>2%
feed for poultry	-	8	-	6
feed for pigs and piglets		7	1	4
feed for calves and cattle		4	-	1
soybean meal	1	2	-	2
total	1	21	1	13

The all analyzed samples in this work were unlabelled and originated from Serbian feed producers. The presented data demonstrate that the GMO soybean was used for feed and it was detected in 46% of samples. The similar study conducted in the tree year's period in Poland showed that the GM soybean is commonly used in animal feeding (61%) *(Kwiaten et al., 2007)*. The frequent presence of GM soybean, which is not declared on the label, was revealed by *Costa and Martinelli (2007)*. Foods and feeds provide some of the most complex matrices for isolation and detection of protein or DNA analyses. The combination of multiple plant substrates and addition of other ingredients such as salts, along with heat processing, may yield unexpected matrix effects.

Novelli et al. (2003) analyzed 88 samples of mixed diets for cattle developed and provided by several agro-industrial companies by screening for the CaMV 35S promoter. In several cases, one method of DNA isolation resulted in an inability to detect the targeted region within the promoter, whereas isolation of DNA by a different method returned positive results, illustrating the requirement to remove PCR inhibitors to ensure accuracy of the assay. In our work the applied method for extraction of DNA (CTAB) was efficacious, and in almost all samples the quality and purity of DNA was satisfy.

The type of DNA extraction significantly affected the standard curves of the PCR assay was reported by *Peano et al. (2004)*. Therefore, the extraction method should be uniform for the standards and any test samples. In the 14 out of 36 positive samples (18%) the GMO content were over 0.9% and according to low should be labelled. The percentage of positive samples in different categories of feed samples varies from 38% in soybean meal samples to 55% in feed for calves and cattle. Similar results reported by *Gryson et al. (2007)* that 7 out of 32 GM feed products analysed in Belgium had GMO contents more than 0.9%.

The comparative feeding studies with broilers and layers conducted by *Chesson and Flachowsky (2003)* in which conventional maize or soybeans were replaced in feeds by transgenic varieties have failed to show differences of any significance in production parameters. No fragments of transgenic DNA or its expressed protein have been found to date in poultry meat or eggs or in any other animal tissues examined. Similar, study of *Cromwell et al. (2002)* indicate that transgenic genetically modified soybean meal is equivalent in composition and nutritional value and will result in similar growth performance to conventional grains for growing-finishing swine.

Labelling of feeds containing GM ingredients informs farmers and gives them the choice of using such feed for their livestock. However, products such as milk, meat, and eggs, which are derived from livestock fed transgenic feeds, are exempt from EU-labelling laws. Currently, all members of EU and Switzerland have labelling regulations pertaining to GM feed *(ISAAA, 2005)*.

Conclusion

The Roundup Ready soybean was used for feed and it was detected in 46% of feed samples. The all analysed samples in this work originated from Serbian feed producers and presence of GM soybean was not declared on the label. In 18% of samples the GMO content were over 0.9%. On the basis of the results, it would be advisable to control all ingredients and feed products by the authorities responsible for the feed chain.

Utvrđivanje prisustva Roundup Ready soje u hranivima u Srbiji

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Rezime

U radu su dati rezultati proučavanja prisustva genetički modifikovane soje tolerantne na Roundup Ready herbicid u hranivima proizvedenim u Srbiji. Genetički modifikovana soja je nađena u 36 od 78 analiziranih uzoraka. U 18% uzoraka sadržaj Roundup Ready soje je bio preko 0,9%. Prema važećim zakonskim propisima u Srbiji svaki proizvod koji sadrži preko 0,9% GMO mora biti obeležen. Prisustvo GMO soje nije bilo naznačeno na deklaraciji. Dobijeni rezultati ukazuju na potrebu uvođenja programa monitoringa hraniva i komponenti koje ulaze u sastav hraniva na prisustvo genetičke modifikacije.

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WORLD PRODUCTION OF PROTEIN AND MAIN SOURCES OF ITS PRODUCTION

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Abstract: This paper deals with the problems of establishing the vegetable protein world resources, its production sources, structure, increasing rate comparing to the population growth, protein consumption level and prospects of improvement of the population nutrition quality.

Key words: protein, production, crops, vegetable, animal, crops, sources

Food and protein problems on the global and national levels still remain the most relevant. There is no country in the world that has completely solved the food protein problem. The actual annual consumption of protein by one person constituted: in the world - 27.5 kg per year (75.3% of normal), in North America - 36.9 kg (101.1%), Europe - 36.6 kg (100, 3%), Oceania and Australia - 34.8 kg (95.3%), South America - 28.1 kg (77%), Asia - 25.5 kg (69.9%), Africa - 22.3 kg (61.1%). Protein deficiency in population nutrition in the world reached about 25%, it is formed because of the consumption of medium-and low-protein sources, and it can be eliminated using high-protein sources. Unfortunately, even on those continents and in economically developed countries where there is quite a high level of protein consumption; a significant number of people are undernourished or has insufficient level of protein consumption.

To determine the actual state of the food protein production we performed the calculations of its supply volume to the world food resources. In the calculations of world protein production we included 162 protein sources, including 123 vegetable sources, 39 animal protein sources. The main source of information in the calculation is statistics data on global production of food products and protein content in these products. Protein food resources formation research is performed on the long-term study (48 years). Food and protein production are closely related, they are important components of the global food problem solving paradigm. World protein resources suitable for food use by production volume are on the first place in agricultural production. They are formed from sources of vegetable origin (cereals, legumes, protein and oil, nut, fruit, citrus plants, vegetables, storage roots and tubers, etc.), animal origin (products of domestic and wild animals, such as meat, milk), poultry and wild birds (meat, eggs), as well as fish, seafood and aquaculture.

World food and protein resources are formed mainly due to agricultural production. We performed calculations of the main protein food resources production by using UN FAO materials [8]. It was determined that for the analysis period (1961-2008) protein production in the world increased by 3.4 times, the population increased by 2.2 times, or the protein production surpassed the population growth (Table 1).

Table 1. Structure and dynamics of global production of proteins suitable for food use, thousand tons

Protein source	1961	1970	1980	1990	2000	2008	2008 from 1961,in %
Total protein production	164275.8	221680.8	290121.7	376217.8	437491.6	543896.6	331.1
Vegetable proteins	133561.3	181016.4	238955.5	311312.1	358599.6	450660.5	337.4
Grain crops	90410.4	124082.1	164136.1	209362.4	219272.6	268421.0	296.9
Grain legumes	23091.0	31808.4	46098.0	62987.2	86785.9	115476.4	500.0
Protein-oil	8014.7	10672.6	13356.7	19910.0	25468.9	35046.9	437.3
Storage roots and tubers	7649.6	9211.6	8505.8	9539.9	12161.3	12774.3	167.0
Vegetables, melonfield crops, mushrooms	2651.9	2900.0	3975.4	5972.6	10010.1	12647.8	476.9
Fruits, berries, citrus plants, nut plants	1736.1	2330.5	2862.7	3511.1	4835.9	6203.1	357.3
Seaweed	7.6	11.2	20.8	28.9	64.9	91.0	1197.4
Animal proteins	30714.5	40664.4	51166.2	64905.7	78892.0	93236.1	303.6
Meat	12409.7	17494.9	23645.6	31111.5	40528.2	48418.1	390.2
Milk	11837.1	13451.1	16073.7	18472.9	20129.5	24261.6	205.0
Eggs	1792.5	2436.3	3324.7	4627.4	6989.7	8329.4	464.7
Fish and seafood	4675.2	7282.1	8122.2	10693.9	11244.6	12227.0	261.5
Population, mln. people	3082	3709	4453	5283	6082	6705	217.6

World proteins production suitable for food use has a strong tendency to increase: in 1961 – 164.3 mln. tons, in 1970 – 221.7; 1980 – 290.1; 1990 – 376.2, 2000 – 437.5; 2008 – 543.9 mln. tons. During 1961–1970 the production of protein increased by 34.9%, 1970–1980 – 30.8%, 1980–1990 – 29,7%; 1990–2000 – 16.3%; 2000–2008 – 24.3%.

In the structure of world proteins production suitable for food use the part of vegetable proteins makes up 82.9% and animal proteins - 17.1%. Consequently, global protein resources are mainly replenished by vegetable proteins. Among the vegetable protein resources the largest share belongs to grain crops – 59.6%, grain legumes – 25.6, smaller share belongs to protein-oil plants – 7.8%, vegetables, melonfeild plants, mushrooms – 2.8%, storage roots and tubers – 2.8 %, fruits, berries, citrus, nut plants - 1.4%. Calculations showed that 93.8% of vegetable proteins comes from 25 major crop species, including cereals – 9 species, legumes – 8 species, protein-oil – 3 species, roots, tubers and other – 5 species. Thus, among the crops-suppliers of protein the leading role belongs to cereals, legumes and protein-oil crops. The contribution of 4 major crops (wheat, soybeans, corn, rice) to the global resources of vegetable protein is 70.1%, 5 crop – 74.4%, 10 crops – 84.9%, 20 crops – 92%, 25 crops – 93.8%, other crops – 6.2% (Table 2).

Table 2. The volume and structure of global protein production of 25 major crops, thousand tons

Plant	1961	1970	1980	1990	2000	2008	2008 from 1961, in %
Total vegetable proteins	133561.3	181016.4	238955.5	311312.1	358599.6	450660.5	337.4
Wheat(*Triticum vulgare*)	28906.4	40707.2	58106.9	78777.1	78531.8	93142.7	322.2
Soybean(*Glycine max*)	10000.4	16342.6	30471.2	40996.5	61335.8	87992.9	879.9
Corn(*Zea mays*)	20295.5	26317.3	39662.3	48817.9	60436.9	81448.5	401.3
Rice(*Orysa sativa*)	15742.2	23409.6	29765.3	39410.2	46114.8	53431.0	339.4
Barley(*Hordeum sativum*)	8327.3	13847.9	18490.9	21154.5	16108.8	19390.3	160.8
Rape(*Brassica napus*)	898.9	1675.5	2689.1	6107.1	9879.1	14464.0	1609.1
Peanut(*Arachis hipogeae*)	3706.2	4745.1	4493.2	6215.2	9415.7	10314.3	278.3
Sorghum(*Sorguhum vulgare*)	4543.4	6302.4	6582.4	6703.5	6700.1	8126.3	178.9
Cotton plant(*Gossypium hirsutum*)	3133.7	4044.8	4676.3	6087.5	5985.3	7621.6	243.2
Sunflower (*Heliantus annus*)	1261.2	1868.5	2540.1	4246.3	4974.4	6736.5	534.1
Potato (*Solanum tuberosum*)	5140.5	5662.9	4568.8	5065.9	6572.9	6596.9	128.3
Beans(*Phaseolus vulgaris*)	2582.5	3081.7	3998.9	4746.8	5030.5	5934.9	229.8
Millet (*Panicum miliaceum*)	2878.8	3793.3	2907.9	3566.3	3352.1	4420.7	153.6
Oats(*Avena sativa*)	4710.9	5293.5	4433.4	4511.6	3107.5	3119.9	66.2
Kasava(*Manihot esculenta*)	855.1	1183.1	1489.6	1982.2	2320.1	3028.4	354.2
Chick-pea (*Cicer arietinum*)	1544.1	1582.4	1228.1	1838.4	2293.5	2633.8	170.6
Pea(*Pisum sativum*)	1461.9	1869.7	1979.6	3608.6	2389.9	2358.7	161.3
Rye(*Secale cereale*)	3862.1	3155.3	2969.2	4621.5	2493.9	2218.8	57.5
Sweet potato(*Ipomoea batatas*)	1472.9	2047.6	2205.9	2086.3	2506.5	2092.4	142.1
Triticale (*Triticale*)	0	0	20.7	556.6	1156.7	1822.7	8805.3
Cow pea (*Vigna unduiculata*)	198.4	322.7	284.4	524.8	814.8	1406.6	709.0
Tomatoes(*Lycohersicon esculentum*)	193.3	251.2	421.2	686.7	975.1	1296.5	670.7
Horse beans(*Vicia vaba*)	1181.6	1164.3	1222.4	1251.0	1121.9	1159.5	98.1
Cabbage(*Brassica oleraceae*)	280.8	334.1	465.6	550.8	871.0	1114.6	396.9
Lentil (*Lens culinaris*)	212.0	258.7	328.9	700.0	946.8	1024.0	483.0
25 crops,Altogether	123390.1	169261.4	226002.3	294813.3	333475.9	422896.5	342.7
Other crops	10171.2	11755.0	12953.2	16498.8	25163.7	27764.0	273.0

The largest source of vegetable proteins in the world food protein resources consists of grain crops. This can be explained by the unique combination of proteins, carbohydrates, vitamins and other substances in food grain, by their extensive use in the bread, pasta and other products production. Calculations showed that in this group of crops the main supplier of vegetable proteins was wheat – 93.1 million tons, corn – 81.4 million tons, rice – 53.4

million tons, barley – 19.4 million tons, sorghum – 8.1 million tons, millet – 4.4 million tons, oats – 3.1 million tons, rye – 2.2 million tons, triticale – 1.8 million tons and other crops. For 48 years average annual increase of protein for these crops was 9.3%, but the part of cereals in the plant protein global resources decreased from 66.8% to 59.3%.

Production of proteins from grain legumes, including vegetable legumes, in the world for 1961-2008 increased from 23091.0 to 115476.4 thousand tons or by 5 times, including for grain legumes – from 22602.8 thousand tons to 114,076.1 thousand tons, or 5.1 times, grain vegetable legume – from 488.2 thousand tons to 1400.3 thousand tons, or 2.9 times. In the grain legumes group the soybean protein accounted for 76.9%, peanuts – 8.9%, kidney beans – 5.65, chickpea – 2.8%, pea – 2.1%, cow pea – 1.2%, horse beans – 1.0%, lentils – 0.9%, pigeon pea – 0.8%, other crops – 0.4%. The average annual increase of grain legumes protein reached 8.5% and was significantly greater than the increase in population. During this time their protein part in the world's resources has increased from 14.1 to 21.2%.

During the analyzed period protein production from protein-oil crops increased from 8014.7 thousand tons to 35046.9 thousand tons, or 4.4 times. In this group of protein crops the part of rapeseed constituted 41.3%, cottonseed – 21.7, sunflower – 19.2, olives – 9, tung nuts – 2.5, sesame – 1.9%, linseed – 1.1, other plants – 3.1%. The percentage of protein from this crops group in the world's protein resources has increased from 4.5 to 6.4%. Average annual growth of production of such protein was 9.3% and was higher than the population growth.

World production of nut-fruited crops protein increased from 392 thousand tons to 1881.3 thousand tons, or 4.8 times, including acajou (cashew) – from 49.5 thousand tons to 639.9 thousand tons, or 12, 9 times, almonds – from 140.9 to 411.6 thousand tons, or 2.9 times, walnut – from 101.9 thousand tons to 353.5 thousand tons, or 3.5 times, hazel – from 30.3 thousand tons to 174.6 thousand tons, or 5.8 times, pistachios – from 4.1 thousand tons to 113.4 thousand tons, or 27.7 times, sweet chestnut – from 20.8 thousand tons to 44.1 thousand tons, or 2.1 times, Brazilian walnut – from 8.5 to 11.2 thousand tons, or by 31.8%, other nuts – from 55.4 thousand tons to 666.6 thousand tons, or 12 times. The average annual increase of nut-fruited crops protein reached 8.1%.

Production of protein, received from the storage roots and tubers increased from 7649.6 thousand tons to 12,774.3 thousand tons, or 1.7 times. In this group of crops potato protein part constituted 51.6%, kasava – 23.7, sweet potato - 16.4, yam – 5.7, taro – 1.8%, other crops – 0.8%. The average annual increase of their protein reached 1.4%, which is less than the increase in population. The percentage of proteins from storage roots and tubers in the global resources of protein increased from 1.7% to 2.3%.

Production of protein from vegetables, melons (contain not a lot of protein, but a lot of vitamins) and fungi increased from 2651.9 thousand tons to 12,674.8 thousand tons, or 4.8 times, including cabbage - from 280.8 thousand tons to 1114.6 thousand tons, or 4 times, tomatoes – from 193.3 thousand tons to 1296.5 thousand tons, or 6.7 times, cauliflower – from 71.2 thousand tons to 486.7 thousand tons, or 6.8 times, cucumber – from 66.8 thousand tons to 354.6 thousand tons, or 5.3 times, eggplants – from 77.3 thousand tons to 425.1 thousand tons, or 5.5 times, carrot – from 64.3 thousand tons to 328.6 thousand tons, or 5.1 times, etc. The average annual growth of protein from this group of crops reached 8.0%. The percentage of protein from vegetables, melons and mushrooms in the global protein resources increased from 1.6 to 2.3%.

During the analyzed period (1961-2008) protein production from harvests of fruits, berries, citrus and nut-fruited crops increased from 1736.1 to 6203.1 thousand tons, or 3.6 times. The part of bananas reached 1088.5 thousand tons or 17.5%, oranges, tangerines, lemons, grapefruits - 878.2 thousand tons, or 14.2%; apples, cherries, plums, pears, apricots, peaches – 599, 4 thousand tons, or 9.7%; mangos, avocados, pineapples, papayas – 369.1 thousand tons, or 6%, strawberries, raspberries, currants, gooseberries, cranberries, blueberries-63.1 thousand tons or 1%, dates – 133.9 thousand tons, or 2.2%. The part of protein from this group in the world protein resources remains almost the same. The average annual increase of protein from this food group reached 5.5%.

On three continents the protein consumption norm has been already achieved, or it is close to it. The population of most countries (2/3 from the total number) consumed less protein than the scientifically-based norm, 1/3 of countries consumed the amount of proteins, which is within the norms limits. We found out that by increasing daily protein consumption to the scientifically-based norm and by optimization of the protein structure we can increase the population life span. Thus, in the world when consuming proteins in the amount of 75 g per day (plant protein – 61% and animal protein – 39%), the average life span reaches 65 years, including the developed countries - 101 g per day (44% and 56% respectively) – 73 years, industrial developed counties – 106 g per day (40% and 60% respectively) – 77 years, European Union countries – 109 g per day (40 and 60% respectively) – 78 years, developing countries – 68 g per day (69% and 31%) – 62 years, badly developed countries – 56 g per day (80% and 20% respectively) – 53 years (Table 3).

Table 3. Influence of the daily protein consumption level per person and the protein structure on the average life span in the countries groups with different levels of economic development

Countries	Protein consumption, g per day			Part of total protein consumption, %		Average life span, years
	total	including vegetable proteins	including animal proteins	vegetable proteins	animal proteins	
1. In the world, total amount	75	46	29	61	39	65
2. Developed countries	101	44	57	44	56	73
Industrially developed countries	106	42	64	40	60	77
European Union	109	44	65	40	60	78
3. Developing countries	68	47	21	69	31	62
Badly developed countries	56	45	11	80	20	53

In most countries the optimal level of protein consumption per person has been already achieved, but in many countries there is evident animal protein deficit. According to forecasts, the world population by the year 2050 will reach 9.2 billions of people, or will increase 1.4 times, that, according to our calculations, will require a significant increase of food resources production (Table 4).

Table 4. Forecast of the population growth and world food resources production

Indicators	2008 p.	2050 p.	2050 from 2008, %
Population, mln. people	6705	9224	137,6
Food production, mln. tons	6378	12732	199,6
crops cultivation	5226	9952	189,5
stockbreeding	1112	2780	250,0

To ensure full-value nutrition for so many people it is necessary to increase food production 2 times, including crops production 1.9 times, and stockbreeding 2.5 times.

Conclusions

In the world there is a steady increase in production of proteins, suitable for food use. Vegetable protein production increase outpaces the population growth. There is a real opportunity to increase food production to the total volume of the population needs. The basis of food security, particularly of its critical component - protein, is vegetable protein food resources, which are formed in the agricultural sector. Increase in high protein resources is a strategic direction, conceptual way, and an important factor in the protein deficiency elimination in the population nutrition worldwide.

Produkcija proteina u svetu i glavni izvori dobijanja proteina

Anatoly Babich, Alina Babich-Poberezhna

Rezime

Ovaj rad se odnosi na probleme formiranja svetskih rezervi biljnih proteina, izvora dobijanja, strukture, povećane potrošnje u odnosu na rast populacije, nivoa potrošnje proteina i perspektiva za poboljšanje kvaliteta ishrane populacije.

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GRAIN YIELD AND QUALITY OF TRITICALE AND BARLEY OBTAINED IN COMPARATIVE PRODUCTION

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Abstract: Grain yield and quality of small grains differ among species, and are affected by many factors, primarily by genotype, agro-ecological conditions and the applied production technology. The study was carried out in the area of Bijelo Polje (Montenegro), with the aim to establish differences in grain yield and protein content between triticale and barley crops, grown under the same agro-ecological conditions. The study lasted two years (2005-2007), on a weakly calcified soil, where both plant species were represented by the following two different cultivars of each: winter triticale cultivars Kg-20 and Favorit, and winter barley cultivars Grand and Rekord. The results of the study show that the winter triticale cultivar Kg-20 had the highest grain yield and the best grain quality. These two plant species are mostly used as livestock feed and these results point to a proper choice of plant cultivars for growing in certain areas, especially in family farms whose main activity is animal husbandry. Value of the investigated material, especially of triticale, could be in its growing as a forage crop, as well as in breeding programs aimed to increase its grain yield and quality.

Key words: winter barley, winter triticale, grain yield, protein content.

Introduction

Among other small grains and forage crops, barley takes a very important place because its kernel is an important raw material in beer production, and it also represents a quality component in the nutrition of domestic animals. The cultivars of distich barley with large and unified grains, and with grain albumin content of 12% are important for the beer industry. Barley grain is richer with important amino acids (lysine), and it also has higher albumin content when compared with maize *(Stojanović et al. 1997)*. In Montenegro barley is grown on 6000-7000 hectares with the average yield of 2000 kg ha^{-1}. Winter cultivars of barley are grown in flat areas, while spring cultivars are grown mostly in mountainous areas.

Triticale is a new species of small grains, which was made by the intergeneric hybridization of wheat and rye. In triticale numerous positive characteristics of its parents are merged, and therefore it has a versatile potential for use *(Zečević et al. 2005)*. According to many researchers' opinion triticale is a prosperous new plant species and all of them emphasize its high genetic potential for grain yield and its nutritive value *(Knežević et al., 2007; Zečević et al., 2009)*. As published earlier, triticale shows relatively good resistance to powder mildew and stem rust *(Milovanović et. al. 1995)*. Triticale species is characterized by a high potential to suppress weed growth in crop *(Kiec, 2003; Knežević et al., 2008)*. Good production technology of cereal crops and optimal nitrogen plant nutrition are positively correlated with increasing biomass formation, grain yield and quality *(Knežević et al., 2007; Zečević et al., 2009)*.

Triticale also has the ability to form photosynthetic surface very quickly, it has very modest requirements about the soil, and it has a satisfactory yield on higher altitudes. Thanks to the facts mentioned above, the areas sown by triticale are in constant increase, so it is grown on over 40000 hectares in our country *(Milovanović and Perišić, 2002)*. The aim of the investigation was to establish differences in grain yield and protein content between triticale and barley crops, grown under the same agro-ecological conditions and with the same scientific farming measures applied.

Material and Methods

The study was carried out in the area of Bijelo Polje (Montenegro) in the vegetation seasons 2005/06 and 2006/07. The trials were set on the alluvium soil type, in random complete block design with four repetitions, and included two triticale cultivars (Kg-20 and Favorit) and two barley cultivars (Grand and Rekord) created in the Centre for Small Grains in Kragujevac. There also was the control variant included, and one type of fertilizing with nitrogen (80 kg ha^{-1}). Elementary plots, which were 10 m^2 each, were sown manually with 450 seeds per m^2 in barley, and 550 seeds per m^2 in triticale. Ploughing and pre-seeding cultivation of the soil were done in a classic way. Before ploughing, mineral fertilizer (NPK 15:15:15) was applied in the dose of 400 kg ha^{-1}. Additional fertilizing was done with nitrogen fertilizer (KAN - 27%) in tillering stage. Harvest was done manually in the stage of full maturity. During the harvest hectolitre mass was measured, as well as 1000 grain mass, crude protein content, and grain yield with 14% of moisture. All observed parameters were determined by standard methods, and grain yield of elementary plots recalculated to kg per hectare.

Meteorological conditions of the studied period. Meteorological conditions of the investigated period are given in table 1 and 2. Weather conditions of the environment have a great influence on growth and development rhythm, especially temperatures and rainfall. Having in mind a higher rainfall amount during autumn, winter and spring months, and not so high temperature in the period of grain filling, one can say that 2005/06 was more suitable for germinating, shooting and winter surviving, as well as for grain yield forming, in regard to the season 2006/07.

Table 1. The average monthly air temperatures during the investigated period (T^0C)

Year	Months									
	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July
2005/06	9.7	3.3	1.2	-2.6	0.4	4.2	11.1	14.6	16.8	19
2006/07	11.4	2.6	0.3	1.8	4.8	7.0	11.5	16.0	19.8	21.9

Table 2. The average rainfall during the investigated period (mm)

Year	Months									
	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July
2005/06	85.2	116.1	182.5	36.6	87.7	180.8	58.4	78.6	119	52.9
2006/07	39.5	74.6	182.5	92.9	45.8	104.7	15.7	60.8	76.9	23.9

Results and Discussion

Grain yield and quality of barley and triticale are basically the most important indicators of their production success. Grain yield, which is always the main production goal, was pretty satisfactory. The two-year average grain yield of triticale was 5725 kg ha^{-1}, while barley grain yield was 4396.87 kg ha^{-1}, which makes the difference of 1328.13 kg ha^{-1} in favour of triticale (Table 3).

Table 3. The yield and quality of the kernel of winter sorts of barley and triticale

Plant species	Year	Cultivar	1000 grain mass (g)	Hectoliter mass (kg)	Protein content (%)	Grain yield (kg/ha)
Barley	2006	Grand	43.00	63.40	12.75	4937.50
		Rekord	45.10	63.30	12.45	4060.00
		average	44.05	63.35	12.60	4498.75
	2007	Grand	44.00	62.50	12.88	4640.00
		Rekord	42.00	62.30	12.62	3950.00
		average	43.00	62.40	12.75	4295.00
Lsd 0.05					0.480	172.97
0.01					0.620	231.04
Triticale	2006	KG-20	36.20	69.40	13.50	6210.50
		Favorit	34.80	68.70	13.40	5687.50
		average	35.50	69.05	13.45	5949.00
	2007	KG-20	36.70	66.80	14.22	5779.50
		Favorit	33.00	65.90	13.89	5222.50
		average	34.85	66.35	14.06	5501.00
Lsd 0.05					0.560	181.04
0.01					0.750	241.80

The cultivars Grand and Kg-20 had significantly higher grain yield in comparison with the cultivars Rekord and Favorit. Among the studied triticale and barley cultivars, the winter triticale cultivar Kg-20 had the highest two-year average value of grain yield (5995 kg ha^{-1}), which was significantly higher in comparison with other studied. This cultivar, also, had the highest average protein content (13.86%) which could have an important role in spreading of its production (Table 3). Grain yield and protein content can be increased by increasing NP fertilizer rates *(Aguirre et al. 2006; Pawlonka and Skrzyczyńska, 2004)*. These results are in accordance with the results of *Milovanović (1993)* and *Jestrović et al. (1989)*, who estimated hilly areas as suitable for reaching high grain yield of triticale.

Considering agro-climatic characteristics of the area, which caused later sowing and longer vegetation, as well as the average grain yield of barley and triticale obtained in recent years, these results were satisfactory for this area. In order to reduce yield losses caused by late sowing, low temperature during winter and early spring time, very important is application of optimal scientific farming measures, for example: soil cultivation, sowing term and seeding rate which is very important for plant tillering as an efficient factor of weed suppression *(Walter et al., 2002)*. Our results are similar or a bit lower than results of previous investigations *(Malešević and Pržulj, 1999; Talbot, 1993)*.

The results of our research are in accordance with a large number of data from the literature which emphasize that the main characteristic of triticale is the large kernel. In the average, the hectolitre mass of the sort Kg-20 was significantly bigger in comparison with the other tested cultivar of triticale, and in comparison with the hectolitre mass achieved with the cultivars of winter barley included in this research in both tested years. The data from the table 3 show that the year itself had an important influence on values of the tested characteristics, because both studied species with their genotypes had significantly greater value of hectolitre mass in the first year in comparison with the second year of the study. The dependence of hectolitre mass on the weather conditions has been emphasized in an earlier investigation *(Jelić et al. 1998)*. In the first year, all the genotypes included in the study achieved 1000 grain mass which was high-significantly greater in comparison with the one in the second year of investigation, and that was influenced by suitable climatic conditions which contributed to a better grain filling in the first studied year. The obtained results are in accordance with the ones shown by *Milovanović (1993)*.

Crude proteins content of the studied genotypes varied depending on the environmental conditions, above all on rainfall amount. The all investigated genotypes had higher protein content in the second year than in the first year of the study. Weather conditions in the first year contributed to achieving higher grain yield and therefore it was expected that protein content of grains would be lower in that year. This is in accordance with the general genetic rule that protein content of grains and grain yield are negatively correlated. The average protein content of barley was 12.67% and the one of triticale 13.75%, which put this plant species in the first plan regarding nutrition of domestic animals.

Advantages of triticale crops above barley are expressed in its modest requirements for scientific farming measures, outstanding tolerance to stressful environmental conditions, pests and diseases *(Milovanović et al., 1995)*. Relatively better conditions for plant production in 2005/06 influenced higher grain yield of the investigated genotypes than the average one. Having in mind that genetic potential for grain yield of both studied species is higher, one can conclude that a special attention should be paid to appropriate production technology and choice of genotypes, which could contribute to further increase of grain yield.

Conclusion

Under the same agro-ecological conditions and with the same scientific farming measures, grain yield of winter triticale was higher in highly significant manner comparing with winter barley. Proteins content of grains depended on annual agro-ecological conditions and in both plant species it was higher in the less suitable year for production, i.e. when grain yield was lower. Proteins content and hectolitre mass were higher in triticale in comparison to barley. The cultivars with the highest grain quality and yield were triticale Kg-20, and barley Grand cultivar.

According to all studied parameters of grain quality and yield, triticale showed significantly higher values in comparison to barley. Since both plant species are used in animal feeding, the advantage should be given to triticale, especially when it comes to more modest scientific farming and worse soil quality, but this certainly does not exclude necessity of growing barley for these purposes.

The results could be very useful for practical agricultural production, as well as for popularization of triticale as a new plant species.

Prinos i kvalitet zrna ozime tritikale i ječma ostvaren u uporednoj proizvodnji

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Rezime

Prinos i kvalitet koje strna žita postižu su različiti i uslovljeni većim brojem faktora, prvenstveno genotipom, agro-ekološkim uslovima i primenjenom tehnologijom proizvodnje. Istraživanja koja su izvedena u okolini Bijelog Polja (Crna Gora), imala su za cilj utvrđivanje razlika u prinosu i sadržaju proteina kod tritikale i ječma koji su gajeni u istim agro-ekološkim uslovima. Ispitivanja su trajala dve godine (2005-2007.), na slabo karbonatnom zemljištu. Ispitivane su po dve sorte i to Kg-20 i Favorit – sorte ozime tritikalee i Grand i Rekord – sorte ozimog ječma. Ostvareni rezultati istraživanja pokazuju da je sorta ozime tritikalee Kg-20 bila najprinosnija i sa najboljim kvalitetom zrna. S obzirom da se ove dve biljne vrste najvećim delom koriste za ishranu stoke, to bi ovi rezultati ukazivali na izbor kulture koja će se gajiti u određenom području, posebno za domaćinstva koja se bave stočarstvom. Vrednost ispitivanog materijala, prvenstveno tritikale, bi

mogla biti kako kroz njegovo gajenje kao krmne kulture tako i kroz proces oplemenjivanja u cilju povećanja prinosa i kvaliteta zrna.

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GROWING OF FORAGE CROPS IN THE FUNCTION OF SOIL PROTECTION FROM DEGRADATION

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Original scientific paper

Abstract: Nutrition and used plants growing system affect a lot on keeping of organic matter level in soil, and with that on content of accessible nutrients for plants and micro organisms. With higher portion of forage crops in growing systems it could be positively influenced on nutrients balance in soil, soil structure and soils' microbial world. In paper is analyzed effect of forage crops growing (soya, alfalfa, red clover, birds foot trefoil, perennial ryegrass, tall fescue) at fertilization with organic (manure) and mineral fertilizer, and by appliance of seed inoculation with compatible nitrogen fixing bacteria, on microorganism groups which are participating in processes of humus creation (total number of microorganisms, number of actynomicetes and fungi). Control was fertilizing only with mineral fertilizer. At the end of two years researching period total number of microorganisms was significantly increased at all plant species and at all fertilizing levels. Number of actynomicetes was extremely low at perennial legumes on the beginning of vegetation. In same time, their complete absence, at the all levels of fertilization, was determined at red clover and birds foot trefoil, while at the end of vegetation their number was significantly higher in compare to other species. Number of fungi was quite balanced at all plant species, but it was noticed their increase after entering of organic matter. Based on gained results, it could be concluded that growing of forage crops in the function of soil protection from further degradation and in procedure of biological reclamation is highly important.

Key words: forage crops, fertilization, soil biogenity.

Introduction

Knowledge as a part of science and professional practice about forage crops and their usage has remarkable importance in livestock production. Although, forage crops are the strongest link between fertile soils and meat, milk, wool and other livestock products. Unfortunately, utilization of their production potential is quite low (alfalfa 33.8%, red clover 38.7%), *Đukić et al., (2007*). Fodder crops are best grown in dense formations, as on that way they use the best vegetation space, and with root they could affect on improvement of soil production characteristics, *Vučković, (1999*). Besides that, some species of forage crops (annual and perennial legumes) have ability to live in symbiosis with specific group of bacteria which are fixating atmospheric nitrogen. Considering fact that perennial forage legumes stay on field up to five years (alfalfa, red clover) and that process of nitrogen fixation is happening almost continuous, in ground remains considerable quantity of fixated nitrogen, from 250 to1.250 kg N ha^{-1}, *Bothe et al., (1983)*, what is really important from the aspect of increase and preservation of soil fertility. Thus, soya as annual legume lives in symbiosis with slow-growing species *Bradyrhizobium japonicum* and fixes up to 180 kg N ha^{-1}, *Rhizobium leguminosarum trifoli* with a red clover ties from 45 to 670 kg N ha^{-1}, *(Jarak et al., 2007; Vojinović et al., 1989)*, while species *Rhizobium meliloti* with alfalfa ties around 50 to 460 kg N ha^{-1}, what could be in Serbia, from around 200.000 ha under alfalfa, amounted around 600.000 t of pure nitrogen per one year *(Šutić and Radin, 2001; Lukić, 2000).* Except these competitiveness advantages of forage crops growing, also important fact is that forage crops have modest requirements related to soil production characteristics, so they could be grown on poorer lands with worse structure (red clover, grasses).

Under pressure of soil degradation and contamination with different pollutants, it is obvious that level of ecological awareness could be developed constantly. That certainly implies care of each span of ground, basic environment for plants growing and developing (plants could represent direct food source in people alimentation or fodder). Based on fact, that yearly in Serbia are losing around 6.000 ha of agricultural land, and that mine industry is dominant in this process, it is understandable existence of legal obligation for soil reclamation (technical and biological). That implies implementation of series of measures, starting from technical reclamation (field levelling) to technical and technological measures in conduction of biological reclamation, with main goal to form soil cover suitable for plant production, or some other purpose important from the aspect of soil protection as natural resource, *Cvijanović et al. (2007).*

Main goal of paper is determination of dynamic change of basic soil biogenity elements during forage crops growing in conditions of different fertilization and appliance of bacterial inoculates.

Materials and Methods

Researches were done on experimental parcel size of 1,5 ha during the period 2007-2008., at the part of RB "Kolubara" d.o.o. Lazarevac ground areas, which were prepared for biological soil reclamation. On the previously prepared anthropogenically soil next forage crops were sown: perennial legumes (alfalfa, red clover and birdsfoot trefoil), annual legume soya and grasses (perennial ryegrass, tall fescue). During experiment next systems of fertilization were applied: 1) only mineral fertilizer, NPK (15:15:15) in the quantity of 1.224 kg ha^{-1}; 2) mineral fertilizer, NPK (15:15:15) in the quantity of 1.224 kg ha^{-1} combined with nitrogen fixing bacteria; 3) mineral fertilizer, NPK (15:15:15) in the quantity of 1224 kg ha^{-1}, in combination with 30 t ha^{-1} of manure and nitrogen fixing bacteria.

Before sowing, seed were treated with various kind of nitrogen fixing bacteria which are compatible with specified plant species: a) symbiotic nitrogen fixing bacteria (*Rhizobium meliloti* for alfalfa, *Rhizobium trifoli* for red clover, *Bradyrhizobium japonicum* for soya), b) associative nitrogen fixing bacteria (*Azotobacter chroococcum and Azotobacter vinelandi* for grasses).

Cells of diazotrophs were incubated in selective liquid nutritious substrates and then put on sterile peat which was carrier. Soil samples for microbiological analysis were taken from rhizosphere (ground area on the distance of 0,5 cm from root) during initial phenophases of plants development and after cultures harvesting. Number of micro organism was determined by indirect method of dilution, with insemination on selective substrates *(Pochn J. et al., 1954; Tardeaux, 1962).*

Results and Discussion

Soil fertility considers content of accessible nutrients in ground, and it depends from many physical, chemical and biological processes, as from their interactions too. According *Molnar (1999),* qualitative and quantitative changes of humus in anthropogenic soils are to slow and they could be estimated only by long-lasting experiments. Mineralization of applied organic matter into the soil, primarily depends on the ability of micro organisms to dissolve it, *Kögel-Knabner (2002).* By entering of microbial inoculates into the soil, especially nitrogen fixing bacteria, is positively acted on plant growth and on present microbial world. Nitrogen fixing bacteria except fixating of atmospheric nitrogen, also produce growth matters (hormones, vitamins, antibiotics, etc.), and affect on plant germination, as on development of useful groups of micro organisms. Numerous of entered microbes are reproduced rapidly, have higher enzymatic activity, and negatively impact on certain groups of pathogenic micro organisms, because it comes to competitive relations for available space and nutrient. Probably, paths of humification are same in different soils, but intensity of some paths' parts are diverse. During decomposition of organic matter, essential products are partially mineralized, and partially assimilated by the microbes. Besides, affection of physical and chemical characteristics, really important is number and content of microbes' world, *Kögel-Knabner (1993).* Quantum of biomass, as dynamics of its' changes is good parameter of condition of organic matter in soil. Total number of micro organisms is talking about general condition of soil, in other words about its' fertility potential. In researches was determined various total number of microbes, what depends from used system of fertilizing and plant species. At all plant cultures, except soy, was determined increase of total number of microbes at the end of vegetation, at all levels of fertilizing. The highest number was estimated at perennial legumes. According that important fact it could be concluded that they are quality material for biological reclamation and sanitation of degraded surfaces, because of rapid bio-cover creation and their ability for symbiotic relations with nitrogen fixing bacteria, *Cokić at al., (2004).* The highest number, observing all plant species, was determined after usage of second fertilization system (Table 1).

Table 1. The total number of micro organisms in the rhizosphere (10^6 g^{-1} soil)

Fertilization		Alfalfa	Red clover	Birdsfoot trefoil	Perennial ryegrass	Tall fescue	Soya	Average
1.	Beginning of vegetation	61,1	117,8	60,1	58,1	80,5	315,1	115,5
	End of vegetation	310,3	537,9	294,3	395,2	141,5	248,8	621,4
2.	Beginning of vegetation	119,4	106,4	58,5	64,9	114,9	524,4	322,8
	End of vegetation	225,8	190,9	367,5	241,4	118,6	282,5	237,8
3.	Beginning of vegetation	96,6	35,1	53,3	97,8	107,4	530,8	153,5
	End of vegetation	366,9	305,3	380,4	154,3	162,2	273,5	260,4
Avr.	Beginning of vegetation	92,4	86,4	57,3	73,6	54,3	456,7	
	End of vegetation	301,4	344,7	320,7	263,6	140,8	268,3	

	F test	LSD 5%	LSD 1%
A – plant species	2.017,137**	6,981	9,220
B - vegetation	2.367,238**	9,873	13,04
C - fertilization	26.051**	13,96	18,44

Fungi and actynomicetes are groups of micro organisms which have really developed enzymatic system, so they could decompose complex organic compounds and they are active participants in process of humification. Actynomicetes have great importance in biomass of micro organisms and during the transformations of organic matter. More important is their active surface, because they initiate deepest changes in grounds' organic and mineral ingredients over their touch surface.

Small representation of actynomicetes as decomposers of resistant compounds shows low biodegradation of cellulose, starch and lignin. According results, it was determined very low number of this microbes group at perennial legumes at beginning of vegetation. In same time at red clover and bird's foot trefoil was determined their complete absence in all levels of fertilization, while at the end of vegetation their number is much higher than at other species. Regarding fact that this is soil without differentiated humus layer, it could be understandable their small presence at the beginning of vegetation. Number of fungi is increasing during the entry of organic matter, so it is obvious their higher number at all plant species during the second system of fertilization. These results are comparative with total number of microbes. Although organic manure was ploughed, due to higher content of monosaccharide in mat, its' decomposition was slower (Table 2).

Table 2. The total number of Actinomycetes in the rhizosphere (10^4 g^{-1} soil)

Fertilization		Alfalfa	Red clover	Birdsfoot trefoil	Perennial ryegrass	Tall fescue	Soya	Average
1.	Beginning of vegetation	1	0	0	8,8	50,5	48,7	18,2
	End of vegetation	30,5	104,1	113,5	30,1	45,7	8,5	55,4
2.	Beginning of vegetation	1,2	0	0	11,9	16,7	66,3	16,0
	End of vegetation	55,6	94,9	153,1	38,1	34,5	73,5	74,9
3.	Beginning of vegetation	7,2	0	0	17,8	16,0	43,9	14,1
	End of vegetation	57,3	80,9	100,2	19,3	31,8	14,1	50,6
Avr.	Beginning of vegetation	1,0	0	0	12,8	27,7	52,9	
	End of vegetation.	47,8	93,3	122,3	29,1	37,3	32,0	

	F test	LSD 5%	LSD 1%
A – plant species	42,645**	2,179	2,879
B - vegetation	89,446**	2,179	2,879
C - fertilization	22,931**	2,517	3,324

In Table 3. are presented results of dynamics of fungi number. In distinction to actynomicetes, fungi are more numerous. Number of observed group is quite a unified at plant species. The largest number is estimated under appliance of second system of fertilization. Like with other examined parameters greater number is determined at the end of vegetation.

Difference in number of actynomicetes and fungi could be explained with fact, that absence of actynomicetes is probably conditioned by acid soil reaction. At the beginning of experiment is estimated pH value in the amount of 5,67 in KCl. How actynomicetes are group of micro organisms which requires neutral, or low-alkaline reaction, that ecological conditions for their development were not adequate.

Table 3. The total number of fungi in the rhizosphere (10^4 g^{-1} soil)

Fertilization		Alfalfa	Red clover	Birds foot trefoil	Perennial ryegrass	Tall fescue	Soya	Average
1.	Beginning of vegetation	15,6	16,7	24,9	43,9	60,1	46,5	39,6
	End of vegetation	60,9	43,4	36,4	62,3	52,2	20,3	45,9
2.	Beginning of vegetation	26,0	18,5	10,4	91,9	57,9	39,9	40,8
	End of vegetation	48,0	57,6	24,1	48,9	65,8	31,6	46,0
3.	Beginning of vegetation	40,6	6,8	41,7	52,2	61,7	51,9	42,9
	End of vegetation	63,9	30,2	33,4	55,7	12,7	38,4	39,1
Avr	Beginning of vegetation	27,4	13,9	25,7	62,7	59,9	46,1	
	End of vegetation	57,6	43,7	31,3	55,6	43,6	30,1	

	F test	LSD 5%	LSD 1%
A – plant species	1,805	-	-
B - vegetation	49,261**	1,463	1,932
C - fertilization	9,267**	1,689	2,231

Conclusion

According gained results, main conclusion is that forage plants could be grown on soils which were prepared for biological reclamation, what would prevent further soil degradation. All researched parameters had increased values at the end of vegetation, what was main goal of experiment (to increase biological activity of soil), and higher values were achieved at fertilization with mineral fertilizers in combination with nitrogen fixing bacteria. In rhizosphere of perennial legumes were determined higher values of researched parameters. These knowledge are really important, as they are perennial crops, what means that processes of humification and dehumification are constant and with higher intensity.

For usage of swaths as hay in animal alimentation, it is necessary to determine content of heavy metals and other parameters important for animal nutrition.

Gajenje krmnog bilja u funkciji zaštite zemljišta od degradacije

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Rezime

Povećanim udelom krmnih biljaka u sistemima gajenja pozitivno se utiče na bilans hraniva u zemljištu, strukturu zemljišta i zemljšni mikrobni svet. U radu je ispitivan efekat gajenja soje, lucerke, crvene deteline, žutog zvezdana, visokog vijuka i engleskog ljulja pri đubrenju sa organskim i mineralnim đubrivom, te primenom inokulacije semena kompatibilnim azotofiksatorima, na grupe mikroorganizama koji učestvuju u procesima obrazovanja humusa. Kontrola je bilo đubrenje samo mineralnim đubrivom. Ukupan broj mikroorganizama na kraju dvogodišnjih istraživanja značajno se povećao kod svih biljnih vrsta, po svim nivoima đubrenja. Brojnost aktinomiceta, na početku vegetacije, je bila veoma niska kod višegodišnjih leguminoza, dok je u isto vreme kod crvene deteline i žutog zvezvdana utvrđen njihov potpuni izostanak pri svim nivoima đubrenja. Međutim, na kraju vegetacije njihov broj je bio značajno veći nego kod drugih vrsta. Brojnost gljiva je bila dosta ujednačena kod svih biljnih vrsta, uz konstataciju da je zapaženo njihovo povećanje sa unošenjem organske materije. Na osnovu dobijenih rezultata može se zaključiti da je gajenje krmnog bilja u funkciji zaštite zemljišta od dalje degradacije, kao i u postupku biološke rekultivacije veoma značajno.

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LEGUMES AS PRE-CROP IN ORGANIC PRODUCTION OF POTATO IN REPUBLIC OF MACEDONIA

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Original scientific paper

Abstract: During September 2004 to August 2005 has been carried out study on organic produced potato on farms in individual property. As pre-culture has been sown common vetch as cost efficient and on market available Macedonian selection Pobeda. After "green manure" practicing on the same plots were set up potato cv. Amorosa. After 1st gathering potato dimensions an average are increasingly and yield in total as well. Chemical analysis pointed out on significant potato quality improved; total carbohydrate content at manure plots is amounted 17.05 %/100 mg d.m. while at control 13.37 %/100 g d.m. Protein content is recorded 2.06 %/100 mg d.m. higher than control 1.42 %/100 g d.m. Regarding vitamin C, cannot be considered relevant conclusions because of non-regulars within analyzed parameter. General conclusion is referred to potato tendency of potassium using, which insoluble soil forms became available resulted by symbiotic azotofixation between common vetch that hosted *Rhizobium leguminosarum var.viciae* and extracted specific matters.

Kye words: legumes, organic production, potation, Republic of Macedonia

Introduction

Organic farming is the fastest growing segment of Macedonian agriculture, following the world trend not only in potato production. In 2004 the Coordinative and Certificate Body are established under the umbrella of Ministry of Agriculture, Forestry and Water Economy. In 2005 BIOSAN is started as Organic Farmer Association to certified nearly 100 small- and moderate-sized farms marketing organic products. Most organic potatoes are sold directly to consumers at farmers markets and farm stands, but some are directed toward the high-value restaurant and specialty-store market. Some are sold as organic potato seed and generally demand a much higher value in the marketplace, usually two to three times that of conventional potatoes.

Organic production is based on soil husbandry practices that build reservoirs of plant nutrients, increase the level of organic matter and biological activity in the soil, and improve soil structure. A key practice on organic farms is crop rotation out of potatoes into green manures *(Arnout van delden, 2001)* that build the soil. But also, there are some other practices include adding recycled organic matter to the soil such as compost and livestock manures. Pesticides are avoided as much as possible because they can disrupt beneficial biological activity.

Material and Methods

Cropping and Sample Characteristic. Common vetch variety Pobeda was sown on alluvial soil type under controlled conditions last 20 years with seeding rate 120-kg ha^{-1}. At the end of September 2005, trials on 9 organic farms spreader abroad Macedonia (Skopje-2 places; Kavadarci-3 places, Probistip-5 places) were established. Before culture starting to bud forming, aboveground green mass was ploughed and leaved for 45 days to be mineralized. Aiming of comparison, control plots were also set up.

Chemical analyses: Method of Fractionation of Carbohydrates, Nitrogen and Ascorbic Acid. Plant samples for chemical analyzing are taken after the II^{nd} potato gathering (11. September 2006). Total and soluble carbohydrate content are determined according Dubois et al. method, nitrogen content on the base of total nitrogen (% N x 6,25) and vitamin C according Tillmans method. Obtained results are statistically analyzed (x, Sx,Ω, CV).

Results and Discussion

Qualitative tuber evaluation. Potato tuber cropping on green manure plots were achieved and average greater dimensions; after the 1^{st} gathering 10.03x5.79 cm by yield per plant 1.96 kg and yield in total 30.53 t ha^{-1} compared with control 21.52 t ha^{-1} (Figure 1). After the 2^{nd} gathering, tubers were more increased its dimensions 12.45x6.67 cm with an average yield per plant 2.26 kg and total yield 38.47 t ha^{-1} compared with control 23.01 t ha^{-1} (Figure 2).

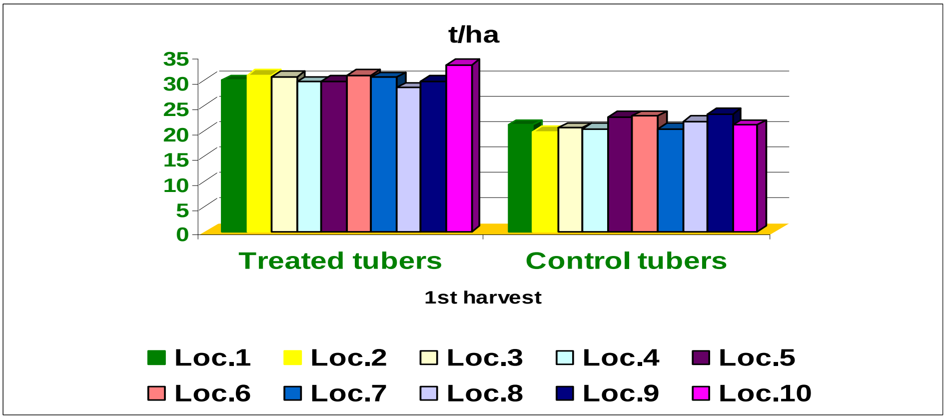

Figure 1. Analysis of morphological potato characteristics 1st harvest (n=30)

Generally considered is needed to stress the fact "banded" soil potassium and phosphorous" as well, after realization of symbiotic nitrogen fixation became available to plant roots influencing on potato growth and quality.

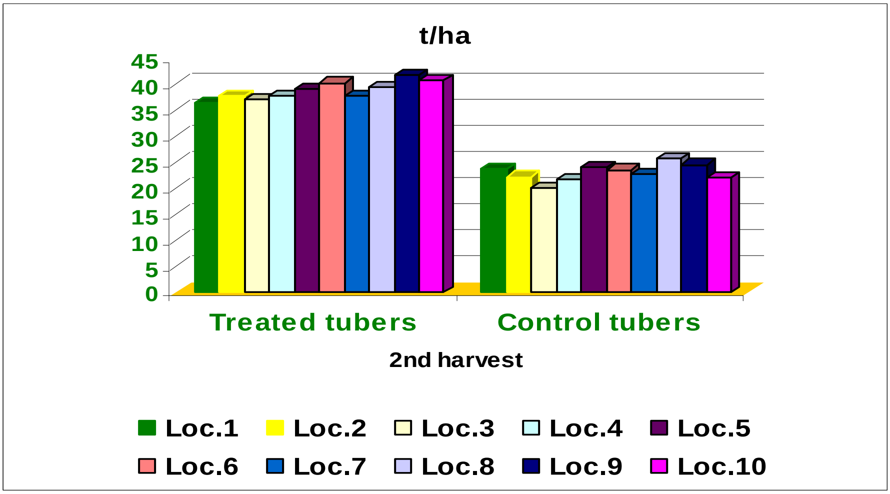

Figure 2. Analysis of morphological potato characteristics 2nd harvest (n=30)

Quantitative tuber evaluation. Potato tuber evaluation is done through energy source analyzing synthesized total carbohydrate and light digestible and adsorbed (soluble) content. Tubers harvested from treated with green manure plots have more quantity of carbohydrates 17.05%/100 g DM than control 13.37%/100 g DM. (Figure 3) *(Kratovalieva et al., 2006).* Summer temperature are going often over

40°C and plants are exceed unfavourable weather conditions and being more adaptable; actually sugar hydrolysis is intensifying and resulted by increased soluble sugar content 6.78%/100 g DM (Figure 4).

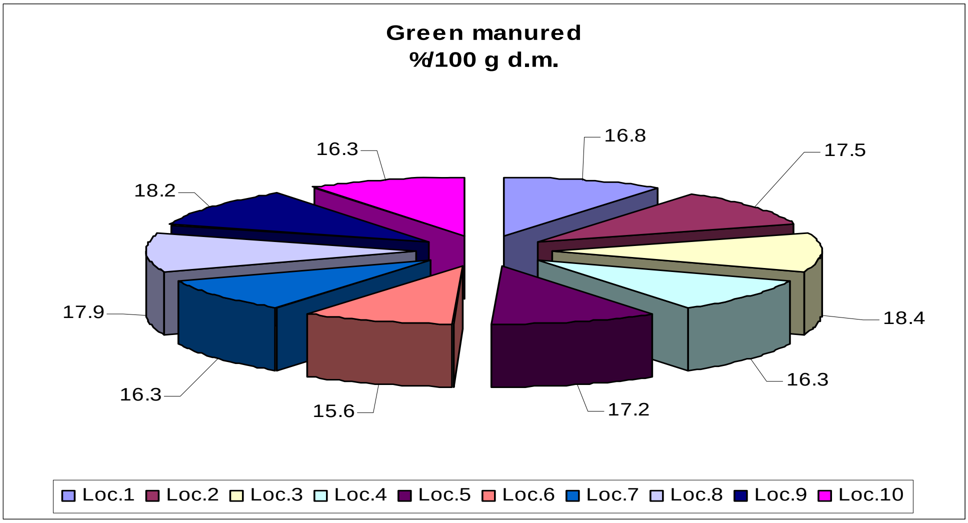

Figure 3. Total carbohydrate content (%/100 g DM) (n=3)

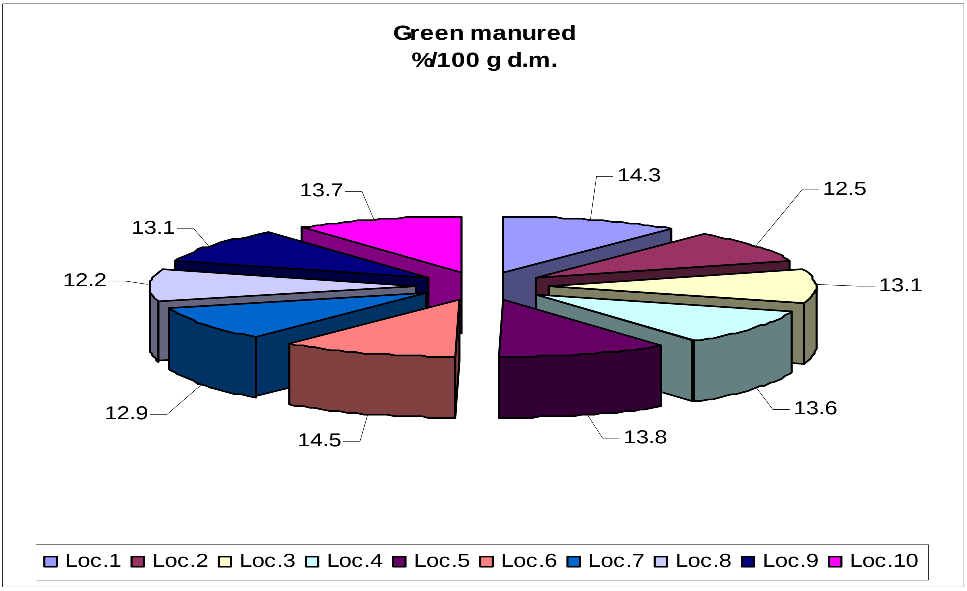

Figure 4. Total carbohydrate content (%/100 g DM) (n=3)

Protein content at tubers harvested from "green manure" plots are significantly increased 2.06% compared with control ones 1.42% (Fig. 5 and 6). This fact is not so unexpectedly regarding mineralization of legume organic mass (*Smith et al. 1987; Kratovalieva et al., 2007)* (Figure 5).

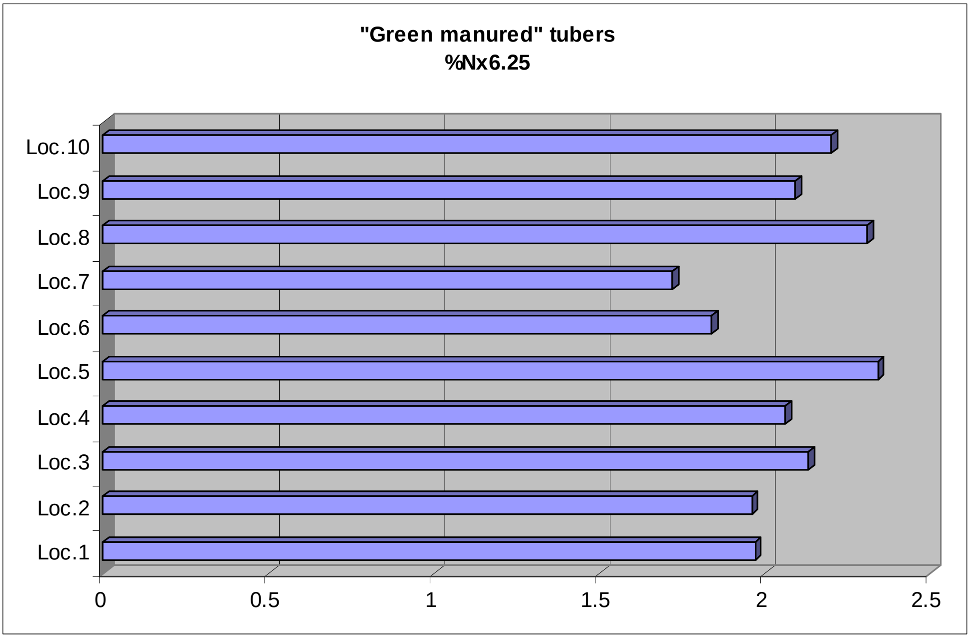

Figure 5. Protein content at green manure potatoes (n=3)

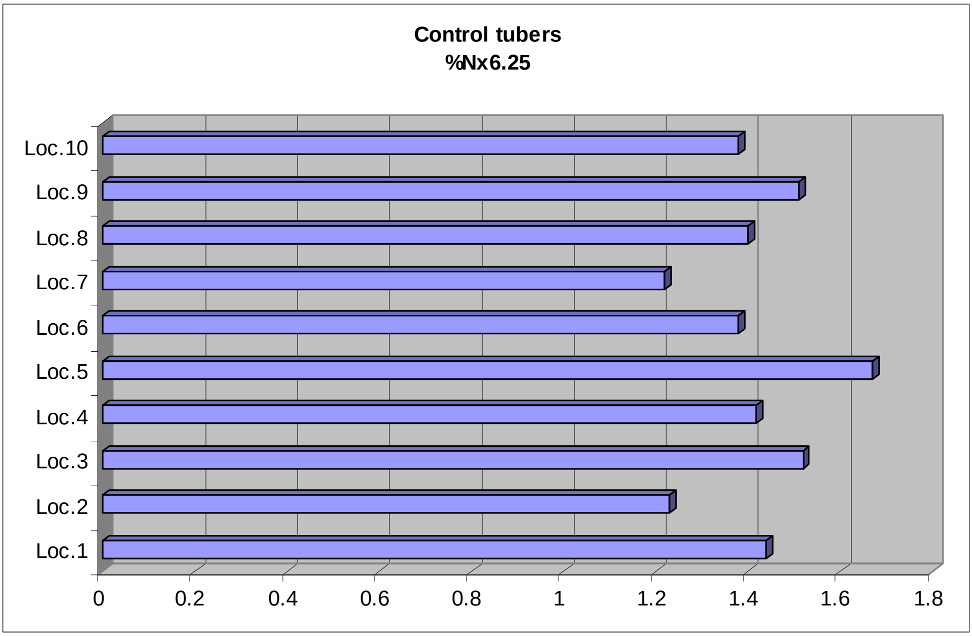

Figure 6. Protein content at control plots (non manured) potatoes (n=3)

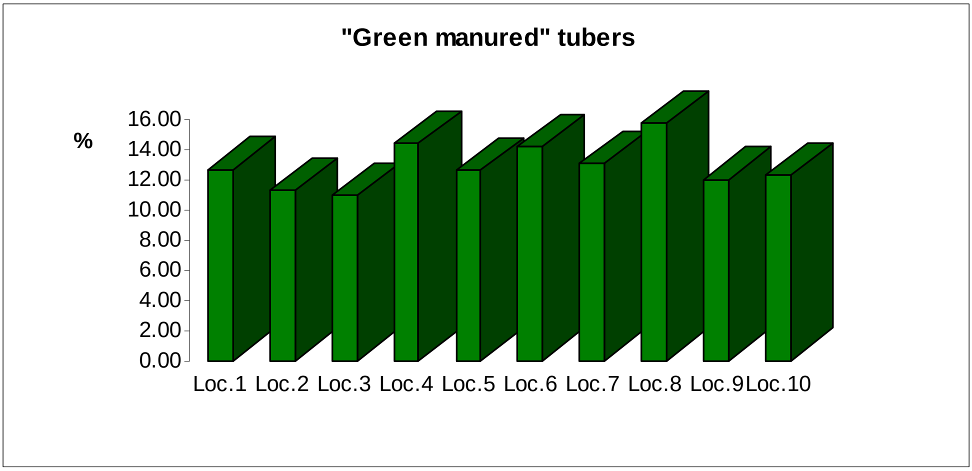

Figure 7. Vitamin C concentration at green manure potatoes (n=3)

Vitamin C as antioxidative parameter as powerful against free radicals is varied very much at researched tubers; an average concentration at manure tubers is lower 12.93% than control 13.87%, but not only at all samples. Variations are appeared from farm to farm and can be considered as parameter independently of green manure practicing as one tool in organic potato production and corresponded with literature data *(Marecek, 2001)* (Figures 7 and 8).

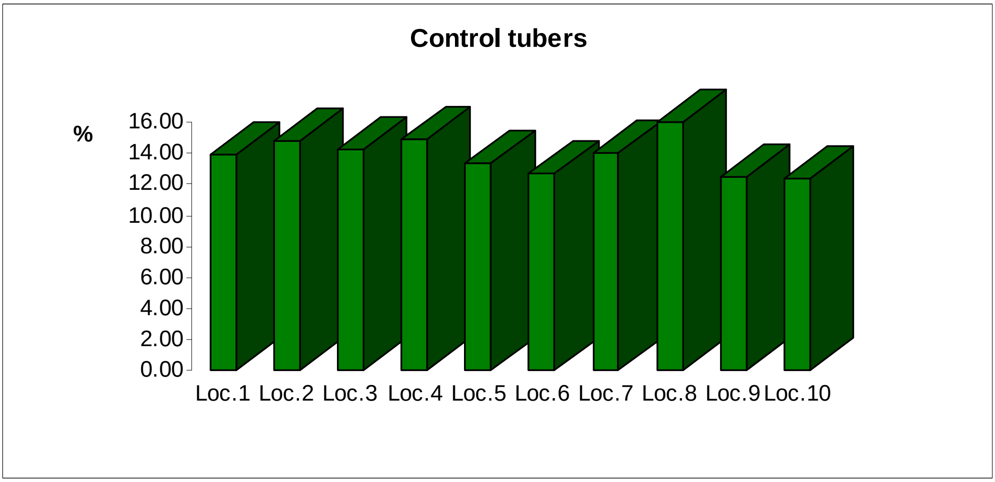

Figure 8. Vitamin C concentration at control (non manured) potatoes (n=3)

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FERTILITY OF SALINE SOILS UNDER PASTURES AND MEADOWS IN VOJVODINA

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Original scientific paper

Abstract: There are two types of saline soils Vojvodina - solonchak and solonetz. Together, they cover an area of 115,000 ha in the province. At 25 sites marked as solonchaks on the soil map of Vojvodina, soil profiles were dug in order to study their main chemical properties. The soils' surface horizons were analyzed. The soils were found to be highly alkaline, with pH values of up to 10.78. Such extremely high pH values can be attributed to the amount and type of salt found in the soil (especially sodium carbonate), the degree of alkalinity, and, partly, the $CaCO_3$ content. The surface horizon of solonchak with the highest salt content is an unfavorable environment for organic matter production, i.e. the development of vegetation, as evidenced by the very low values of humus content obtained in our analyses. The supply of plant-available phosphorus in the soils under study was found to be medium, while that of potassium was optimal. The average hay yield obtained from extensive pastures and meadows is low. The production of plant biomass for livestock nutrition on saline soils in Vojvodina could be increased at a relatively low cost by applying a top dressing of mineral nitrogen fertilizer and harrowing the soil in the spring.

Key words: pastures, meadows, soil fertility, solonchak, solonetz

Introduction

Saline soils in the province of Vojvodina are represented by two types of soil – solonetz and solonchak *(Škorić et al., 1985)*. Solonchak covers about 25 000 ha in the province, while solonetz is found on around 90 000 ha *(Benka and Salvai, 2005)*. These are agricultural soils of low fertility that are mostly utilized as pastures or meadows as part of extensive agriculture. The main limiting factors preventing their use as pastures or meadows through intensive farming systems are the high groundwater salinity and the low fertility of these soils *(Miljković, 2005)*. Fertilizer application has been proven to be the best method for rapidly increasing the productivity of meadows and pastures *(Lazarević et al., 2009)*.

The objectives of this paper were to determine fertility levels of soils categorized as solonchak on the Soil Map of Vojvodina R 1:50 000 *(Nejgebauer et al., 1971)* and provide a recommendation on how to increase these levels and by virtue of this fresh weight production and quality (by increasing the proportion of grasses and legumes of higher quality).

Materials and methods

Disturbed soil samples were taken from the surface horizon at a number of sites from the Soil Map of Vojvodina R = 1:50 000. According to the domestic soil classification (*Škorić et al., 1985*), the 16 sites studied in this paper have halomorphic soils of two types: solonchak (profile number: 14 – Trešnjevac, 16 - Bački Brestovac, 17 - Mali Stapar, 18 - Kljajićevo and 21 - Rančevo) and solonetz (profile number: 1 - Žabalj1, 2 - Žabalj2, 3 - Despotovo, 5 - Novi Bečej Matej, 6 - Novi Bečej Konice, 7 - Novi Bečej Slano kopovo, 10 - Ogar, 11 - Kula, 12 - Ruski Krstur, 19 - Stanišić, and 24 - Gornji Breg).

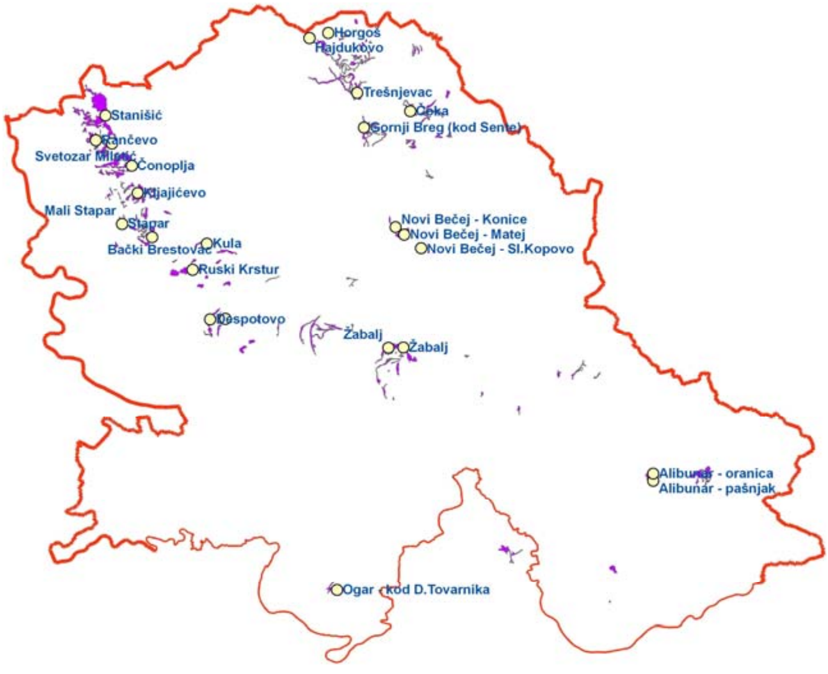

Figure 1. Sites from which the samples were taken

In order to study soil properties in the laboratory, disturbed soil samples were taken from surface horizons using a soil knife. Laboratory work and data processing were carried out in the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops in Novi Sad.

A number of chemical soil properties were determined in surface horizon:
- pH value was determined potentiometrically in a suspension of soil in H_2O or KCl;
- $CaCO_3$ content was determined volumetrically using Scheibler's calcimeter;
- Humus content was measured according to Tjurin;
- Total nitrogen content was determined with a CHNS analyzer;
- Levels of readily available (ammonium lactate) phosphorus and potassium were measured spectrophotometrically (P) or by flame photometry (K)
- Exchangeable Sodium Percentage (ESP = adsorbed Na ^{x} 100/CEC; CEC is Cation Exchange Capacity)
- Cation Exchange Capacity (CEC), soil extraction with sodium acetate and ammonium acetate, concentration Na^+ in extract was determined with a flame photometer - "Evans".

Results and discussion

The active acidity values (pH in H_2O) of solonchak (Tab.1) covered a narrow range - from 10.71 to 10.78 pH units. These are very high values, characteristic of highly alkaline soils *(Živković, 1991)*. Sodic solonchaks have a highly alkaline reaction even at the time year when their salt content is at its lowest *(Jakovljević and Pantović, 1991)*.

In solonetz, active acidity values (tab.2) were somewhat lower, and they had been even lower some time back, as reported by *Belić et al. (2004)*. The finding that the active acidity of solonetz was lower than that of solonchak is in agreement with the way saline soils progress from solonchak through solonetz to solod in the course of their formation *(Nešić and Hadžić, 2004)*.

Carbonates were found to be present in solonchak in high amounts, which reduces the availability of certain micronutrients to plants. In the solonetz profiles, the average carbonate levels were lower, and in some of the profiles, the carbonates had leached down to the deeper layers of the soil.

Humus content is one of the key elements of soil fertility. The solonchak soil profiles we studied had extremely low humus contents in the surface horizon (Tab.1), which is linked with the high alkalinity of the soil. In such conditions, humus leaches down to the subsurface horizon, outside the active rhizosphere. The low levels of humus, a structure-forming soil component and a source of plant nutrients, is the reason why these soils have poorer structure and lower fertility.

The average humus levels in the surface horizon of the solonetz profiles (tab.2) were near-optimal, which makes this environment more suitable for the production of organic matter, i.e. plant biomass for livestock nutrition.

In line with the low humus content, the total nitrogen levels of solonetz and, especially, solonchak were low as well. It is well known that nitrogen is the essential component of crop yield and that cultivated plants respond to fertilization with this macronutrient most rapidly. A top dressing of mineral nitrogen fertilizer applied in the spring would lead to a significant increase in biomass yields on pastures and meadows developed on saline soils in Vojvodina. Fertilizer application should be preceded by the agronomic practice of harrowing in order to aerate the soil and improve its physical properties.

Phosphorus and potassium are the two macronutrients that are most commonly added to the soil in the course of crop production. In the present study, the average supply of phosphorus in solonchak and solonetz was found to be medium, while that of potassium was optimal to high.

The ESP (Exchangeable Sodium Percentage) values found in the surface horizon of solonchak indicate that the pedogenesis of this soil has progressed beyond the initial stage of salinization and that alkalization is well under way as the soil is gradually transforming from solonchak to solonetz.

Table 1. Values of the main chemical properties of the surface horizon of solonchak

Locations / Values	**pH**		**$CaCO_3$** %	**Humus** %	**Total N** %	**P_2O_5** mg/100g	**K_2O** mg/100g	**ESP** %
	u KCl	u H_2O						
Trešnjevac	10,24	10,76	29,54	0,46	0,025	5,70	12,07	15,19
Bač. Brestovac	10,17	10,78	14,77	1,95	0,098	15,80	27,26	31,42
Mali Stapar	10,28	10,71	25,32	1,49	0,109	7,10	16,48	15,92
Kljajićevo	10,24	10,74	20,26	1,73	0,118	19,50	25,00	24,98
Rančevo	10,24	10,72	20,61	1,53	0,106	15,40	23,46	20,76
average	10,23	10,74	22,10	1,44	0,091	12,70	20,85	21.65
StDev	0,04	0,03	5,59	0,57	0,04	5,99	6,35	6.74

Table 2. Values of the main chemical properties of the surface horizon of solonetz

Locations / Values	**pH**		**$CaCO_3$** %	**Humus** %	**Total N** %	**P_2O_5** mg/100g	**K_2O** mg/100g	**ESP** %
	u KCl	u H_2O						
Žabalj 1	5,32	6,67	0,00	7,71	0,513	4,20	25,83	8,47
Žabalj 2	8,22	10,06	1,38	2,25	0,138	4,40	23,19	22,75
Despotovo	8,6	10,55	6,41	1,95	0,101	8,95	16,08	15,24
Novi Bečej 1	5,30	6,59	0,00	4,68	0,299	3,10	31,87	2,93
Novi Bečej 2	7,10	9,47	0,49	1,22	0,075	3,50	19.00	8,24
Slano kopovo	8,18	10,09	0,69	2,24	0,137	6,80	19,60	11,80
Ogar	7,37	8,50	1,26	2,25	0,164	4,30	36,82	3,61
Kula	10,52	10,93	28,64	1,51	0,110	28,42	57,44	13,61
Ruski Krstur	8,90	10,30	12,65	4,69	0,301	29,60	56,47	26,62
Stanišić	9,06	10,26	25,74	1,65	0,091	16,50	51,56	19,47
Gornji Breg	8,07	9,33	29,70	5,43	0,342	18,00	26,05	7,11
average	7,88	9,34	9,72	3,23	0,206	11,62	33,08	12.71
StDev	1,56	1,49	12,37	2,08	0,140	10,00	15,38	7.72

Conclusion

In Vojvodina, saline soils (solonchak and solonetz) are regarded as non-arable agricultural land and are used exclusively as pastures and meadows in extensive agriculture. This is due not only to their poor physical properties and salinity but also to their low fertility, which is reflected in high alkalinity and low levels of humus and total nitrogen.

We are of the opinion that the production potential of these soils could be increased significantly by applying a top dressing of mineral nitrogen fertilizer in the spring and harrowing the soil. With a relatively small investment, this would increase the yield of green plant biomass and would also improve biomass quality through an increased proportion of higher-quality grasses and legumes.

Plodnost zaslanjenih zemljišta pod pašnjacima i livadama u Vojvodini

J. Vasin, M. Belić, Lj. Nešić, P. Sekulić, T. Zeremski Škorić, J. Ninkov, S. Milić

Rezime

Zaslanjena zemljišta - slatine u Vojvodini su zastupljena preko dva tipa zemljišta solonjec i solončak na oko 148.000 ha. Na 25 lokaliteta koji su na pedološkoj karti Vojvodine označeni kao solončaci otvoreni su pedološki profili u kojima su ispitana osnovna hemijska svojstva. U radu su prikazani rezultati analiza površinskih horizonata zemjišta. Ispitivana zemljištu su sa izraženom alkalnošću (vrednosti pH jedinica su i do 10.76). Ekstremno visoke pH vrednosti (preko 9) se mogu dovesti u vezu sa količinom i vrstom soli (pogotovo sa natrijum karbonatom), stepenom alkalizacije, a u izvesnoj meri i sa sadržajem $CaCO_3$. Površinski horizont solončaka sa najvećim sadržajem vodorastvorljivih soli je nepogodna sredina za produkciju organske materije, tj. razvoj vegetacije, na šta ukazuju analizama dobijene vrlo niske vrednosti sadržaja humusa. Sadržaj makrohraniva za biljke je na na niskom i srednjem nivou za fosfor i optimalnom za kalijum. Prosečan prinos sena sa ekstenzivnih pašnjaka je nizak 0.5-1.5 t ha^{-1}, a sa livada do 2 t ha^{-1}. Sa relativno niskim ulaganjima u cilju povećanja proizvodnje organske biljne materije za stočnu ishranu sa zaslanjenih zemljišta u Vojvodini, a s

obzirom na nemogućnost primene obrade zemljišta, može se preporučiti prolećno drljanje i prihranjivanje primenom azotnih mineralnih đubriva.

Ključne reči: livade, pašnjaci, plodnost zemljišta, solončak, solonjec

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M. D. Petrović[1], Z. Skalicki[2], V. Bogdanović[2], M. M. Petrović[3]

[1]Faculty of Agronomy, 32000, Čačak, Republic of Serbia
[2]Faculty of Agriculture, 11080, Belgrade-Zemun, Republic of Serbia
[3]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
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