

## ENHANCEMENT OF BIOMASS PRODUCTION OF BIRDSFOOT TREFOIL, SAINFOIN AND SUBTERRANEAN CLOVER BY MIXED CROPPING WITH PERENNIAL RYEGRASS

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### Abstract

The productivity of three leguminous crops birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis viciifolia* Scop.) and subterranean clover (*Trifolium subterraneum* L.) was evaluated in green house grown alone or mixed with *Lolium perenne* L. perennial ryegrass in 2013 and repeated in 2014. Each leguminous crops were grown alone (100%) or mixed with ryegrass at (50:50). Birdsfoot trefoil or sainfoin was also grown with subterranean clover along with perennial ryegrass grass (33:33:33). Higher productivity was found with the treatments of mixtures, i.e., for dry aboveground biomass, by 20.4%, and for dry root biomass, by 25.4%, respectively. More stable productivity of plant biomass was obtained in mixtures.

**Key words:** Subterranean clover, Mixtures, Dry biomass, Root biomass, Sustainable yield index.

### Introduction

Considerable evidences have now been available regarding the benefits associated with mixed cropping of legumes with grasses for the development of environmentally friendly agriculture (Kusvuran *et al.*, 2014a; 2015; Luscher *et al.*, 2014). Mixed cropping are more effective over pure with regard to the use of resources for plant growth and nitrogen transfer from legume to grasses (Mahapatra, 2011; Kusvuran *et al.*, 2014b; Jani *et al.*, 2015, 2016). Under unfavorable condition mixed crops can overcome better than mono cropping (Porqueddu *et al.*, 2003; Peyraud *et al.*, 2009; Bostan *et al.*, 2013). They are more productive than pure stands and obtained forage had more balanced nutritive value (Vasilev *et al.*, 2005; Chourkova, 2007; Albayrak *et al.*, 2011; Saruhan *et al.*, 2012; Kusvuran *et al.*, 2014c; Stevanovic *et al.*, 2015). In addition, Nikolova & Kertikov (2008) found that population density of the insect pest complex in stands of pea-triticale mixture was considerably lower, as compared to pure cultivation of winter forage pea.

The annual drought resistant legume *Trifolium subterraneum* L., well known as subterranean clover has the ability for self-sowing (Yakimova & Yancheva, 1986). It is widely distributed in Middle and Northern Europe, and America (Nichols *et al.*, 2012). Subterranean clover has practical applicability under the climatic conditions of Bulgaria (Vasilev, 2006, 2009; Vasileva *et al.*, 2011; Vasilev & Vasileva, 2012a, 2012b; Ilieva *et al.*, 2015). Resistance to winter, tolerance to summer drought and self-sowing at the end of spring is an advantage in comparison to white clover (Porqueddu *et al.*, 2003; Mihovski & Goranova, 2007). In our previous studies, subterranean clover was found a suitable component for mixtures with commonly used grasses for forage production (cocksfoot, tall fescue, wheatgrass) (Ilieva & Vasileva, 2011; Vasileva & Vasilev, 2012a, 2012b; Vasileva, 2015). Similarly, birdsfoot trefoil and sainfoin were also found valuable components for mixtures (Demdoum *et al.*, 2010; Chourkova, 2010, 2014). The present report describes the productivity of mixtures of birdsfoot trefoil, sainfoin and subterranean clover with perennial ryegrass.

### Material and Methods

The experiment was carried out in green house under semi controlled condition at the Institute of Forage Crops, Pleven, Bulgaria in 2013 and repeated in 2014. Three leguminous crops sainfoin (*Onobrychis Adans.*), birdsfoot trefoil (*Lotus corniculatus* L.) cv. "Targovishte 1" and subterranean clover (*Trifolium subterraneum* ssp. *brachycalicinum*) cv. "Antas" were sown in pots separately (100 %) or mixed with perennial ryegrass (*Lolium perenne* L.) cv. "IFK - Harmoniya" (50:50%). In another set subterranean clover was also grown with mixture of birdsfoot trefoil and perennial ryegrass (33:33:33%) or sainfoin and perennial ryegrass (33:33:33%). Plastic pots (6 l) were used filled with soil (leached chernozem subtype) (6 l). Seeds of birdsfoot trefoil, subterranean clover and perennial ryegrass were sown at the depth of 1-1.5 cm while sainfoin was sown at the depth of 3 cm. Treatments were replicated four times and arranged in a completely randomized plot design.

Plants were harvest twice (two cuts) to evaluate the productivity of leguminous crops. Plants were uprooted and roots were washed and blot dry. Fresh and dry above and below ground biomass (g/pot) were recorded. For dry biomass shots and roots were dried at 60°C and total productivity was calculated (dry aboveground biomass + dry root biomass). For the assessment of productivity, sustainable yield index (SYI) was used (Singh *et al.*, 1990).

$$SYI = (Y_m - S_d) / Y_{max},$$

where:  $Y_m$  – mean yield,  $S_d$  – standard deviation and  $Y_{max}$  – the maximum yield

These characteristics were compared to the characteristics of pure legumes (birdsfoot trefoil, sainfoin and subterranean clover). Data of two years was averaged and statistically analyzed using software SPSS (2012).

## Results and Discussion

All mixtures tested showed higher productivity of dry aboveground biomass as compared to pure crops (Table 1). The productivity of dry aboveground biomass from the pure crops was similar in values. When compare the mixtures of birdsfoot trefoil with the pure birdsfoot trefoil an increased yield was obtained by 22.5% for the two components mixture with perennial ryegrass, and by 27.0% for the three components mixture with perennial ryegrass and subterranean clover, respectively.

Dry aboveground biomass productivity for the mixtures of sainfoin was higher by 10.7 and 15.8% higher as compared to pure grown sainfoin. Similar findings were observed in other study, where crested wheatgrass was used as a grass component (Vasileva, 2011). The promotion in dry aboveground biomass productivity for the mixture of subterranean clover with perennial ryegrass was by 22.1%. There is report of suppression the productivity of dry aboveground biomass in mixtures with some grass components. So, it was with a mixture of birdsfoot trefoil with wheatgrass (Vasileva, 2011). Perennial ryegrass although destructive as a species had no adverse effect on the productivity of dry biomass in all mixtures tested in our study.

The amount of dry root biomass of legume components in mixtures was found higher as compared to pure crops, in the birdsfoot trefoil's mixtures. We assume it was due to the peculiarity of this species to fix small amount of nitrogen from the atmosphere (Carlsson & Huss-Danell, 2003). The main factors for the formation of plant biomass in sainfoin according to Hardarson &

Atkins (2003) are nitrogen from the soil and from nitrogen fixation.

When the proportion of legume components was higher as in three components mixtures of sainfoin the productivity of dry root biomass higher with pure cultivated sainfoin by 18.0%. Mixtures of subterranean clover with perennial ryegrass produced higher dry root biomass. In the mixtures productivity of plant biomass was higher by 20.4% more dry aboveground biomass and by 25.4% more dry root biomass as compared to pure crops. Total productivity of plant biomass in mixtures was significantly higher (Fig. 1). In birdsfoot trefoil's mixtures – by 25.2 and 29.3% for two and three components mixtures and there were no significant differences between them. For sainfoin - the productivity of mixtures of sainfoin with perennial ryegrass increased by 7.7% higher as compared to pure sainfoin and twice more in the mixture with perennial ryegrass and subterranean clover. In the mixtures there was 22.7% more plant biomass obtained over pure crops. Total plant biomass productivity from two components mixtures was increased by 17.3% and almost twice as compared to three components mixtures (by 30.7%). In an analogous study with grass component tall fescue it was found that total productivity from the mixtures had been over 30.0% than pure cultivated crops (Vasileva, 2014). A large amount of plant biomass (aboveground and root biomass) could be explained with higher nitrogen use efficiency in mixtures (Vasileva *et al.*, 2015). Successful competition between components for available inorganic nitrogen in the mixtures resulted in higher nitrogen use efficiency in the system.

**Table 1. Productivity of dry above ground and dry root biomass.**

| Treatments                    | Ratio, % | First cut                   | Second cut | Average             |
|-------------------------------|----------|-----------------------------|------------|---------------------|
|                               |          | g/pot                       |            |                     |
| <b>Above ground Biomass</b>   |          |                             |            |                     |
| Birdsfoot trefoil             | 100      | 2.89                        | 3.02       | 2.96                |
| B. trefoil + Ryegrass         | 50:50    | 3.66                        | 3.59       | 3.63                |
| B. trefoil + Subcl + Ryegrass | 33:33:33 | 3.72                        | 3.80       | 3.76                |
| Sainfoin                      | 100      | 2.78                        | 2.66       | 2.72                |
| Sainfoin + Ryegrass           | 50:50    | 3.09                        | 2.93       | 3.01                |
| Sainfoin + Subcl + Ryegrass   | 33:33:33 | 3.29                        | 3.01       | 3.15                |
| Subclover                     | 100      | 3.43                        | 2.01       | 2.72                |
| Subclover + Ryegrass          | 50:50    | 4.22                        | 2.42       | 3.32                |
| <b>SE (P=0.05)</b>            |          | 0.16                        | 0.20       | 0.13                |
| <b>Root biomass</b>           |          |                             |            |                     |
| Birdsfoot trefoil             | 100      | 1.27                        | 2.18       | 1.73                |
| B. trefoil + Ryegrass         | 50:50    | 1.46                        | 3.01       | 2.24                |
| B. trefoil + Subcl + Ryegrass | 33:33:33 | 1.62                        | 2.96       | 2.29                |
| Sainfoin                      | 100      | 2.81                        | 3.09       | 2.95                |
| Sainfoin + Ryegrass           | 50:50    | 2.91                        | 3.28       | 3.10                |
| Sainfoin + Subcl + Ryegrass   | 33:33:33 | 3.21                        | 3.74       | 3.48                |
| Subclover                     | 100      | 1.98                        | 0.97       | 1.48                |
| Subclover + Ryegrass          | 50:50    | 2.41                        | 1.14       | 1.78                |
| <b>SE (P=0.05)</b>            |          | 0.25                        | 0.36       | 0.25                |
| <b>Average for</b>            |          | <b>Above ground biomass</b> |            | <b>Root biomass</b> |
|                               |          | <b>g/pot</b>                |            |                     |
| Pure crops                    |          | 2.80                        |            | 2.05                |
| Mixtures                      |          | 3.37                        |            | 2.57                |
| Two components mixtures       |          | 3.32                        |            | 2.37                |
| Three components mixtures     |          | 3.46                        |            | 2.88                |
| <b>SE (P=0.05)</b>            |          | 0.14                        |            | 0.17                |

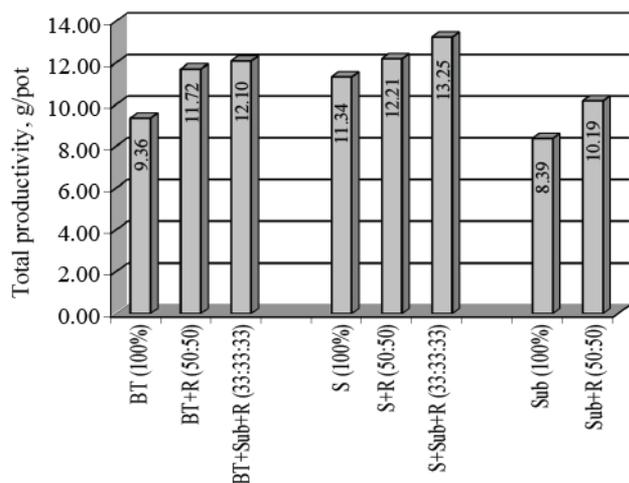


Fig. 1. Total productivity (dry aboveground biomass + dry root biomass) of mixtures.

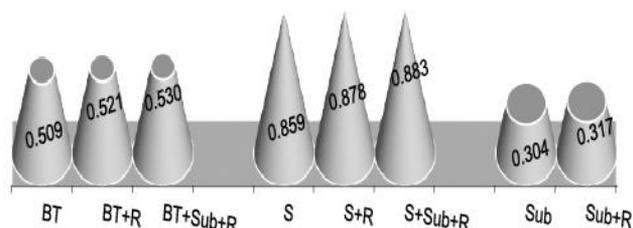


Fig. 2. Sustainable yield index (SYI) of the total productivity. SE (P=0.05) = 0.001

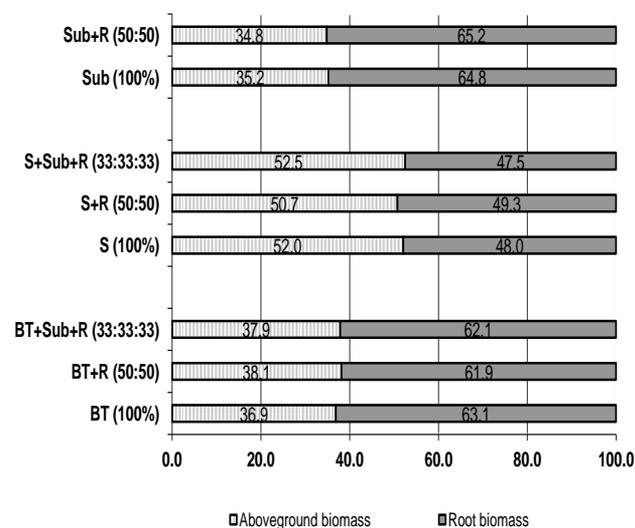


Fig. 3. Aboveground and root mass proportion in the total productivity, %.

In grasses total accumulated nitrogen was result only of the uptake of nitrate nitrogen through the roots by leaf nitrate reductase and efficiency of their use was lower. In legumes nitrogen comes from nitrogen fixation. The relations between components affected the sustainable yield index of the biomass productivity of plants. Sustainable yield index values were significantly higher for the all mixtures studied (Fig. 2).When the proportion of aboveground and root biomass in the total productivity was assessed (Fig. 3) it was observed that in pure crops the proportion of aboveground biomass was the lowest for

subterranean clover (35.2%) and the highest (52.0%)for sainfoin. This is determined by the morphology of the crops. The proportion of aboveground biomass in mixtures varied between 34.8% in mixture of subterranean clover with perennial ryegrass to 52.7% in mixtures of sainfoin with subterranean clover and perennial ryegrass.

**Conclusions**

Productivity of dry aboveground biomass in two components mixtures of subterranean clover with perennial ryegrass was by 22.1% higher than pure subterranean clover. Dry root biomass productivity in three components mixtures of birdsfoot trefoil and sainfoin (with perennial ryegrass and subterranean clover) was by 32.4% and 18.0%, respectively, higher as compared to pure birdsfoot trefoil and sainfoin. More stable were productivity of plant biomass in mixtures and sustainable yield index showed higher values.

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**References**

Albayrak, S., M. Turk, O. Yuksel and M. Yilmaz. 2011. Forage yield and the quality of perennial legume-grass mixtures under rainfed conditions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 39: 114-118.

Bostan, C., M. Butnariu, M. Butu, A. Ortan, A. Butu, S. Rodino and C. Parvu. 2013. Allelopathic effect of *Festuca rubra* on perennial grasses. *Rom Biotech Lett.*, 18: 8190-8196.

Carneiro, J.P. 1999. Avaliação de luzernas anuais em solos ácidos, Estudo do efeito de alguns factores com vista ao melhoramento deplantas. Doutoramento em Engenharia Agronomica. Universidade Técnica de Lisboa.

Carlsson, G. and K. Huss-Danell. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant Soil*, 253: 353-372.

Chourkova, B. 2007. Botanical composition and productivity of birdsfoot trefoil in mixtures with Meadow grasses in Bulgaria. *J. Balkan Ecol.*, 10: 57-61.

Chourkova, B. 2010. Study of introduced Meadow grasses in mixtures with birdsfoot trefoil under the agro-ecological conditions of Troyan. *Biotechnol. Anim. Husb.*, 26: 429-434.

Chourkova, B. 2014. Productivity and botanical composition of a mixed sward of birdsfoot trefoil and red fescue depending on the term of sowing and proportion of components. *Int. J. Agric. Innov. Res.*, 3: 276-280.

Demdoun, S., F. Munoz and I. Delgado. 2010. Forage production of a collection of sainfoin over a three year period. In: The contributions of grasslands to the conservation of Mediterranean biodiversity. *Options Méditerranéennes*, 92: 101-104.

Hardarson, G. and G. Atkins. 2003. Optimizing biological N<sub>2</sub> fixation by legumes in farming system. *Plant Soil*, 252: 41-54.

Ilieva, A. and V. Vasileva. 2011. Study on nodulation and nitrate reductase activity in some mixtures. *J. Mount. Agric. Balkans*, 14: 513-530.

Ilieva, A., V. Vasileva and A. Katova. 2015. The effect of mixed planting of birdsfoot trefoil, sainfoin, subterranean clover, and tall fescue on nodulation, and nitrate reductase activity in shoots. *J. Global Agric. Ecol.*, 3: 222-228.

- Jani, A.D., J.M. Grossman, T.J. Smyth and S. Hu. 2015. Influence of soil inorganic nitrogen and root diameter size on legume cover crop root decomposition and nitrogen release. *Plant Soil*, 393: 57-68.
- Jani, A.D., J.M. Grossman, T.J. Smyth and S. Hu. 2016. Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. *Renew. Agric. Food Syst.*, 31: 214-229.
- Kusvuran A., E. L. Parlak and T. Saglamtimur. 2015. Biomass yield of faba bean (*Vicia faba* L.) and its mixture with some grasses (Poaceae). *Turk. J. Agric. Natur. Sci.*, 2: 178-184.
- Kusvuran, A., Y. Ralice and T. Saglamtimur. 2014a. Determining the biomass production capacities of certain forage grasses and legumes and their mixtures under mediterranean regional conditions. *Acta Adv. Agric. Sci.*, 2: 13-24.
- Kusvuran, A., M. Kaplan and R.I. Nazli. 2014b. Intercropping of Hungarian vetch (*Vicia pannonica* Crantz.) and barley (*Hordeum vulgare* L.) under different plant varieties and mixture rates. *Legume Res.*, 37: 590-599.
- Kusvuran, A., M. Kaplan and R.I. Nazli. 2014c. Effects of mixture ratio and row spacing in Hungarian vetch (*Vicia pannonica* Crantz.) and annual ryegrass (*Lolium multiflorum* Lam.) intercropping system on yield and quality under semiarid climate conditions. *Turk. J. Field Crops*, 19: 118-128.
- Lelièvre, F. and F. Volaire. 2009. Current and potential development of perennial grasses in rainfed mediterranean farming systems. *Crop Sci.*, 49: 2371-2378.
- Luscher, A., I. Mueller-Harvey, I.F. Soussana, R.M. Rees and J.L. Peyraud. 2014. Potential of legume-based grassland-livestock systems in Europe: A review. *Grass Forage Sci.*, 69: 206-228.
- Mahapatra, S.C. 2011. Study of grass-legume intercropping system in term of competition indices and monetary advantage index under acid lateritic soil of India. *Amer. J. Exp. Agric.*, 1: 1-6.
- Mihovski, T.S. and G. Goranova. 2007. Western European varieties of white clover (*Trifolium repens* L.) under condition of Bulgaria. *J. Balkan Ecol.*, 10: 407-410.
- Nichols, P.G.H., C.K. Revell, A.W. Humphries, J.H. Howie, E.J. Hall, G.A. Sandral, K. Ghamkhar and C.A. Harris. 2012. Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop Pasture Sci.*, 63: 691-725.
- Nikolova, I. and T. Kertikov. 2008. Study on the number of pest insects on winter forage peas (*Pisum arvense* L.) depending on the farming system and the period of sowing. *Plant Sci.*, 45: 222-225. (In Bulgarian)
- Peyraud, J.L., A. Le Gall and A. Luescher. 2009. Potential food production from forage legume based systems in Europe: an overview. *Irish J. Agric. Food Res.*, 48: 115-135.
- Porqueddu, C., G. Parente and M. Elsaesser. 2003. Potential of grasslands. *Grassland Sci. Eur.*, 8: 11-20.
- Saruhan, V., I. Gul, A. Kusvuran and F. Aydin. 2012. Effects of sewage sludge used as fertilizer on heavy metal contents of bird's-foot trefoil (*Lotus corniculatus* L.) and soil. *Asian J. Chem.*, 24: 866-870.
- Singh, R.P., S.K. Das, Bum Rao and N.M. Reddy. 1990. Towards sustainable dry land agricultural practices. *Central Research Institute Dryland Agriculture*, Hyderabad, India. 106 p.
- SPSS Version 20.0. SPSS Inc. 233 S. Wacker Drive Chicago, IL.
- Stevanovic, P., S. Vuckovic, V. Popovic, J. Ikanovic, L. Zivanovic, M. Tabakovic, R. Vujic and Z. Latic. 2015. Influence of the mineral fertilization on morphological and productive characteristics of the *Lotus corniculatus* on pseudogley. *Wulfenia*, 22: 190-204.
- Vasilev, E. 2009. Chemical composition of subclovers forage (*Tr. subterraneum* L.) and crude protein yield in pasture mixtures with grasses. *J. Mount. Agric. Balkans*, 12: 329-341.
- Vasilev, E., V. Vasileva, Ts. Mihovsky and G. Goranova. 2005. Assessment of legume based mixture swards constrained by the environmental conditions in Central North Bulgaria - COST Action 852. In: *Sward dynamics, N-flows and forage utilisation in legume-based systems*. (Eds.): Wachendorf, M., A. Helgadottir and G. Parente. pp. 177-180 Grado, Italy.
- Vasilev, E. 2006. Productivity of subterranean clover (*Tr. subterraneum* L.) in pasture mixtures with some perennial grasses for the conditions of Central North Bulgaria. *Plant Sci.*, 43: 149-152.
- Vasileva, V. 2011. Study on productivity of perennial legume crops in mixtures. *J. Mount. Agric. Balkans* 14: 296-307.
- Vasileva, V. 2014. Productivity of aboveground and root mass in mixtures. *J. Mount. Agric. Balkans*, 17: 956-969.
- Vasileva, V. 2015. Morphological parameters and ratios in some mixtures with subclover. *Sci. Internat.*, 3: 107-112.
- Vasileva, V. and E. Vasilev. 2012a. Study on productivity of some legume crops in pure cultivation and mixtures. *Agric. Consp. Sci.*, 77: 91-94.
- Vasileva, V. and E. Vasilev. 2012b. Dry mass yield from some pasture mixtures with subterranean clover (*Trifolium subterraneum* L.). *J. Mountain Agric. Balkans*, 15: 1024-1033.
- Vasileva, V., E. Vasilev and M. Athar. 2011. Nodulation and root establishment of two clover species grown in pasture mixtures with wheatgrass. *FUUAST J. Biol.*, 1(1): 1-4.
- Vasileva, V., K. Kocheva, J. Mincheva, G. Georgiev, A. Ilieva and C. Porqueddu. 2015. Physiological analysis of growth and nitrogen metabolism of intercropped pasture species subterranean clover (*Trifolium subterraneum* L.) and cocksfoot (*Dactylis glomerata* L.) supplemented with different inorganic nitrogen. *J. Plant Nutr.*, (In Press).
- Yakimova, Y. and H. Yancheva. 1986. Phytocenological and ecological characteristics of some annual clovers in the Strandja region. *Plant Sci.*, 23: 47-53 (In Bulgarian).

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