

EVALUATION OF COMPETITIVE ABILITY OF PERENNIAL RYEGRASS IN MIXTURES WITH RED CLOVER AND LUCERNE SUBJECTED TO DIFFERENT LEVELS OF N

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Original scientific paper

Abstract: Grass-legume mixtures represent complex communities of grasses and legumes that make better use of resources and have a number of positive traits in comparison to monoculture. The aim of our research was to examine competitive ability of perennial ryegrass in mixtures with lucerne and red clover (50/50, 70/30) in condition of fertilization with three different nitrogen levels (0, 50, 100 kg ha⁻¹). Dry matter yield of perennial ryegrass in mixtures with red clover was higher compared to mixtures with lucerne. Nitrogen had a very significant impact on perennial ryegrass production. Added N significantly increase DM production of grass treatment with 100 kgN ha⁻¹.

The values, of relative yield above 1 (RY >1), aggressivity index and competitive balance index, above 0 (Ag > Cbg > 0) indicated higher competitive ability of perennial ryegrass in relation to legumes. Competitive ability of ryegrass was higher in 70/30 mixtures and mixtures with lucerne. N fertilization increases competitive ability of ryegrass and decreases competitive ability of legumes.

Key words: aggressivity, competition, grass-legume mixtures, species balance, yield

Introduction

Grass-legume mixtures are a complex systems of highly productive species which contribute to better utilization of natural resources and achieving higher and stable yields.

Each species in the mixture has a special role contributing to the good functioning of the community. Species differ in their morphological and physiological characteristics, which enables them to exploit resources in different ways, i.e. to exploit the environment more efficiently than pure crops. Therefore, due to the different depth and development of the root system of grass and legumes, the utilization of water and mineral substances in the mixture is more efficient than in pure crops. Different shape and leaf exposures allow more efficient use of sunlight. As a consequence of this, there is a higher yield of mixtures relative to monoculture (*Annicchiarico and Tomasoni, 2010*). According to *Sturludottir et al. (2014)*, examined mixtures were 7, 9 and 15% more productive than the most productive monoculture. In research of *Helgadottir et al. (2018)* these differences were higher and mixtures performed yields that were higher than the yields of monocultures for 36 and 39%.

Legumes are very important component of a system. Leguminous plants adopt nitrogen through nitrogen fixation from the atmosphere and enrich the soil. In this way they meet their nitrogen needs and part of the needs of non-leguminous species (*Mulder et al., 2002*). The amount of nitrogen that legumes in the monoculture can fix is around 350 kgN ha⁻¹ year⁻¹ in alfalfa (*Medicago sativa* L.), 373 kgN ha⁻¹ in red clover (*Trifolium pratense* L.) and 545 kgN ha⁻¹ year⁻¹ in white clover (*Trifolium repens* L.). Growing in the mixtures with legumes, grasses take a larger fraction of their nitrogen from N₂ fixation (about 80%), depending on the management, dry matter yield and location (*Huss-Danell et al., 2007*). Part of the fixed nitrogen is released by legumes into the root zone making it available to other non-leguminous species. *Brophy and Heichel (1989)* have found that the lucerne releases 4.5% of the total fixed nitrogen, while other species release significantly more. Thus, *Louarn et al. (2015)*, by examining the mixture of alfalfa and white clover with tall fescue and perennial ryegrass, have found that the amount of transferred nitrogen to grasses ranges from 43 to 72 kgN ha⁻¹ year⁻¹. From an ecological point of view it is very important, since reduces the need for the use of nitrogen mineral fertilizers.

Leguminous plants are a rich source of protein which increases the nutritional value of the mixture relative to the grass monoculture (*Sturludottir et al., 2014, Brink et al., 2015*). In addition, they increase digestion and consumption (*Peyraud et al., 2009*).

Grass-legume mixtures as a very competitive community, does not leave enough space for weeds development and enhance their suppression (*Bijelić et al., 2017a, Helgadottir et al., 2018*).

Persistence of plants in swards mostly depends of species ability to cope with a complex of environmental and agro-technical conditions. According to this to maintain mixture structure and advantages it is necessary to balance between different species in the mixtures. To meet production targets producers use some agrotechnical measures like N fertilization which together with other management

practices can have depressive effect on growth and persistence of legumes in the crops. In research of *Enriquez-Hidalgo et al.* (2015), application of N fertilizer reduced the potential of white clover to compete with the associated grass. It depressed clover content for 41% and clover yield while it increased yield of accompanied grasses in the mixtures. *Leto et al.* (2008) in their study stated that fertilization with N gave 29% higher grasses DM yield, 9% higher grasses content and decreased legume contribution. Similar conclusions were made in the research by *Nyfelner et al.* (2009) and *Bijelić et al.* (2017b). *Nyfelner et al.* (2009) were summarize that for achieving maximal yield mixtures which were fertilized with 50 kgN ha⁻¹ have to have the legume content of 50 – 70%. Increasing the amount of nitrogen reduces the percentage of the legumes necessary for achieving maximal yield.

Therefore, maintenance of an appropriate content of species in grass-legume mixtures for achieving the maximum yield is a great challenge hence occupies the attention of many researchers beside agronomist. Thus, breeders propose a novel framework for improving forage mixtures by breeding. They suggest that breeding programs should be based not only on agronomic traits but also on interaction traits for sustainable production (*Litrico and Violle, 2015*).

The objectives of our research were to (i) estimate forage yield of perennial ryegrass-legume mixtures sown in two different seeding ratios (50/50, 70/30) as well as dry matter yield and competitive ability of each species in the mixtures and (ii) to study the effect of N fertilization on already mentioned parameters with intention to determine the level N fertilizer at which mixtures will achieved a satisfactory yield without losing the contribution of any of the components.

Material and methods

The experiment was established at the experimental field of the Institute for Animal Husbandry in spring 2014. The design of the experiment was split-plot with forage mixtures as a main plot and the fertilizer treatments as the subplot. All plots were randomly organized in three replications. Plot size was 2.0 x 5.0 m. Three species were used for the composition of forage mixtures: red clover (cv. K-39), lucerne (cv. NS Banat) and perennial ryegrass (cv. Calibra). This species were combined in two different ratio as the % of sowing rate of pure crop: perennial ryegrass + red clover (50/50 and 70/30); perennial ryegrass + lucerne (50/50 and 70/30). Seeding rates were 25 kg ha⁻¹ for perennial ryegrass and 20 kg ha⁻¹ for legumes. All stands were sown at 20 cm spacing between rows. In the experiment, three nitrogen rates were tested, 0, 50 and 100 kgN ha⁻¹. Nitrogen was added in the form of ammonium nitrate in stage of stem elongation. Dry matter yield was recorded over three cuts in each two production years. Whole plot was harvested and measured fresh weight. Herbage samples of 1 kg fresh mass were taken from each plot, oven dried at 60°C for 48 h and weighed for calculation of DM yield.

The proportion of legumes, ryegrass and weeds were assessed by separation each one from 1m², measuring weight proportion and calculation. The level of competitiveness in the mixture is expressed by relative yield total (RYt) and relative yield of species (RY). To estimate relative yield total and relative yield of each species in the mixtures, equations defined by *McGilchrist and Trenbath* (1971) and *De Wit and Van Den Bergh* (1965) were used:

$$RYT=(RYg+RYl)/2$$

$$RYg=Ygl/Ygg, \text{ for perennial ryegrass,}$$

$$RYl=Ylg/Yll, \text{ for legumes,}$$

where Ygl and Ylg represents yield of perennial ryegrass and legumes in the mixtures, respectively; Ygg and Yll are the yield of perennial ryegrass and legumes in their monocultures. If RY=1 means that tested species competing against plants of its own species as well as with other species. If RY>1 means that tested species competing better with other species than with its own species. An RY<1 means that tested species is less competitive than other species.

How aggressively species act in the mixture were quantify by aggressivity index. For calculation of perennial ryegrass aggressivity equation of *McGilchrist and Trenbath* (1971) were used:

$$Ag=0.5(RYg-RYl)$$

when Ag=0, Ag>0 and Ag<0, than perennial ryegrass are as aggressive as, more aggressive and less aggressive than legumes in the examined mixtures.

Competitive balance is an index of competitive ability of one species in the mixture. The equation of the competitive balance was first established by *Wilson* (1988) and modified by *Williams and McCarthny* (2001). It uses natural logarithm of the yield of the both species in the mixtures. The formula for Cbg calculation is as follows:

$$Cbg=\ln(RYg/RYl)$$

if Cbg=0 the perennial ryegrass is competitive as legumes, if Cbg<0, perennial ryegrass is less competitive than legumes and if Cbg>0 perennial ryegrass is more competitive than legumes.

Data for the dry matter forage production, species competition and aggressivity which were obtained from four mixtures set in three replication and fertilized with three nitrogen levels in two years were analyzed by two-way ANOVA. Prior to the analysis, Shapiro-Wilk test of normality was done like as Levene's test for testing homogeneity of variances. Differences among means were

determined with LSD at the probability level of 0.05. General linear models for ANOVA was used (SPSS 2011).

Results

Significant differences were observed in total dry matter production of two mixtures. Additionally, dry matter production of their components differed significantly among examined mixtures and treatments of N fertilization for grass and seeding ratio for legumes (Table 1). The mixture of the perennial ryegrass and red clover produced about 18% higher forage yield for both species ratios than the mixture of perennial ryegrass-lucerne. The highest yield of 10.8 t ha⁻¹ provided 50/50 red clover mixture while the lowest 70/30 lucerne mixture of 7.8 t ha⁻¹ (Fig. 1a). Generally, the yield of the mixtures decreased with an increase of perennial ryegrass proportion in the mixtures. Although N fertilization had no significant impact, it had depressive effect on DM production, especially the level of 50 kgN ha⁻¹ (Fig. 1b).

Dry matter yield of perennial ryegrass in red clover mixtures was higher by 17.6% compared to lucerne mixtures (Fig. 1c). Since grasses respond well to nitrogen fertilization this factor had a very significant impact on perennial ryegrass production. Regarding that added N let to a marked increase of grass DM production relative to N unfertilized mixtures (Fig. 1d). The response to N fertilization was the most prominent in treatment with 100 kgN ha⁻¹ even by 107.3%.

The observed significant variations of DM legume production occur only as a consequence of the seeding ration. For both mixtures 50/50 ratio had higher DM legume production according to 70/30 mixtures (Fig. 1e). Although the other two factors did not have a significant impact, the yield of legumes in red clover mixtures was higher while N fertilization, conversely, reduced DM production of legumes. This reduction is particularly noticeable in the 50 kgN ha⁻¹ treatments. (Fig. 1f).

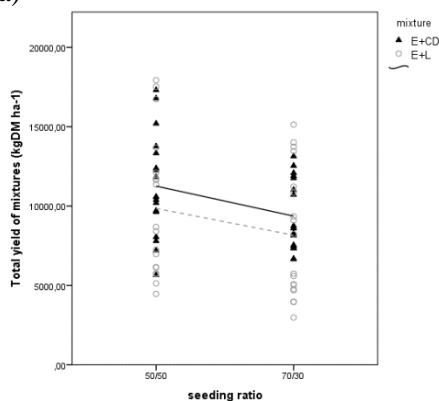
Table 1. Statistical significance for dry matter production of mixtures and its components (DMm, DMg, DMI), relative yield total (RYt), relative yield of grass (RYg) and legumes (RYl) in mixtures, index of grass aggressivity (Ag) and index of competing balance of grass (Cbg).

Treatments	DMm	DMg	DMI	RYt	RYg	RYl	Ag	Cbg
Mixture	p<0.05	p<0.05	ns	p<0.05	ns	ns	ns	ns
Seeding ratio	ns	ns	p<0.01	p<0.01	ns	p<0.01	ns	p<0.05
N fertilization	ns	p<0.01	ns	p<0.01	p<0.01	ns	p<0.01	p<0.01
Mixture x Seeding ratio	ns	ns	ns	ns	ns	ns	p<0.05	ns
Mixtures x N fertilization	ns	ns	ns	ns	ns	ns	ns	ns
N fertilization x Seeding ratio	ns	ns	ns	ns	ns	ns	ns	ns
Mixture x Seeding ratio x N fertilization	ns	ns	ns	ns	ns	ns	ns	ns

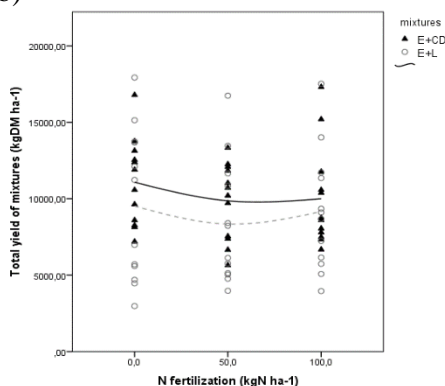
ns- non significant; significant at $p \leq 0.05$; significant at $p \leq 0.01$;

There were significant differences among the different level of N fertilization, seeding ratio and mixtures for the tested values of RYt, RYg, RYl, Ag and Cbg. Both mixtures and seeding ratio showed $RYt < 1$ what means that all species in the mixtures compete for the same resources. RYt was significantly higher in red clover mixtures like in seeding ratio of 50/50. N fertilization significantly increased values of RYt. For control treatment and treatment with 50 kg N ha^{-1} RYt was under 1. However, fertilization with 100 kg ha^{-1} increased values of RYt above 1.

a)



b)



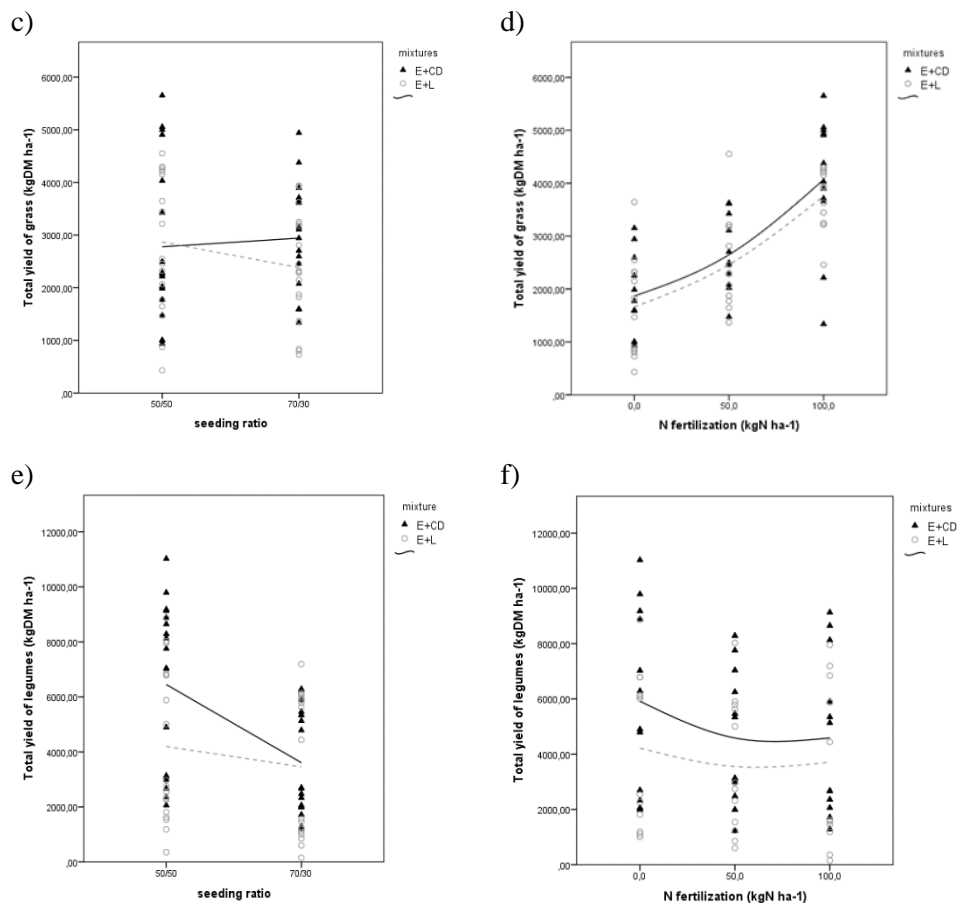


Figure 1. Impact of N fertilization and seeding rate on total dry matter yield of a mixtures (a, b), grass component (c, d), and legumes component (e, f).

As well as RYt, RYg was higher in red clover mixtures and 50:50 seeding ratio. The average RYg, across mixtures, was significantly higher for nitrogen treatments compared to control. In control values of RYg were under the 1 while in fertilized treatments indices values ranged from 1.14 – 1.62. The corresponding RY of legumes were under 1 for all examined factors. Seeding ration of 50/50 showed significant increase in RYl. Significant changes of the aggressivity index values are visible only in the fertilization treatment. N fertilization significantly increased aggressivity of perennial ryegrass in mixtures with red clover and lucerne. The treatment of 100 kgN ha⁻¹ gave the highest aggressivity 0.66. For all mixtures it

showed values that were over 0 which indicates a greater aggressivity of the grass in regard to leguminous plants.

Table 2. Effect of examined factors on relative yield total (RYt), relative grass yield (RYg), relative legume yield (RYl), aggressivity index of grass (Ag) and index of competitive balance (Cbg)

Factors	RYt	RYg	RYl	Ag	Cbg
Mixture					
E+CD	0.95a	1.27	0.64	0.31	0.75
E+L	0.83b	1.10	0.55	0.27	0.73
Seeding ratio (%)					
50/50	0.98a	1.23	0.72a	0.26	0.54b
70/30	0.80b	1.13	0.47b	0.33	0.94a
N fertilization (kgN ha ⁻¹)					
0	0.70b	0.79c	0.66	0.09c	0.28b
50	0.83b	1.14b	0.52	0.31b	0.83a
100	1.14a	1.62a	0.60	0.48a	1.10a

E-perennial ryegrass; CD-red clover; L-lucerne; Means with the different letters are significantly different at the 0.05, according to the LSD test.

Among the tested mixtures, Cbg showed the significant highest value in 70/30 perennial ryegrass-red clover mixture. As for aggressivity, N fertilization showed positive impact on Cb. The highest value of Cb had treatments with 100 kgN ha⁻¹ and the lowest treatments without fertilizer. For all treatments values were above 0 what means that ryegrass was more competitive than legumes.

Discussion

The data from the experiment indicate that dry matter production of binary perennial ryegrass-red clover mixtures was higher than that of perennial ryegrass-lucerne mixtures. In research of *Halling et al. (2004)* mixtures of perennial ryegrass and red clover yielded more compared to perennial ryegrass-lucerne mixtures in first examined year. In second year lucerne mixtures significantly outyielded other mixtures. Also, in research of *Gokkus et al. (2009)* in first experimental year mixtures of red clover and smooth brome grass gave higher yield than lucerne-smooth brome grass. Although the remaining two factors had no significant impact, N fertilization reduced the yield of the mixtures as well as the increase in the content of the perennial ryegrass in seeding ratio. Given that the legumes have the greatest impact on biomass production in grass-legume mixtures, this reduction of yield can be the result of reduced nitrogen fixation and the contribution of legumes in the mixture under the N fertilization (*Enriquez-Hidalgo et al., 2015*) and increased grass biomass, whereby causing a reduction in the space available for the growth of legumes (*Kai-yun et al., 2015*). Contrary, according to

Sengul et al. (2003) N fertilization enhance the DM yield in all mixture combination.

Evenness in grass-legume mixtures is very important for achieving high yields. In research of *Nyfele et al. (2009)* equal stand mixtures yielded more than mixtures dominated by one species. Also, *Kyriazopoulos et al. (2013)* found higher yields in 50/50 mixtures than in 75/25 mixtures (*D. glomerata: T. subterraneum*). In our results mixtures with seeding ration 50/50 yielded more than mixtures with 70/30 species ratio.

Synchronization of N supply and demands in grass-legume mixtures is an important factor for achieving sustainable grassland production (*Lüscher et al., 2014*).

N fertilization increased significantly the herbage production of perennial ryegrass in contrast to the yield of two legumes. This result is in agreement with researches of *Youlcu et al. (2010)* and *Kia-yun et al. (2015)*. Added nitrogen through the fertilization negatively affects symbiotic fixation in legumes, what encourages the grass components to occupy a wider space in the mixtures subtracting scope for legumes development. Further, this resulted in greater grass proportion and greater yield (*Soussana and Tallec, 2010*).

The presence of legumes in mixtures had generally more positive effect on forage yield than the presences of other groups of plants (e.g. forbs, grasses) (*Lambers et al., 2004*). In research of *Nyfele et al. (2009)* the stands were given highest yield when the clover proportion was ranged between 30 and 80% depending on the year and the N fertilizer treatment. Legumes DM yield were effected only by the seeding ratio. 50/50 mixtures had higher legumes DM yield than 70/30 mixtures. Decrease in yield from 50/50 to 70/30 ratio is greater for red clover than for lucerne. Similar results have been reported by *Kyriazopoulos et al. (2013)*.

The index of relative yield total for mixtures gives indication whether between species in mixtures exists some kind of avoidance of competition, no competition or antagonism (*Williams and McCarthy, 2001*). All mixtures regardless of species and seeding ratio had RYt under 1 what means that there is strong competition for available resources. Fertilizers can change competitive relationships of a species, leading to variations in the outcome of competition (*Gao et al., 2005*). N fertilization at level of 100 kgN ha⁻¹ increase that value above 1 that is provided sufficient resources for the species not to compete with each other.

The relative yield of each species corresponds to productivity of that species in the mixture relative to productivity of that species in sole crop. Values of this indicator were for grass above 1 and for legume under 1. That means that red clover and lucerne produced lower DM yield in mixture with ryegrass than in monoculture. This points to a better competitive ability of perennial ryegrass what is documented with Cbg>0 and Ag>0. Furthermore, ryegrass behaves much more competitive and aggressive in the 70/30 mixtures. Also, *Kyriazopoulos et al.*

(2013) in their research, have proven greater aggressiveness of the *Dactylis glomerata* in 75/25 mixtures with *Trifolium subterraneum*. N fertilization favoured competitive ability and aggressivity of ryegrass particularly level of 100 kgN ha⁻¹.

Conclusion

From this study we can confirmed that mixtures of red clover and ryegrass can achieve higher yields than lucerne-ryegrass mixtures in first two years of production in existing agroecological conditions. Regardless of the species, 50/50 seeding ratio mixtures revealed higher yields compared to 70/30 mixtures. Perennial ryegrass behaves like a superior competitor to red clover and lucerne under given circumstances. Nitrogen fertilization strengthens the competitive ability of the perennial ryegrass which adversely affects the yield of legumes in the mixture and the yield of the mixture themselves. Generally, we can conclude that in this conditions, cultivation of the mixture would be justified and sustainable in sowing grass to legume ratio 50/50 and without the use of N mineral fertilizers.

Ocena konkurentne sposobnosti engleskog ljulja u smešama sa crvenom detelinom i lucerkom đubrenim različitim količinama azota

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Rezime

Travno-leguminozne smeše predstavljaju složene zajednice trava i leguminoza koje bolje koriste prirodne resurse i imaju niz pozitivnih osobina u odnosu na monokulturu. Cilj našeg istraživanja je bio da se ispita konkurentna sposobnost engleskog ljulja u smešama sa lucerkom i crvenom detelinom (50/50, 70/30) u uslovima đubrenja sa tri različita nivoa azota (0, 50, 100 kg ha⁻¹). Prinos suve materije engleskog ljulja u smeši sa crvenom detelinom bio je veći u poređenju sa smešom sa lucerkom. Azot je imao značajan uticaj na prinos engleskog ljulja. Dodati azot je značajno povećao prinos engleskog ljulja, naročito doze od 100 kgN ha⁻¹. Vrednosti relativnog prinosa iznad 1 ($RI > 1$), indeksa agresivnosti i indeksa konkurentne ravnoteže iznad 0 ($Ag > Cbg > 0$) ukazuju na veću konkurentnu sposobnost engleskog ljulja u odnosu na mahunarke. Konkurentna sposobnost engleskog ljulja bila je veća kod 70/30 smeša i smeša sa

lucerkom. N djubrenje povećava konkurentsku sposobnost ljujla i smanjuje konkurentsku sposobnost leguminoza.

Acknowledgement

The authors thank to the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project "Implementation of new biotechnological solutions in breeding of cattle, sheep and goats for the purpose of obtaining biologically valuable and safe food", TR 31053.

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