NODULATION AND ROOT ESTABLISHMENT OF TWO CLOVER SPECIES GROWN IN PASTURE MIXTURE WITH WHEATGRASS

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Abstract

A field trial was conducted during 2007-2010 at the Institute of Forage Crops, Pleven, Bulgaria to study the nodulation of subterranean clover (*Trifolium subterraneum* L.) and white clover (*Trifolium repens* L.) in some pasture mixtures using wheatgrass (*Agropyron cristatum* L.) as grass component. The treatments tested were: Subterranean clover (100%), White clover (100%), Subterranean clover + Wheatgrass (50%:50%), White clover + Wheatgrass (50%:50%), Subterranean clover + White clover (50%:50%), Subterranean clover + White clover + Wheatgrass (25%:25%:50%). It was found that subterranean clover in a mixture with wheatgrass formed 11.9% more nodules and by 11.0% higher root dry mass than in pure sward. White clover nodulated better in pure sward as compared to mixture and subterranean clover showed better nodulation than the white clover. The results obtained in the present study also indicated that wheatgrass in a mixture with white clover inhibited the root dry mass production by 22.2%. In the triple mixture (wheatgrass, subterranean clover and white clover) no significant differences were observed in characteristics of nodulation and dry mass production.

Introduction

Legume-rhizobial symbiosis is a significant source of nitrogen and plays an important role in ecological and agronomic structure of sustainable agriculture (Dita *et al.*, 2006). Often legumes are grown in mixtures with grasses. The choice of suitable components is important to create sustainable and balanced mixture (Peeters *et al.*, 2006). Advantage of grass-legume mixtures is the ability to supply grass component with nitrogen through symbiotic nitrogen fixation (Botha, 2002; Nyfeler *et al.*, 2005). Nodulation and nitrogen fixation in mixture can be variable over the years and is related to the proportion of legumes in the sward (Kristensen *et al.*, 1995).
White clover is a nitrogen-fixing crop resistant to grazing and has an high nutritive value which makes it suitable for pasture mixtures (Elgersma & Hassink, 1997; Frame *et al.*, 1998; Hepper & Lee, 1979; Kleen *et al.*, 2006; Leung & Bottomley, 1994). Subterranean clover is an annual drought resistant legume with winter-spring type of development and selfsowing ability (Yakimova and Yancheva, 1986, 1987). It is widespread component in pastures of temperate areas of middle and northern Europe and America. However, it is a relatively new crop for Bulgaria. The studies to date show its ability to successfully overcome the difficult winter period conditions of Danube plain which are crucial for its possible establishment in perennial grass mixtures (Vasilev, 2006, 2009). In pot study on nodulation of birdsfoot trefoil in mixture with white clover, birdsfoot trefoil formed 25-27% more nodules than pure growing, and subterranean clover depressed the nodulation of white clover by 52.5% (Vasileva & Ilieva, 2009). There are sporadic studied conducted in Bulgaria on nodulation and symbiotic nitrogen fixation in forage legumes in mixtures with non-nitrogen-fixing pasture crops. The aim of this study was to investigate the nodulation and root establishment of subterranean and white clover in some grass-legume and legume mixtures.

Materials and Methods

The trial was conducted out at the experimental field of Institute of Forage Crops, Pleven Bulgaria during t 2007-2010. The experiment was initiated in the fall of 2007 using subterranean clover (*Trifolium subterraneum* L.) cv. Clear, white clover (*Trifolium repens* L.) cv. Milka and wheatgrass (*Agropyron cristatum* L.) cv. Svejina. The seed of these species were obtained from the forage production unit of our Institute. The size of plot was 6 m^2 . The sowing was done manually keeping line spacing of 11.5 cm. The sowing rate was kept at : subterranean clover 2.5 kg/da, white clover 1.1 kg/da and wheatgrass 2.5 kg/da. The experiment was conducted in a randomized block design with 4 replications of each treatment. The treatments were tested were: Subterranean clover (100%), White clover (100%), Subterranean clover + Wheatgrass (50%:50%), White clover + Wheatgrass (50%:50%), Subterranean clover + White clover (50%:50%), Subterranean clover + White clover + Wheatgrass (25%:25%:50%).

Local agronomical practices and caring technologies in use for growing swards were adopted without fertilization. The fields were set for sheep (Pleven breed Black headed) grazing for two times in 2008 and four times in 2009. Soil monoliths were taken (20/30/40 cm) from each replicates in the beginning of flowering

stage of the legumes in the second and third year. Nodule number were counted per plant and dry root mass was recorded after drying at 60 ^{o}C (Beck *et al.*, 1993). The experimental data were averaged and statistically processed, using software SPSS for Windows 2000.

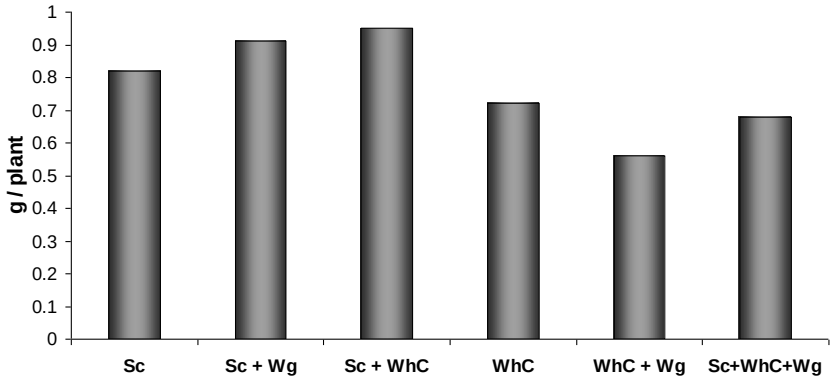

Fig. 1. Dry root mass of subterranean clover and white clover in mixtures SC- Subterranean clover; Wg- Wheatgrass; WhC- White clover

Table 1. Nodulation of subterranean clover and white clover in mixture

Treatments		**Number of nodules/plant**
Subterranean clover 100%	42	to subterranean clover
Subter. clover + Wheatgrass (50%:50%)	47	+11.9
Subter. clover + White clover (50%:50%)	3	-92.8
SE (P=0.05)	13	
White clover 100%	28	to white clover
White clover + Wheatgrass (50%:50%)	18	-35.7
Sub. clover+Wh. clover +Wheatgrass (25%:25%:50%)	27	-3.57
SE (P=0.05)	3	

Results and Discussion

Legumes depending on the availability of mineral nitrogen effectively regulate nodulating ability and their nitrogen-fixing efficiency (Nyfeler *et al.* 2006; Sebastia *et al.*, 2004). Elgersma and Hassink (1997) found that the uptake of mineral nitrogen in a mixture is more as compared to in pure sward and also affects the number of nodules formed. It was observed that subterranean clover in mixture with wheatgrass formed 11.9% more nodules as compared to pure growing (Table 1). Biological and morphological characteristics of the species in mixture, and mutual tolerance between them were different. In mixture there are different relationships between the plants, affecting their performance (Popov, 1971). Mutually inhibitory relationships was found between subterranean clover and white clover in regard to nodulation and it was almost absent in subterranean clover.
Ledgard and Steele (1992) found that in mixtures with some legumes, the grass component inhibited the process of nodulation. In our study wheatgrass depressed the nodulation of white clover by 35.7%. It is highly aggressive species with active growth in the spring and autumn, slower in summer and dominated over white clover (White, 1983). The data showed that white clover nodulated better as a pure crop than in the mixture. This may be due to the weaker sensitivity to mineral nitrogen levels in mixture. It is assumed that symbiotic nitrogen fixation in white clover is regulated by nitrogen need rather than the availability of carbohydrate reserves in the heel (Seresinhe *et al.*, 2008). This may be due to the cyclical nature of the dominance of legume and grass in mixture, depending on levels of soil nitrogen (Ledgard and Steele, 1992).

Root dry mass was an accompanying characteristic of nodulation. In the mixture of subterranean clover with wheatgrass, it was by 11.0% greater than in pure sward (Figure 1). In the mixture of subterranean clover with white clover, the root dry mass of subterranean clover was 15.8% lower compared to pure stand. This observation is in conformity with Vasileva and Vasilev (2011) who observed a 43% decrease in root dry mass of subterranean clover in mixture with white clover grown in pot conditions. In another study Vasileva (2011) found that root dry mass of white clover in mixture with wheatgrass was by 48% higher than that of pure white clover but does not affect dry mass production. The results obtained in the present study also indicate that wheatgrass in a mixture with white clover inhibited the root dry mass production by 22.2%. In the triple mixture (wheatgrass, subterranean clover and white clover) no significant differences were observed in characteristics of nodulation and dry mass production.

Conclusion

Subterranean clover in a mixture with wheatgrass formed by 11.9% more nodules and by 11.0% higher quantity of dry root mass as compared to the pure sward. White clover nodulated better in pure sward as compared to mixture and Subterranean clover showed better nodulation as compared to white clover.

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