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The impact of soil liming on the productivity of grass-legume mixture of red clover *(Trifolium Pratense* L.*)* and italian ryegrass *(Lolium Italicum* L.*)*

Dalibor Tomić, Vladeta Stević, Dragan Đurović, Đorđe Lazarević
University of Kragujevac, Faculty of Agronomy – Čačak, Serbia

Abstract: Grass-legume mixtures are of great importance in the production of high quality forage, both in terms of factory farming and free range livestock. The paper investigates the effect of cultivation of red clover (pure crop and mixed with Italian ryegrass) and liming application (control – without CaO; 3t ha^{-1} CaO) on acid soil with a pH of 4.8, to green forage yield, hay yield, the share of red clover, Italian ryegrass and weed in the total hay yield. Growing of red clover in mixture with Italian ryegrass, achieved significantly higher yield of green forage and hay, in relation to the pure crop of red clover. Irrespective of the method of cultivation, liming significantly affected the increase of green forage and hay yield. In the variant with red clover and Italian ryegrass, the red clover proportion in total hay yield decreased as a result of the application of liming to increase the share of Italian ryegrass**.**

Key words: red clover, Italian ryegrass, liming, forage yield

Introduction

Production of the sufficient amount of high quality forage is one of the prerequisites for the intensification of livestock production. This production is mainly based on the natural meadows and pastures, and partly on the growing fields of red clover, alfalfa, mixtures of grasses and legumes and others. In the Republic of Serbia under the grassland is over 27% of agricultural land (SGS 2011). In the period of 2001-2005, the average hay yield on meadows ranged

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from 1.5-2.0 t ha^{-1} (SGS 2006). The absence of agro-technical measures is the reason for the low and unstable yields and poor quality forage (Dubljević 2007, Djukić *et al*. 2008). Grasslands with different floristic composition are significantly different in productivity and growing legumes and grasses in mixture provides more profitable production and better forage quality (Nešić *et al*. 2007). The introduction of legumes in feed mixtures decreases the use of nitrogen mineral fertilizers, thus reducing the possibility of loss of nitrogen from the soil by leaching or emission in the form of gases (Ledgart *et al*. 1999), which affects the preservation of the environment (Janzen and McGinn 1991). By Winther and Jensen (1999) symbiotic N_2 fixation in legumes is a fundamental process for maintaining soil fertility and continuous productivity of organic growing systems. Share of weeds in the total yield of grass-legume mixtures is much smaller in relation to the pure crop of grasses or legumes (Sleugh *et al*. 2000).

Soil acidity is one of the factors which makes it difficult growing of many cultivated plants, both legumes and grasses (Edmeades *et al*. 1981; Wheeler 1998). It is known that soil pH affects all phases of plant growth, disease resistance, resistance to low temperature, the lifetime of the crop, forage yield and quality. In addition to a lack of calcium, acid soils are characterized by a high prevalence of easily mobile forms of Al, Fe, Mn, and low contents of available P, K and Mo (Su and Evans 1996). According to McKenny et al. (1993) red clover shows greater tolerance to Al, as compared to other legumes, but it is sensitive to the toxic effects of Mn. Satisfactory yields of crops on acid soils can be achieved if the repair is carried out by introduction of lime fertilizers (Grewel and Williams 2003). Since acid soils occupy significant area in the Republic of Serbia, the aim of the study was to examine the impact of liming on the red clover yield grown as a pure crop and in mixture with Italian ryegrass.

Materials and methods

The experiment was established in the period 2011-2012 in Čačak (43°54'39.06" N, 20°19'10.21" E, 246m a.s.l.), on alluvial soil, with acid reaction (pH_{H2O} 4.8), which contains 3.18% of the organic matter, 0% $CaCO_3$, 22.08 mg P_2O_5 and 30.0 mg K_2O in 100 g of soil. Along with tillage, 300 kg ha^{-1} $N_{15}P_{15}K_{15}$ was incorporated into the soil. The experiment was set up in a completely randomized block design with three replications, with a plot size of $5m^2$ (5x1m). The researches comprised two method of cultivation: crop, red clover (cv. K-39, Institute for Forage Crops, Kruševac) and red clover mixed with Italian ryegrass (Tetraflorum – Slovenian tetraploid species). Levels of calcification included control (without CaO) and 3 t ha^{-1} CaO. Entering the lime material was performed by the surface application immediately before the presowing preparation. Sowing was done on row spacing of 20 cm (Italian ryegrass was sown in the same rows

with red clover), with the amount of seed of 18 kg ha^{-1} of red clover in the pure crop and in mixture of 12 kg ha^{-1} of red clover and 12 kg ha^{-1} of Italian ryegrass. Mechanical weed control was performed on two occasions. The crop was grown without irrigation. Mowing was done in the budding phase. Tests were carried out on the first cut in 2011 and in the first and second cut in 2012 (because of extreme dry periods there was no second cut in 2011 and third cut in 2012).

The mean annual air temperature in 2011 was 12.37 °C (0.4°C higher than the ten-year average), and the total amount of rainfall 374.5 mm (305.5 mm lower than the ten-year average) (Table 1). Total rainfall during the growing season 2012 (April-October) was 212.2 mm.

Tab. 1. The amount and distribution of rainfall by month (P) and mean monthly temperatures (T) for 2011, 2012 and the period 1992-2002.

Month		I	II	III	VI	V	VI	VII	VIII	XI	X	XI	XII	$\bar{x}$ and $\sum$
1992-2002	P(mm)	30.7	38.9	42.5	51.2	56.4	88.4	82.6	51.6	74.9	57.6	52.8	52.7	680.3
	T (ºC)	0.5	3.1	7.6	11.7	17.9	21.3	22.6	23	16.8	12.2	6.1	0.8	11.97
2011	P(mm)	22	29	31	15.5	95.5	47	30.5	9.5	42	21	2.5	29	374.5
	T (ºC)	0.7	0.7	7.6	13.1	16.7	22	23.8	23.7	21.6	11.8	3.7	3	12.366
2012	P(mm)	60	70	10	47	68	38	22	0	7.2	30	-	-	-
	T (ºC)	1.8	2.5	6.8	12.2	17.3	24.1	26.6	25.4	20.9	13.8	-	-	-

Green forage (GF) yield was determined by measuring the total weight of the plot immediately after cutting at the optimum stage of growth and plant development and recalculated to GF yield in t ha^{-1}. After drying the samples (1000 g) at 65°C, the hay yield (t ha^{-1}) and weight share (%) of italian ryegrass, red clover and weeds were determined.

The results were subjected to a single-factor analysis of variance (ANOVA) using the SPSS 4.5 software. Significant differences between mean values were tested by the LSD test.

Results and discussion

Irrespective of soil liming, significantly higher yield of forage on the mixture treatment (red clover with Italian ryegrass), compared with the pure crop of red clover, was reported in 2011 (25.2%) and the first cut in 2012 (54.8%) (Table 2.). By growing grasses in mixture with legumes, the soil becomes more biogenic and significant amount of nitrogen is fixed by the bacteria of the genus Rhizobium, which is available for the plants to use (Wheeler 1998). Increased biological value also affects the increase of mineralization of organic matter in the soil (Wheeler 1998, Clay *et al*. 1993), which contributes to more rapid growth and achievement of higher yields.

Tab. 2. Impact of the method of cultivation of red clover RC (RC -crop, RC + IR - in mixture with Italian ryegrass) and soil liming (O - without liming, Ca - with liming) on the green forage yield (t ha^{-1}) in the period 2011-2012.

		2011	2012		Σ
		I cut	I cut	II cut	
Method of cultivation	RC + IR	9,43 a	44.53 a	10.9 b	64.86
	RC	7,53 b	28.77 b	15.3 a	51.6
Soil liming	O	7.53 b	34.1 b	14.51 a	56.14
	Ca	9.24 a	39.2 a	11.69 b	60.13
RC + IR	O	8,39 b	39.2 b	11.67 b	64.26
	Ca	10,47 a	49.87 a	10.13 b	70.47
RC	O	6,67 b	29.0 c	17.34 a	53.01
	Ca	8,02 a	28.53 c	13.25 b	49.8

The values denoted with different small letters within columns for method of cultivation and liming are significantly different (P<0.05) in accordance with the LSD test;

According to Mandić *et al*. (2004) the highest biological value of several studied grass – legume mixtures were determined under the mixture of red clover and Italian ryegrass. Italian ryegrass is an ideal species for growing in mixture with red clover (Simić *et al*. 2011). However, in the second cut in 2012 a significantly lower yield of green forage was reported in the mixture treatment compared to the pure crop of red clover. This is a consequence of the greater tolerance of red clover, in relation to Italian ryegrass, to the drought conditions that occurred during the second growth in 2012. According to Garwood and Williams (1967) white clover *(Trifolium repens* L.*)* is more tolerant to the drought conditions compared to the Italian ryegrass, because of the better nitrogen provision (due to the process of nitrogen fixation), whose adoption from the soil is reduced in terms of water scarcity.

Application of liming significantly increased the yield of green forage in both ways of growing in 2011 as well as in the mixture treatment in the first cut of 2012. Intake of calcium in the soil decreases its acidity, which also affects the increace of microbial activity of the soil (Agarwal *et al*. 1972) and higher nitrogen mineralization (Edmeades *et al*., 1981, Hojito *et al*. 1987). By reducing the acidity of the soil, there are favorable conditions provided for the development of symbiotic nitrogen-fixers. Jarak *et al*. (2002) suggest that the abundance and microbial activity of the symbiotic nitrogen-fixers of perrennial legmes is limited by the low pH value. On acid soils survival of the nodulating bacteria is difficult and the reproduction is slow, thus decreasing the yield of legumes (Nutman 1976). Soil liming helps to reduce the amount of easily mobile and toxic form of Al, Fe and Mn and increase the amount of readily available P in the soil for clovers and Italian ryegrass (Wheeler 1998). In the second cut of 2012 on the treatment with a single crop of red clover, due to the large impact of

drought, a significant reduction in green forage yield was reported on the treatment with liming.

Application of soil liming significantly increased the hay yield of Italian ryegrass in 2011 and in the first cut in 2012, while in 2012 the second cutting the lack of rainfall reduced the effect of this measure (Table 3.). Red clover hay yield on the mixture treatment decreased significantly with the application of soil liming. This is a consequence of increasing the yield of Italian ryegrass whose competitiveness is slightly more evident compared with clovers (Thomas 1984). Hay yield of red clover grown in pure crop in 2011 and in the second cut in 2012 increased significantly by liming application. On the mixture treatment generally a lower weed hay yield was reported in relation to the pure stand of red clover. According to Živanović-Katić (2004) in rare crops weeds occur widely, consume nutrients and water as well as decrease the yield of crops, while dense crops "suffocate" weeds and prevent their mass occurrence.

Tab. 3. Impact of the method of cultivation of red clover RC (RC -crop, RC + IR - in mixture with Italian ryegrass) and soil liming (O - without liming, Ca - with liming , on the hay yield (t ha^{-1}) of Italian ryegrass, red clover and weed in the period 2011-2012.

			2011	2012		∑
			I cut	I cut	II cut	
Italian ryegrass	RC + IR	O	1.88 b	7 b	2.62	11.5
		Ca	2.7 a	7.9 a	2.99	13.59
Red clover	RC + IR	O	0.740 c	2.04 b	1.85 c	4.63
		Ca	0.253 d	0.40 c	0.60 d	1.25
	RC	O	0.559 b	4.64 a	5.08 a	10.28
		Ca	2.384 a	4.11 a	3.89 b	10.38
Weed	RC + IR	O	0.355	0.08 c	0.023	0.46
		Ca	0.478	0.14 bc	0.017	0.63
	RC	O	0.721	0.98 ab	0.097	1.8
		Ca	0.6	1.40 a	0.31	2.31

The values denoted with different small letters within columns for method of cultivation and liming are significantly different (P<0.05) in accordance with the LSD test;

On the mixture variant, the Italian ryegrass share in total hay yield increased significantly in all the cuttings by the application of soil liming (Table 4.). While, with the use of lime, the share of red clover in total hay yield of all the cuttings significantly reduced. The reason for this can also be more conspicuous competitiveness and faster growth of Italian ryegrass compared to red clover. By the application of soil liming on natural grasslands in type of *Poetum violaceae*, Prentović *et al*. (2007) found a significant increase in height of plants from the grass family. In the variant with a single crop of red clover, the application of soil liming had no significant effect on the change of its share in

the total yield in any cut. According to Mc Kenny *et all*. (1993), red clover is characterized by somewhat higher tolerance to acid soils than the most of the other legumes.

Tab. 4. Impact of the method of cultivation of red clover RC (RC -crop, RC + IR - in mixture with Italian ryegrass) and soil liming (O - without liming, Ca - with liming) on the proportion of red clover, Italian ryegrass and weeds in hay (%) in the period 2011-2012.

			2011	2012	
			I cut	I cut	II cut
Italian ryegrass	RC + IR	O	62,6 b	76.3 b	57.0 b
		Ca	78,13 a	90.8 a	83.1 a
Red clover	RC + IR	O	24,24 b	22.8 b	42.4 b
		Ca	7,2 c	6.3 c	16.3 c
	RC	O	68,6 a	82.4 a	98.1 a
		Ca	79,8 a	74.7 a	92.9 a
Weed	RC + IR	O	13,16 b	0.9 c	0.6
		Ca	14,67 b	2.9 bc	0.5
	RC	O	31,4 a	17.6 b	1.9
		Ca	20,2 ab	25.3 ab	7.1

The values denoted with different small letters within columns for method of cultivation and liming are significantly different (P<0.05) in accordance with the LSD test;

For the mixture treatment, generally lower share of weeds was reported in relation to the pure crop of red clover. The application of soil liming had no significant effect on the share of weeds in the total hay yield in any of the examined variants. However, Živanović-Katić *et al*. (2008) stated that the application of soil liming leads to changes in the floristic composition of plant communities. The authors determined that the largest presence of weeds in barley crop was found on the control variant without liming, in which due to increased soil acidity and the negative impact of increased content of easily mobile Al came to the massive deterioration of plants and reduction of their competitiveness. In this study, red clover and Italian ryegrass relatively well-filed conditions of low soil pH, so that there was no significant thinning of them.

Conclusions

By growing of red clover in mixture with Italian ryegrass, regardless of soil liming, there was achieved a significantly higher green forage and hay yield, in relation to the pure crop of red clover. However, in the second cutting in 2012, significantly higher green forage yield was reported on the treatment with the pure crop of red clover. This is the consequence of much higher tolerance of red clover, as compared to Italian ryegrass, to the drought conditions that occurred

during the second increase in the 2012. Application of liming caused a significant increase in green forage and hay yield in both methods of cultivation.

In the mixture variant, Italian ryegrass share in the total hay yield increased significantly in all cuttings, by using soil liming at the expense of reducing the share of red clover. This is a consequence of the greater competitiveness of Italian ryegrass compared to red clover. In the variant with a single crop of red clover, the use of soil liming didn't have a significant effect on the change of share of red clover in any cut. Generally a smaller proportion of weeds was recorded in the mixture treatment, in relation to the single crop of red clover.

By the application of liming and cultivation of red clover in mixture with fast-growing grasses, such as Italian ryegrass with proper utilization and cropping, the stability in the production of forage can be realized.

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UTICAJ KALCIZACIJE ZEMLJIŠTA NA PRODUKTIVNOST TRAVNO-LEGUMINOZNE SMEŠE CRVENE DATELINE *(Trifolium pratense* L.*)* I ITALIJANSKOG LJULJA *(Lolium italicum* L.*)*

- originalni naučni rad -

Dalibor Tomić, Vladeta Stević, Dragan Đurović, Đorđe Lazarević
Univerzitret u Kragujevcu, Agronomski fakultet – Čačak, Srbija

Rezime

Travno-leguminozne smeše imaju veliki značaj u prozvodnji kvalitetne kabaste stočne hrane, kako u uslovima farmskog držanja, tako i pri slobodnom sistemu ispaše životinja. U radu je ispitivan uticaj načina gajenja crvene deteline (čist usev i u smeši sa italijanskim ljuljem) i primene kalcizacije (kontrola – bez CaO; 3t ha-1 CaO) na kiselom zemljištu pH 4,8 na prinos zelene krme, prinos sena, udeo crvene deteline, italijanskog ljulja i korova u ukupnom prinosu sena. Gajenjem crvene deteline u smeši sa italijanskim ljuljem postignut je značajno veći prinos zelene krme i sena u odnosu na čist usev crvene deteline. Primena kalcizacije je nezavisno od načina gajenja, značajno uticala na povećanje prinosa zelene krme i prinosa sena. Na varijanti sa crvenom detelinom i italijanskim ljuljem, udeo crvene deteline u ukupnom prinosu sena, smanjio se primenom kalcizacije kao posledica povećanja udela italijanskog ljulja.

Ključne reči: crvena detelina, italijanski ljulj, kalcizacija, prinos krme