



Life Sciences Group

## International Journal of Agricultural Science and Food Technology



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**Dates: Received:** 09 February, 2017; **Accepted:** 08 March, 2017; **Published:** 10 March, 2017

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Keywords: Birdsfoot trefoil; Sainfoin; Subterranean clover; Root biomass to aboveground biomass ratio.

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#### **Research Article**

# **Parameters Related to Nodulating Ability of Some Legumes**

#### **Abstract**

Parameters related to nodulating ability of some legumes were studied in a pot trial in the Institute of Forage Crops, Pleven, Bulgaria during the 2013 and 2014 years. Birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis viciifolia* Scop.) and subterranean clover (*Trifolium subterraneum* L.) were pure cultivated and in mixture with perennial ryegrass (*Lolium perenne* L.) in the next ratios: birdsfoot trefoil + perennial ryegrass (50:50%); sainfoin + perennial ryegrass (50:50%); subterranean clover + perennial ryegrass (33:33:33%); sainfoin + subterranean clover + perennial ryegrass (33:33:33%). From the pure cultivated crops, birdsfoot trefoil showed both, the highest nodulating ability and root biomass to nodule number ratio. Birdsfoot trefoil and subterranean clover in mixtures with perennial ryegrass formed more nodules as compared to pure crops by 16.0% and by 24.4%, respectively. Sainfoin showed weak nodulating ability, both, pure and in mixtures. In terms of root to aboveground biomass ratio, the legumes were arranged as follows: subterranean clover, birdsfoot trefoil and sainfoin.

## Introduction

Forage legumes have significant role in agriculture and are often integrated in mixed farming systems to enhance crop yields through their nodulating and nitrogen fixing ability [1], Every year on the global level about 50 million tons nitrogen is fixing in the agro ecosystems [2,3], consider that legume species fix between 15 and 25 kg nitrogen per tone of dry matter.

Legumes often are grown in mixtures where they are stimulated to fix more nitrogen from the air moreover, they are less competitive with grasses for soil nitrogen [4], There is a dynamic relationship between the legume and the grass component, where soil nitrogen absorption by the grass lowers its inhibitory effect on nitrogen fixation. Climate changes force forage crops and mixtures to adapt to the altered conditions [5]. Legumes species that can provide self-sowing and persist continuously in the sward as subterranean clover become of practical importance [6,7], Choosing the right components is important because of their competitiveness depends on the success of the mixture [8,9], In the present work some parameters related to nodulating ability of valuable forage crops as birdsfoot trefoil, sainfoin and subterranean clover alone and in mixtures with perennial ryegrass were studied.

## **Materials and Methods**

The trial was carried out in the greenhouse of Institute of Forage Crops, Pleven, Bulgaria (2013 - 2014) under semi

controlled conditions. Birdsfoot trefoil (Lotus corniculatus L.) cv. "Targovishte 1"; sainfoin (Onobrychis Adans.) – local population; subterranean clover (Trifolim subterraneum ssp. brachycalicinum) cv. "Antas" and perennial ryegrass (Lolium perenne L.) cv. "IFK - Harmoniya" were used. The crops were studied pure and in two and three components mixtures in the next treatments: birdsfoot trefoil (100%); sainfoin (100%); subterranean clover (100%); perennial ryegrass (100%); birdsfoot trefoil + perennial ryegrass (50:50%); sainfoin + perennial ryegrass (50:50%); subterranean clover + perennial ryegrass (50:50%); birdsfoot trefoil + subterranean clover + perennial ryegrass (33:33:33%); sainfoin+ subterranean clover + perennial ryegrass (33:33:33%). Plastic pots were used filled with soil (leached chernozem subtype). The sowing was made on the depth of 1-1.5 cm for birdsfoot trefoil, subterranean clover and perennial ryegrass, and 3 cm for sainfoin. Treatments were four replicated arranged in a completely randomized plot design.

Two cuts for forage were harvested, the root biomass was washed with tap water and the next characteristics were recorded: nodulating ability (nodule number per plant); roots to number of root nodules ratio (dry root biomass, dried at 60°C was devided to number of root nodules); root biomass to aboveground biomass ratio, weight (dried at 60°C). These characteristics were compared to the characteristics of pure legumes (birdsfoot trefoil, sainfoin and subterranean clover). Data were averaged for two years and statistically processed standard error finding using softweer [10].

### **Results and Discussion**

Symbiotic fixations of nitrogen take place in the root nodules [11], in our study birdsfoot trefoil and subterranean clover formed the most number of nodules (Table 1).

According to [4,12], legumes effectively regulate the processes of nodulation and nitrogen fixation in mixtures depending on the accessibility of mineral nitrogen. Taking into account the nodulation in mixtures of birdsfoot trefoil, we can see that there are no significant differences for its mixtures with ryegrass and these with ryegrass and subterranean clover. In both mixtures nodulation was higher as compared to pure birdsfoot trefoil by 16.0% and 17.6%, respectively.

The major factor influenced the nodulation in mixture is the competitiveness of legume with subsequent grass component [13,14] consider that the uptake of mineral nitrogen in mixture is more as compared to pure growing of legumes and this reflect on the nodule number. However, the competitiveness for soil nitrogen may have beneficial effect on the stimulating of nitrogen fixing processes because mineral nitrogen inhibits N-fixation process. The positive interaction in mixture of alfalfa and timothy (1:1 and 1:2) was found [15]. Timothy involving as a component increased the proportion of fixed nitrogen from alfalfa.

Sainfoin as compared to other legumes tested formed less number of nodules, pure, as well in mixtures. In mixtures with ryegrass and in these with ryegrass and subterranean clover, nodule number not differs significantly from the pure sainfoin.

Relatively less amount of fixed nitrogen in comparison with other legumes was found in sainfoin from many authors [16–19], As a possible reason they and others [8,20], showed that this crop needs twice as much  ${\rm CO_2}$  (approximately 20 mol) for 1 mol  ${\rm N_2}$ . Moreover, in sainfoin less amount of assimilates usually were used for the leaf formation as well the leaves have a smaller surface.

Subterranean clover in mixture with ryegrass formed by 24.3% more number of nodules as compared to pure subterranean clover. Apparently, there was positive synergy observed in regard to nodulating ability.

As a whole birdsfoot trefoil, sainfoin and subterranean clover formed higher number of nodules in mixtures and on average there were any significant differences between the mixtures.

Specific nodulating ability follows the tendency of nodulation. This characteristic varied from 0.1269 to 0.1438 in birdsfoot trefoil, from 0.0237 to 0.0269 in sainfoin and showed closed values in subterranean clover (0.0362 - 0.0375) (Figure 1).

Having in a mind the characteristics included in the calculation of root biomass to nodule number ratio, lower values of this ratio indicated better provision of root biomass with root nodules. The roots to number of root nodules ratio was the best found in birdsfoot trefoil grown alone and in subterranean clover grown in mixtures with ryegrass (Table 2)

We assume that it was due to the higher nitrogen use efficiency of subterranean clover under intercropped conditions because of the successful competition between the two species for available nitrogen.

Components included in mixtures have different nitrogen metabolism. In ryegrass 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 subterranean clover nitrogen comes from nitrogen fixation. Root biomass to aboveground biomass ratio is determining by the plant development. Formation of biomass of the plants and its various organs is the result of the assimilation activity of photosynthetic tissues, as well as of the root system functioning [21].

It has been known long ago that grass/legume intercropping systems are highly efficient in relation to productivity than separately grown crops [22–24], Root biomass to aboveground biomass ratio varied from 0.5211 in subterranean clover in mixtures with ryegrass to 1.1091 in sainfoin in the three component mixtures (Figure 2). A large amount of root biomass in mixtures increased the possibility for taking of immobile nutrients which contributed the better plant development as considered [25,26],

Table 1: Nodulating ability of birdsfoot trefoil, sainfoin and subterranean clover, pure and in mixtures.

Treatments	Ratio	Nodules per plant
Birdsfoot trefoil	100	38.1
Birdsfoot trefoil+Ryegrass	50:50	44.2
Birdsfoot trefoil+Subclover + Ryegrass	33:33:33	44.8
Sainfoin	100	14.4
Sainfoin + Ryegrass	50:50	14.8
Sainfoin + Subclover+ Ryegrass	33:33:33	15.1
Subclover	100	29.2
Subclover + Ryegrass	50:50	36.3
SE (P=0.05)		4.67
Average for:		
Pure crops	27.2	
Mixtures	31.0	
Two components mixtures	31.8	
Three components mixtures	30.0	
SE (P=0.05)	1.0	

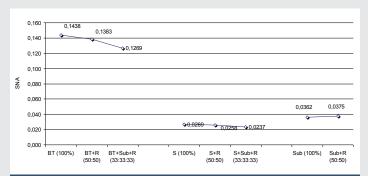


Figure 1: Intraoperative image, Complete section of the pancreas body

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Values of the root to aboveground biomass ratio are rwelated to the quantity of root and aboveground biomass and different type of root system of the components included in the mixtures.

#### Conclusion

Nodulating legumes in pastures are the basis for economic and environmental sustainability of agricultural systems. From the birdsfoot trefoil, sainfoin and subterranean clover studied, birdsfoot trefoil showed the highest nodulating ability and root biomass to nodule number ratio. Birdsfoot trefoil and subterranean clover in mixtures with perennial ryegrass formed by 16.0% and by 24.4%, respectively more nodules as compared to pure grown crops. Sainfoin showed weak nodulating ability, both, pure and in mixtures. In terms of root

**Table 2:** Roots to number of root nodules ratio in birdsfoot trefoil, sainfoin and subterranean clover, pure and in mixtures.

Treatments	Ratio	Root biomass to nodule number ratio
Birdsfoot trefoil	100	0.0453
Birdsfoot trefoil+Ryegrass	50:50	0.0506
Birdsfoot trefoil+Subclover + Ryegrass	33:33:33	0.0511
Sainfoin	100	0.2049
Sainfoin + Ryegrass	50:50	0.2098
Sainfoin + Subclover+ Ryegrass	33:33:33	0.2301
Subclover	100	0.0505
Subclover + Ryegrass	50:50	0.0489
SE (P=0.05)		0.0030
Average for:		
Pure crops	0.1002	
Mixtures	0.1181	
Two components mixtures	0.1031	
Three components mixtures	0.1406	
SE (P=0.05)	0.0920	

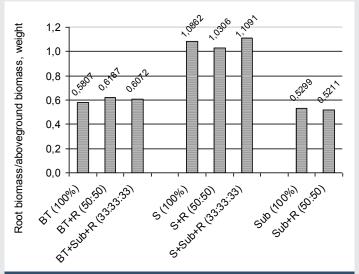


Figure 2: Root/aboveground biomass ratio (weight) in birdsfoot trefoil, sainfoin and subterranean clover, pure and in mixture.

to aboveground biomass the legumes were arranged as follows: subterranean clover, birdsfoot trefoil and sainfoin. The role of mixtures based on legumes for sustainable agriculture was shown.

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