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EFFECT OF ADDITIONAL FERTILIZING WITH NITROGEN ON FORAGE YIELD IN RED CLOVER-ITALIAN RYEGRASS GRASS-LEGUME MIXTURE

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Abstract

The high and stable yields of good quality forage on lawns can be achieved with proper fertilization and rational utilization. The aim of this study was to analyze the impact of the additional application of nitrogen fertilizer in red clover-Italian ryegrass grass-legume mixture on forage yield, hay yield and the proportion of red clover, Italian ryegrass and weeds in the total hay yield. The field experiment was set up 2011th in Čačak (Serbia) on fluvisol soil type, acid reaction (pH_{H2O} 4.8), using a randomized block design with three replications and experimental unit 5x1m. Together with the primary tillage in the autumn, the soil was entered 300 kg ha^{-1} $N_{15}P_{15}K_{15}$. Red clover cv. K-39 and Italian ryegrass cv. Tetraflorum were seeded at 20 cm inter-row spacing, both with 12 kg ha^{-1} seed. The experiment included two treatments: without fertilization (control) and additional application of nitrogen fertilizer (KAN - 27 % N) in the rate of 40 kg ha^{-1} early in the spring 2012th and 2013th. Additional application of nitrogen did not significantly increase yields of forage and hay in all cuts in both years. In the second year, nitrogen fertilization led to a significant increase in the share of Italian ryegrass in the hay, with a proportional decrease of red clover and weeds, in both cuts. This may be due to the pronounced competitiveness and faster growth of Italian ryegrass in relation to red clover, especially in drought conditions. In the third year of cultivation, there were no plants of Italian ryegrass and additional feeding resulted in a significant increase in the proportion of red clover in the hay, at the expense of reducing the share of weeds.

Key words: *additional fertilization, Italian ryegrass, nitrogen, red clover*

Introduction

In the Republic of Serbia (RS) the lawns participate with over 27 % of agricultural land (Djukić et al., 2008). Forage production is mainly realized in natural meadows and pastures and partly in the growing fields of red clover, alfalfa, mixtures of grasses and legumes, etc. Along with efforts to reduce energy consumption, environmental pollution, to intensify sustainable agriculture systems and sustain biodiversity, the possibility of the more frequent introduction of the forage legumes into grass mixtures should be considered. In this way, the use of mineral nitrogen fertilizers is reduced and thus the possibility of loss of nitrogen from the soil by leaching or gas emission (Ledgard et al., 1999). According to Vinther and Jensen (1999), the symbiotic nitrogen fixation at legumes is a fundamental process for maintaining soil fertility of soils and continuous productivity of the organic growing systems. By the cultivation of legumes and grasses in mixture, the more profitable production and better quality forage is achieved (Nešić et al., 2007). The share of weeds in the total yield of grass-legume mixture is significantly lower than the pure crop of grasses or legumes (Sleugh et al., 2000).

In the period of 2001-2005, the average hay yield in the meadows of the RS ranged from 1.5-2.0 t ha^{-1} (SGS, 2006). The most frequent cause of low and unstable sward yield, and low

quality of forage is the lack of application of agro-technical measures (Dubljević, 2007). With the proper fertilization of meadows and pastures with mineral and organic fertilizers, with rational exploitation, under the same conditions, it is possible to achieve the several times increase of the hay yield, while improving the quality of forage (Stevanović et al., 2004; Nešić et al., 2004; Vučković et al., 2004). One of the most important nutrients for achieving high forage yields of natural grasslands is nitrogen. Vitousek and Howarth (1991), Frink et al. (1999), LeBauer and Treseder (2008) point out that nitrogen is usually the limiting factor for high production of natural grasslands.

Soil acidity is one of the factors that complicate the cultivation of many crop plants, both legumes and grasses (Edmeades et al. 1981; Wheeler 1998). On acid soil *Rhizobia* bacteria survival is difficult and reproduction is slow, which results in lower yields of legumes (Nutman, 1976).

The aim of this study was to study the effect of additional nitrogen fertilizing of soil on the forage yield of red clover-Italian ryegrass grass-legume mixture on acid soil.

Materials and methods

The experiment was established in the period 2011-2013 in Čačak (43°54'39.06" N, 20°19'10.21" E, 246m a.s.l.), on alluvial soil, acid reaction (pH_{H2O} 4.8), which contains 3.18% organic matter, 0% $CaCO_3$, 22.08 mg P_2O_5, 30.0 mg K_2O 100 g^{-1} soil (Gupta, 2008). 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 analyses were carried out at grass-legume mixtures of red clover (cultivar K-39, Institute for Forage Crops, Krusevac) and Italian ryegrass (Tetraflorum – Slovenian tetraploid variety). The experiment included two treatments: without fertilization (control) and additional application of nitrogen fertilizer (KAN - 27 % N) in the rate of 40 kg ha^{-1}. Nitrogen fertilization was done in the spring just before the start of the growing season in 2012 and 2013. Sowing was done with row spacing of 20 cm (Italian ryegrass and red clover were sown in the same rows), with the amount of seeds 12 kg ha^{-1} of red clover and 12 kg ha^{-1} of Italian ryegrass. The crop was grown without irrigation.

The mean annual air temperature in 2011, 2012, and 2013 was 12.37°C, 13.12°C and 12.99° respectively and amount of annual rainfall 374.5 mm, 463.5 mm and 582.7 mm respectively (Table 1). The average annual air temperature for the multi-year period (1992-2002) is 11.97°C, and the average amount of annual rainfall 680.3 mm.

The analyses were performed on the first and second cut in the second year of cultivation (2012) and the third cut in the third year of cultivation (2013). Mowing was done in the budding stage. Green forage yield was determined by measuring the total mass of the plot immediately after cutting at the optimum stage of growth and development of the plants. From a measured sample (1000 g) the weight share of grasses (*fam. Poaceae*), legumes (*fam. Fabaceae*) and the other species in the green fodder was determined. After drying the samples at 65°C, the hay yield (t ha^{-1}) was calculated. 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.

Table 1. Precipitation (P) and mean monthly temperatures (t) during 2011, 2012, 2013 and several years average (1992-2002).

Month	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$ i $\sum$
2011													
t (°C)	0.7	0.7	7.6	13.1	16.7	22	23.8	23.7	21.6	11.8	3.7	3	12.366
P (mm)	22	29	31	15.5	95.5	47	30.5	9.5	42	21	2.5	29	374.5
2012													
t (°C)	1.8	-2.5	6.8	12.2	17.3	24.1	26.6	25.4	20.9	13.8	9.5	1.4	13.12
P (mm)	60	70	10	47	68	38	22	0	7.2	30	23.7	87.6	463.5
2013													
t (°C)	3,5	3.8	6,6	13.2	18,2	20,6	23,3	24,1	17,2	14,5	8,9	2,0	12,99
P (mm)	51,0	68,0	65,7	37,0	78,5	61,5	10,0	62,5	87,0	17,0	40,5	4,0	582,7
1992-2002													
t (°C)	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
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

Results and discussion

Growing of grasses in mixture with legumes leads to the increase of the biological value of soil and fixation of significant amounts of nitrogen by bacteria of the genus *Rhizobium* which could be used by the plants (Wheeler, 1998). Increased biological value of soil affects the increase of organic matter mineralization in the soil (Wheeler, 1998), which contributes to the more intensive growth of grasses and achieving higher yields. According to Mandić et al. (2004) the highest biological value of the soil of the several examined grass-legume mixtures has been noted under a mixture of red clover and Italian ryegrass. Italian ryegrass is an ideal species for growing in mixture with red clover (Simić et al., 2011). Additional nitrogen fertilization in the first cut in 2012 did not affect the significant increase of the forage and hay yield of the grass-legume mixture (Table 2). The average forage yield was 42.35 t ha^{-1}, and the average yield of hay 19.17 t ha^{-1}. Additional nitrogen fertilization in the second cut in 2012 and in the first cut in 2013 as well did not significantly affect the increase of forage and hay yield. The total forage yield in the second cut in 2012 in the control treatment was by 70.2% lower, and in the treatment with additional nitrogen fertilization 78.8% lower in relation to the first cut in 2012. In the first cut in 2013 (the third year of production) forage yield was lower than in the first cut in 2012 by 63.8% in the control treatment and by 66.6% lower in the treatment with fertilization. According to Dubljević (2007), natural lawn fertilization with nitrogen is very important because its application increases forage and crude protein yields, potentiates growth and tillering of grasses, increases the density of grass cover, extends vegetation and slows down the aging of plants. According to Xia and Wan (2008), fertilizing the lawn with nitrogen increases the yield of carbon in the aboveground part, directly by entering the life processes of the plants and indirectly, by affecting on the rapid mineralization of organic matter in the soil (Nowinski et al., 2008). Vučković et al. (2004) reported that application of 160 kg N ha^{-1} resulted in the increase of the natural lawn biomass by 153% compared to the control. Ivanovski et al. (2004) point out the positive effects of surface application of manure and mineral fertilizer in spring on the dry matter yield of the grasslands, while not identified significant changes in the chemical composition of the dry matter. Ocokoljić et al. (1983) indicate that the application of nitrogen fertilizers significantly increases the yield and content of proteins, which is, according to Alibegović-Grbić et al. (2004), above all the consequence of increase in dry matter yield. According to Stevanović et al. (2004), Nešić et al. (2004), Vučković et al. (2004), Alibegović-Grbić et al. (2004), Stevens et al. (2004), fertilization may significantly affect the yield and quality of dry matter of natural grasslands, as well as the change in their floristic composition. Poor response of the grass-legume mixture of red clover and Italian ryegrass to additional nitrogen fertilizing of our

research proves that due to leguminous component in the mixture, nitrogen was not a limiting factor for achieving high yields.

Table 2. The effect of additional nitrogen fertilization on yield of forage (GFY) t ha^{-1}, hay yield (HY) t ha^{-1}, the proportion of Italian ryegrass (IR%), red clover (RC%) and weeds (W%) (%) in total forage yield of grass-legume mixtures of red clover and Italian ryegrass.

		GFY	HY	IR%	RC%	W%
First cut 2012	O	39.20	9.03	76.3	22.8	1.46
	N	45.50	10.14	84.4	9.10	2.03
	ANOVA 0.05	ns	ns	ns	**	ns
	cv %	8.7	15.3	8.4	22.6	29
Second cut 2012	O	11.67	4.49	57	42.4	0.48
	N	9.64	3.2	77.9	20.2	1.98
	ANOVA 0.05	ns	ns	**	**	*
	cv %	13.9	29.3	6.4	13.8	27.3
First cut 2013	O	14.20	4.89	-	47.0	53.0
	N	15.20	5.37	-	76.7	23.3
	ANOVA 0.05	ns	ns	-	**	**
	cv %	19	23.8	-	6.4	10.3

** - F test significant at $p<0.01$; * - F test significant at $p<0.05$; ns - F test non-significant

The proportion of red clover in the total hay yield in the first and second cut in 2012 significantly decreased with additional nitrogen fertilization at the expense of increasing the share of Italian ryegrass and weeds share. This is due to a better reaction of Italian ryegrass whose competitiveness are somewhat more pronounced compared to the clover, when fertilized with nitrogen, especially in the drought conditions that occurred during the vegetation period in 2012. According to Garwood and Wiliams (1967) white clover (*Trifolium repens* L.) is more drought-tolerant than Italian ryegrass due to better nitrogen provision (due to the process of nitrogen fixation), whose adoption from the land is reduced in the conditions of lack of water. According to Živanović-Katić (2004), denser crops suppress the weeds and prevent their mass appearance. Natural lawn fertilization with nitrogen leads to a reduction of plant diversity, reducing the presence of legumes and C4 plants and increase the presence of C3 plants (Gough et al. 2000, Stevens et al. 2004). According to Xia and Wan (2008), this phenomenon is the consequence of increased competition of plants, especially for light. Proportion of red clover was significantly higher in the second cut when compared to the first cut in 2012, which is the consequence of its greater tolerance to drought in relation to Italian ryegrass. In the first cut in 2013, the additional nitrogen fertilizing resulted in a significant increase in the proportion of red clover in relation to the control at the expence of reducing the share of weeds. There were no plants of Italian ryegrass, considering that this is the third year of crop, and the red clover crop was sparse and exausted. The additional nitrogen fertilization in such conditions contributed to a better regeneration and more rapid growth of red clover, particularly in the early stages of growth, leading to an increase of its competitive ability in relation to the weeds.

Conclusion

The high and stable yields of good quality forage on lawns can be achieved with proper fertilization and rational utilization. Additional application of nitrogen did not significantly increase yields of forage and hay in all cuts in both years. Weak reaction of red clover-Italian ryegrass mixture on additional nitrogen fertilizing proves that due to the leguminous

component in the mixture, the nitrogen was not a limiting factor for achieving high yields. However, the red clover share in the total hay yield in the first and second cut in 2012 significantly reduced with additional fertilizing with nitrogen at the expence of increase of Italian ryegrass and weed share. This is due to better reaction of Italian ryegrass, whose competitiveness are somewhat stronger compared with red clover, as a responce to fertilizing with nitrogen, especially in the drought conditions that occurred during the vegetation period in 2012. In the first cut in 2013, the additional nitrogen fertilizing affected significantly on the increase of red clover share in relation to the control treatment at the expence of reducing the share of weeds. Given that this is the third year of the crop, there was no Italian ryegrass, and the red clover crop was sparse and exausted. Additional nitrogen fertilization in such conditions contributed to a better regeneration and more rapid growth of red clover, especially in the early stages of growth, leading to an increase in its competitive ability in relation to the weeds.

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