Journal of Applied Sciences

ISSN 1812-5654

OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2016.68.76

Research Article
Botanical Composition Improvement with Subterranean Clover (*Trifolium subterraneum* L.) in Grass Mixtures

Viliana Vasileva

Institute of Forage Crops, 89 General Vladimir Vazov Str, Pleven 5800, Bulgaria

Abstract
Botanical composition of cocksfoot and tall fescue, pure and in mixtures with three subspecies subterranean clover were investigated in field trial in the Institute of Forage Crops, Pleven, Bulgaria (2011-2013). The next ratios were used: Grass+*Trifolium subterraneum* ssp. *brachycalicinum* (50:50%), Grass+*Trifolium subterraneum* ssp. *yaninicum* (50:50%), Grass+*Trifolium subterraneum* ssp. *subterraneum* (50:50%), Grass+*Trifolium subterraneum* ssp. *brachycalicinum*+*Trifolium subterraneum* ssp. *yaninicum*+*Trifolium subterraneum* ssp. *subterraneum* (25:25:25:25%). It was found that inclusion the subterranean clover as a component of mixtures with cocksfoot and tall fescue had positive effect on the botanical composition and decreased the weed infestation in the swards. *Trifolium subterraneum* ssp. *brachycalicinum* in mixture with cocksfoot had faster growing rate as well the biggest part in the sward (31.0%) and reduced the weeds half. *Trifolium subterraneum* ssp. *brachycalicinum* in mixtures with tall fescue had small part and decreased the weeds by 28.1%. Subterranean clover as a legume component contributed to increasing the leaf/stem ratio of cocksfoot and tall fescue.

Key words: Subterranean clover, mixtures, botanical composition, cocksfoot, tall fescue

Received: November 09, 2015 Accepted: December 21, 2015 Published: January 15, 2016

Citation: Viliana Vasileva, 2016. Botanical composition improvement with subterranean clover (*Trifolium subterraneum* L.) in grass mixtures. J. Applied Sci., 16: 68-76.

Corresponding Author: Viliana Vasileva, Institute of Forage Crops, 89 General Vladimir Vazov Str, Pleven 5800, Bulgaria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Interest towards intercropped systems based on co-cultivation of legumes and cereals is rising in recent years due to their role in formation of a system for sustainable and organic farming (Pypers *et al*., 2005; Luscher *et al*., 2014; Kusvuran *et al*., 2014). It has been known long ago that cereal/legume intercropping systems are highly efficient in relation to the using of sources of environment than separately grown crops (Porqueddu *et al*., 2003; Vasilev *et al*., 2005; Peyraud *et al*., 2009; Mahapatra, 2011). The leguminous component in the intercropping system contributes for the increasing of nitrogen content in the system due to its nitrogen fixing ability providing the majority of nitrogen required for them as well for the accompanying grass component (Pypers *et al*., 2005; Peeters *et al*., 2006; Pozdisek *et al*., 2011; Vanlauwe *et al*., 2014).

The permanent changes having occurred in the last ten-year periods in the climate (increase of average yearly temperatures, long droughts in the spring and summer, increase of CO_2 concentration in the atmosphere) present a serious risk to the perennial crops because affect their capacity for several years. This requires to study new herbaceous forage species having pronounced resistance to unfavorable abiotic factors and good adaptive capacity towards the new conditions (Gornall *et al*., 2010; Bostan *et al*., 2013; Mihovski and Kirilov, 2014; Luscher *et al*., 2014; Aranjuelo *et al*., 2014). At present the main interest in directed towards more drought resistant and drought tolerant components. Legumes species that can provide self-sowing and persist continuously in the sward become of practical importance (Carneiro, 1999).

Subterranean clover (*Trifolium subterraneum* L.) is an annual drought resistant ephemeral legume with winter-spring type of development and ability for self-sowing (Yakimova and Yancheva, 1986; Piano *et al*., 1996; Frame *et al*., 1998). It is a widespread component in the pastures and other grasslands of the temperate areas of central and Northern Europe and America (Frame *et al*., 1998; Pecetti and Piano, 1998, 2002; Kyriazopoulos *et al*., 2008). The species is found In Bulgaria in open, dry grasslands in the plains and lowlands (Assyov *et al*., 2012).

An important element of the biology of this species is that its reproductive organs are formed in early may and the seeds ripen before the end of the spring in hedgehog-shaped heads that remain on the soil surface. Heads are 10.0-15.0 mm spherical, loose, appressed on the soil and self-burying (Kozhuharov, 1976). A big part of the seeds germinated during the autumn. Other part germinated during the spring of the next year but because of the winter-spring type of the development the plants took part in the sward during most of the vegetation season, some of them wintering and on the next spring formed seeds (Frame *et al*., 1998; Vasilev, 2006). Substantial part of the formed seeds is solid and germinates after two-three years. This biological specificity turns the superficial soil layer into an original seed bank which makes the species even more flexible one (Pecetti and Piano, 1994). The precipitations during the late summer contribute to emergence of new self-sown plants (Frame *et al*., 1998). So, subterranean clover although an annual species due to its biological ability to self-seeding present in the sward composition at the beginning and end of the vegetation. The annual seed formation and germination is a reason the crop of subterranean clover to be appear as perennial one (Vasilev, 2006).

Investigations on subterranean clover as a component of sown pasture in recent years have shown that it has practical application for local climatic conditions in Bulgaria (Vasilev, 2006, 2009; Ilieva and Vasileva, 2011; Vasileva *et al*., 2011; Vasileva and Vasilev, 2012; Vasileva, 2014, 2015a, b; Ilieva *et al*., 2015). When sown at an appropriate time in the autumn, it establishes a uniform stand before the beginning of the permanent cold spell and grows up early in the spring and forms a dense sward. A marked winter resistance, effective utilization of autumn-winter soil moisture, successful seed formation and self-sowing at the end of spring allow subterranean clover to avoid summer droughts. Subterranean clover is a suitable component for the mixture with grasses. In the present work subterranean clover was studied in mixture with cocksfoot and tall fescue.

Cocksfoot is a deeply-rooted medium to longlived highly productive grass species. It initiates growth early in the spring and due to the deep roots and access moisture in lower soil profiles, grows well during hot summer months as well during the autumn (Jacobs and Siddoway, 2007).

Tall fescue has high resistance to high summer temperatures, withstands dry as well wet soils and is a suitable component for mixtures with hay-pasture direction of use (Katova, 2007).

The aim of this work to study the botanical composition of mixtures of cocksfoot and tall fescue with subterranean clover.

MATERIALS AND METHODS

The trial was conducted at the experimental field (43°23'N, 24°34'E, 230 m altitude) of the Institute of Forage Crops, Pleven, Bulgaria (2011-2013) on podzolized soil subtype without irrigation. Cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* schreb.) pure and in mixture with three subclover subspecies, i.e., *Trifolium subterraneum* ssp. *brachycalicinum*, *Trifolium subterraneum* ssp. *yaninicum* and *T. subterraneum* ssp. *subterraneum* were tested. The trial was carried out on the long plot method with size of plots 70 m^2 four replicated. The variants were as follows: Cocksfoot (100%), cocksfoot+*Trifolium subterraneum* ssp. *brachycalicinum* (50:50%), cocksfoot+*Trifolium subterraneum* ssp. *yaninicum* (50:50%), cocksfoot+*Trifolium subterraneum* ssp. *subterraneum* (50:50%), cocksfoot+*Trifolium subterraneum* ssp. *brachycalicinum*+*Trifolium subterraneum* ssp. *yaninicum*+*Trifolium subterraneum* ssp. *subterraneum* (25:25:25:25%), tall fescue (100%), tall fescue+*Trifolium subterraneum* ssp. *brachycalicinum* (50:50%), tall fescue+*Trifolium subterraneum* ssp. *yaninicum* (50:50%), tall fescue+*Trifolium subterraneum* ssp. *subterraneum* (50:50%), tall fescue+*Trifolium subterraneum* ssp. *brachycalicinum*+*Trifolium subterraneum* ssp. *yaninicum*+*Trifolium subterraneum* ssp. *subterraneum* (25:25:25:25%).

Cocksfoot cv. "Dabrava" and tall fescue cv. "Albena" were used. Subclover for: *Trifolium subterraneum* ssp. *brachycalicinum*-cv. "Antas", *Trifolium subterraneum* ssp. *yaninicum*-cv. "Trikkala" and *Trifolium subterraneum* ssp. *subterraneum*-cv. "Denmark".

The sowing was done on September, 17, 2011 between row space was 11.5 cm and sowing rates: Tall fescue 25 kg ha^{-1}, cocksfoot 25 kg ha^{-1}, subterranean clover -25 kg ha^{-1}. No fertilizers and pesticides were applied during the vegetation. The swards were harvested for forage. To determine botanical composition samples before the cutting was taken from the all replications using 0.25 m^2 quadrate. Leaf/stem ratio of the grasses (pure and in mixtures) based on fresh weight was calculated.

In the present work the data from one cut harvested on June 6, 2012 and two cuts harvested on May 7 and July 10, 2013. Experimental data for leaf/stem ratio were statistically processed using SPSS (2012).

RESULTS AND DISCUSSION

The period of study was characterized as an unfavorable in regard to the agro meteorological conditions. Data for mean air temperature and cumulative rainfall was shown on the Table 1. After the sowing long dry period occur. Extremely high temperatures affected the growth and development of the plants, botanical composition and productivity of the swards. One cut only was harvested.

Botanical composition of the swards is one of the main criteria for their resistance (Mannetje, 2003).

Cocksfoot and mixtures with subterranean clover: Cocksfoot has slowly growing rate during the first year and attacked by weeds (Vasilev, 2000; Kirwan *et al*., 2005; Jacobs and Siddoway, 2007). Regardless the autumn sowing cocksfoot it could not tiller to the frosts appearing. Plant development during the vegetation was slowly and weeds infestation significant (41.0%) (Fig. 1).

Part of the cocksfoot in mixture was affected by the growing rate of subterranean clover.

Despite the unfavorable conditions subterranean clover well developed in all swards tested. Comparing the three subspecies it is apparent that *Trifolium subterraneum* ssp. *brachycalicinum* took 31.0% in the mixture and weed infestation was half reduced as compared to the pure sward.

Table 1: Meteorological conditions for the period of study (2011-2013)

Attribute	First year (°C)	First year (mm)	Second year (°C)	Second year (mm)	Third year (°C)	Third year (mm)
I	-1.0	32.8	-5.2	17.8	0.5	19.0
II	0.2	27.2	8.5	7.6	3.9	60.9
III	6.1	25.7	14.8	46.3	6.3	39.6
IV	11.4	28.2	17.4	85.2	14.0	50.8
V	16.8	79.8	24.1	40.3	19.5	63.7
VI	21.4	33.6	27.8	1.4	21.3	112.4
VII	23.5	50.2	25.8	35.6	22.7	105.8
VIII	23.6	41.3	21.1	21.0	24.9	20.2
IX	22.0	0.0	15.0	56.0	18.4	15.8
X	11.1	50.2	8.1	4.0	12.2	59.2
XI	7.6	0.4	-1.0	56.7	8.7	29.1
XII	3.8	28.6	-5.2	17.8	0.2	2.3
av/sum	12.2	398.0	13.0	416.1	12.7	578.8

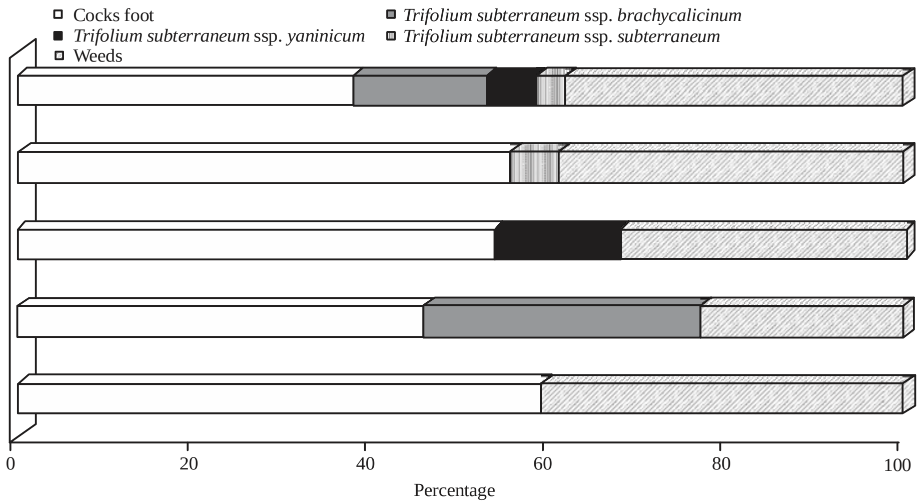

Fig. 1: Botanical composition of cocksfoot pure and in mixture with subterranean clover (one year after sowing)

Similar were results of Porqueddu *et al.* (2003). Significantly smaller was the part of *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum*, 14.3 and 5.5%, respectively and the weeds were 32.3 and 39.0%, i.e. as the control. In the four-component mixture subterranean clovers part was 23.8 and 15.0% accounted for *Trifolium subterraneum* ssp. *brachycalicinum*. Significantly slower growth rates were found for both, *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum* and they suppress the weeds less as compared to *Trifolium subterraneum* ssp. *brachycalicinum*.

Rainfall in the third year of the experiment was unevenly distributed. An early spring drought was occurred and lasts one month (from April 18 to May 16). The average daily and average monthly average maximal temperatures were above the norm. Moisture in the top soil was insufficient for the normal development of the crops as a whole, especially grasses.

Subterranean clover regardless the shallow root system as compared to perennial legumes (alfalfa, birdsfoot trefoil, sainfoin) rapidly developed in the first days of the year suitable for vegetation. Subterranean clover covered the soil surface and due to the prostrate habit retains the moisture which influences the accompanying component in the mixture.

Data for botanical composition of the swards in first cut of the second year showed that the weed infestation was considerably less (Fig. 2). Most weeds were found in the pure sward.

The effect of the subspecies of the clover in the mixture was significant. *Trifolium subterraneum* ssp. *brachycalicinum* which characterized with fast growing rate took part of 28.5%. The other two subspecies subterranean clover formed smaller biomass 15.1 and 11.3%, respectively for *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum* and did not affect the weeds. The part of subterranean clover in four-component mixture was 25.4 and 12.2% of them occupied *Trifolium subterraneum* ssp. *brachycalicinum*.

Since the second half of May when the second cut is forming, rainfall was evenly distributed which favored the development of crops. Subterranean clover's part in the second cut was up 10.7% and there were no significant differences for *Trifolium subterraneum* ssp. *brachycalicinum* and *Trifolium subterraneum* ssp. *yaninicum*. The smallest part was found for *Trifolium subterraneum* ssp. *subterraneum* which is less competitive. As a whole the part of subterranean clovers in the second cut was half reduced as compared to first cut due to the biology of the crop.

Tall fescue and mixtures with subterranean clover: Data for botanical composition of the pure sward of tall fescue during the first year after sowing showed that grass predominated 57.3% (Fig. 3).

Subterranean clover develops less in mixtures with tall fescue and its part for *Trifolium subterraneum* ssp. *brachycalicinum* reached up 11.0%. Smaller was the share for *Trifolium subterraneum* ssp. *yaninicum* (8.8%) and *Trifolium subterraneum* ssp. *subterraneum* (3.7%).

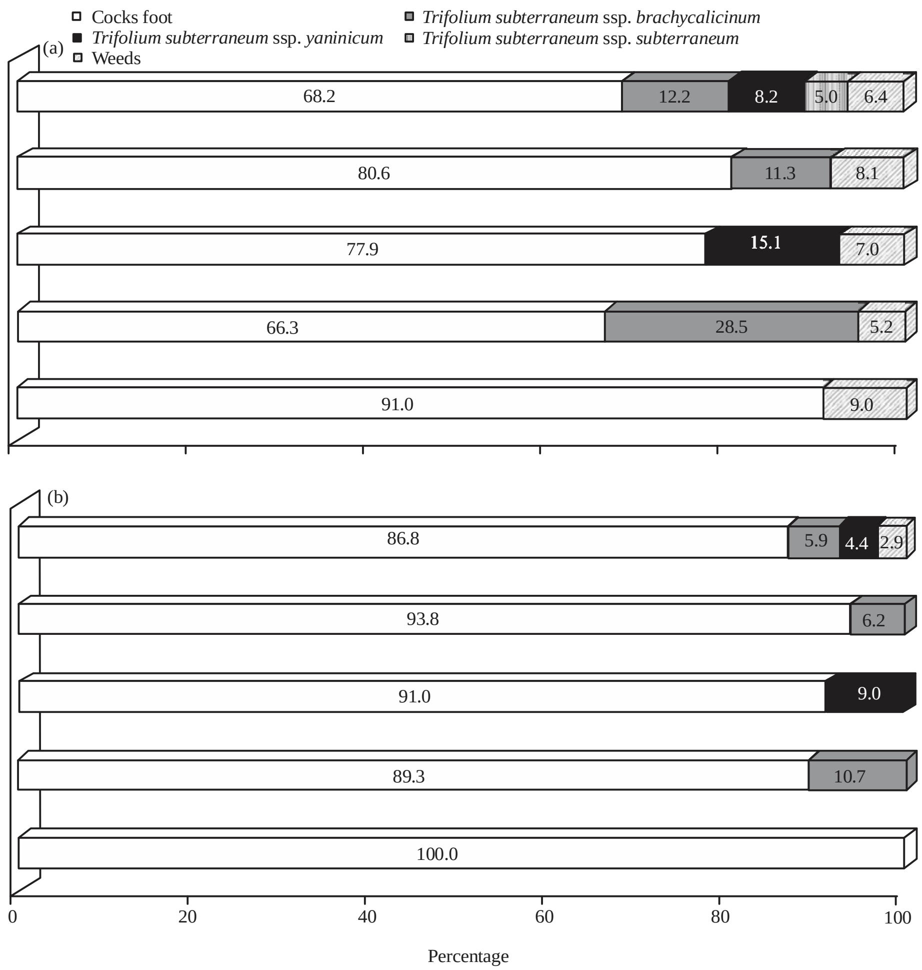

Fig. 2(a-b): Botanical composition of (a) Cocksfoot pure and in (b) mixture with subterranean clover (two years after sowing)

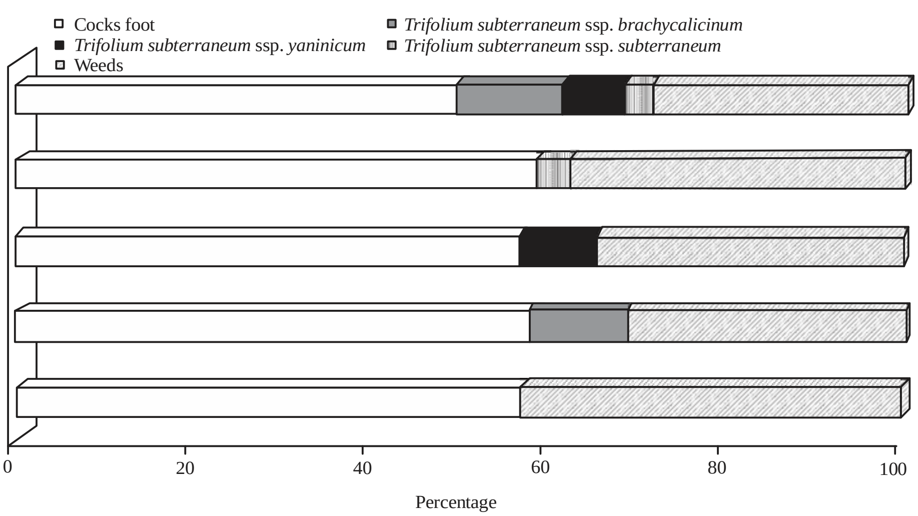

Fig. 3: Botanical composition of tall fescue pure and in mixture with subterranean clover (one year after sowing)

Subterranean clover has no effect on the part of tall fescue in the sward and it remains almost unchanged, varying in narrow limits (50.0-59.0%). In the mixtures with *Trifolium subterraneum* ssp. *brachycalicinum* the reduction

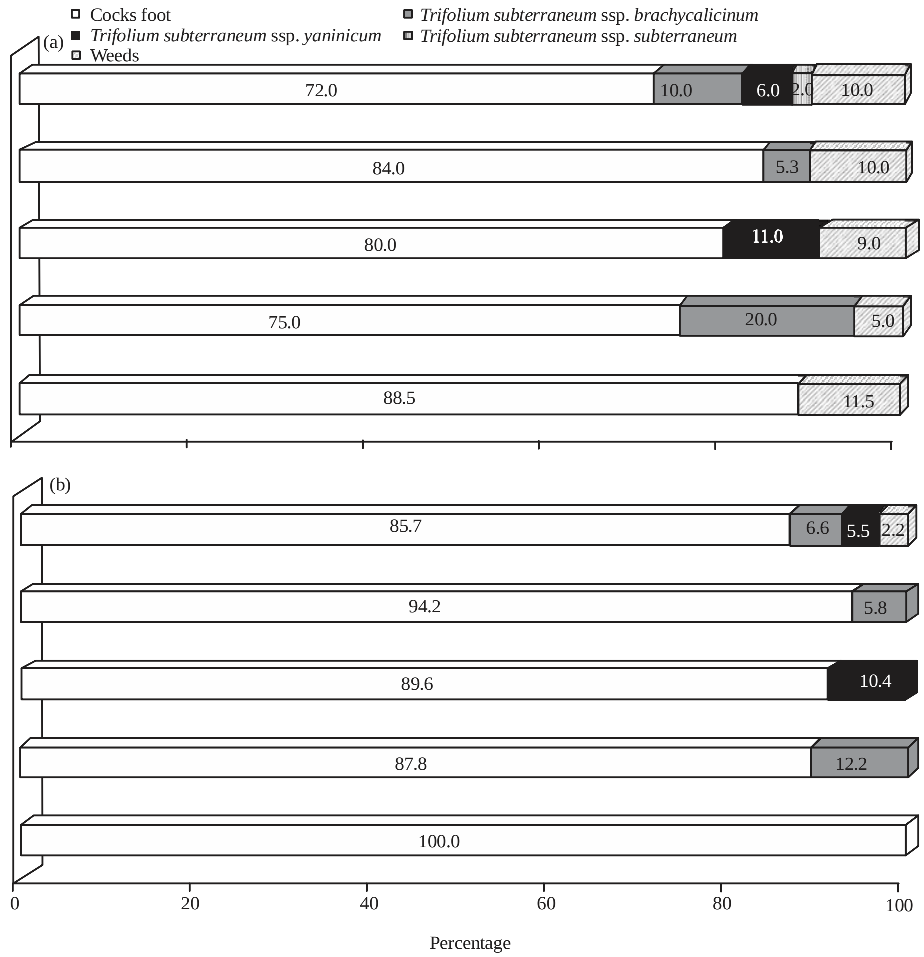

Fig. 4(a-b): Botanical composition of (a) Tall fescue pure and in (b) mixture with subterranean clover (second year after sowing)

of weeds was by 28.1% and almost 50.0% in mixtures with cocksfoot. Nearly, 1/4 falls on the subterranean clovers in the four-component mixture.

Tall fescue (pure) predominated (88.5%) in first cut during the second year after sowing (Fig. 4). Part of *Trifolium subterraneum* ssp. *brachycalicinum* (20.0%) was the highest and weed infestation was half reduced as compared to pure swards. Probably a weaker development of subterranean clover in mixtures with tall fescue was due to the greater competiveness of tall fescue because of its alellopathic potential (Siegel and Bush, 1997; Renne *et al.,* 2004; Marinov-Serafimov *et al.*, 2015).

Regarding the part of other two subspecies-there was the same tendency as about the cocksfoot. *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum* parts were 11.0 and 5.3%, respectively and up 18.0% for the clovers in the four-component mixture.

There were no weed infestation in the second cut. Tall fescue is aggressive against the weeds too. Part of clovers was 12.2, 10.4 and 5.8% for *Trifolium subterraneum* ssp. *brachycalicinum*, *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum*. This part in the four-component mixture was 14.3%.

Pecetti and Piano (1998) consider that in subterranean clover, leaf size contributes to plant competitively. In our study the leaf/stem ratio (fresh weight) of grass components were calculated. This ratio is an important factor affecting quality and forage intake (Smart *et al.*, 1998; Ostrem *et al.*, 2013).

Leaf/stem ratio in the year after sowing was lower due to slowly development of grasses. For cocksfoot leaf/stem ratio varied from 0.44-0.59 and for tall fescue from 0.28-0.36 (Fig. 5). Similar were Shaffer *et al.* (1994) findings for tall fescue.

The values of this ratio were higher in mixtures as compared to pure grasses. More leaf biomass formation from

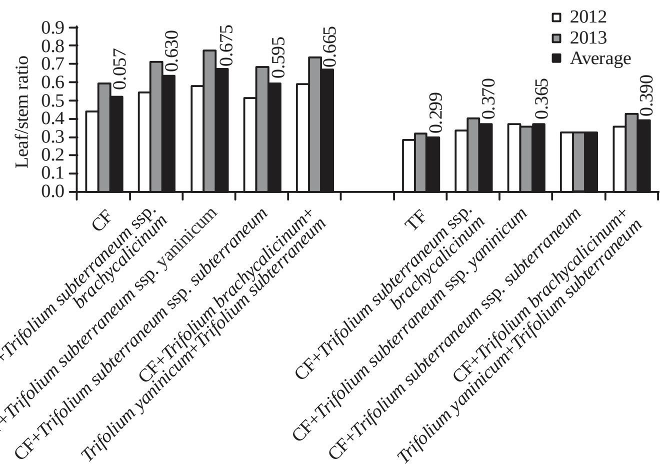

Fig. 5: Leaf/stem ratio of cocksfoot and tall fescue pure and in mixture with subterranean clover

grasses in mixtures with legume component was associated to better nitrogen assimilation (Ledgard and Steel, 1992).

CONCLUSIONS

The inclusion of subterranean clover as a component of mixtures with cocksfoot and tall fescue improved botanical composition and decreased the weed infestation in the swards. *Trifolium subterraneum* ssp. *brachycalicinum* in mixture with cocksfoot had the biggest part in the sward (31.0%) and reduced the weeds half (43.9%) in the year after sowing. *Trifolium subterraneum* ssp. *brachycalicinum* in mixtures with tall fescue had small part and decreased the weeds by 28.1%. Subterranean clover as a legume component contributed to increasing the leaf/stem ratio of cocksfoot and tall fescue and it was higher as compared to this ratio in pure grown grasses.

REFERENCES

Aranjuelo, I., C. Arrese-Igor and G. Molero, 2014. Nodule performance within a changing environmental context. J. Plant Physiol., 171: 1076-1090.

Assyov, B., A. Pertova, D. Dimitrov and P. Vassilev, 2012. Conspectus of the Bulgarian Vascular Flora: Distribution Maps and Floristic Elements. 4th Edn., Bulgarian Biodiversity Foundation, Sofia, ISBN: 9789549959581, Pages: 490.

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

Carneiro, J.P., 1999. Avaliacao de luzernas anuais em solos acidos: Estudo do efeito de alguns factores limitantes com vista ao melhoramento de plantas. Ph.D. Thesis, Univercidade Tecnica de Lisboa, Portugal.

Frame, J., J.F.L. Charlton and A.S. Laidlaw, 1998. Temperate Forage Legumes. CAB International, Wallingford, UK., Pages: 327.

Gornall, J., R. Betts, E. Burke, R. Clark, J. Camp, K. Willett and A. Wiltshire, 2010. Implications of climate change for agricultural productivity in the early twenty-first century. Philosophical Trans. R. Society B, 365: 2973-2989.

Ilieva, A. and V. Vasileva, 2011. Study on nodulation and nitrate reductase activity in some mixtures. J. Mountain Agric. Balkans Agric. Acad., 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.

Jacobs, J. and J. Siddoway, 2007. Tame pasture grass and legume species and grazing guidelines. Plant Materials Technical Note No. MT-63, United States Department of Agriculture (USDA), USA., December 2007.

Katova, A., 2007. Species and varieties of perennial grasses for high quality forage in Bulgaria. Proceedings of the 14th Meeting of the FAO-CIHEM Mountain Pastures Network, May 30-June 1, 2007, Troyan, Bulgaria, pp: 156-161.

Kirwan, L., G. Belanger, J. Finn, M. Fothergill and B. Frankow-Lindberg *et al*., 2005. Higher yield and fewer weeds in four-species grass/legume mixtures than in monocultures: Results from the first year at 20 sites of COST action 852. Proceedings of the 20th International Grassland Congress, June 26, 2005, Sao Paulo, Brazil, pp: 425.

Kozhuharov, S., 1976. *Trifolium Subterraneum* L. In: Flora of the Peopl's Republic of Bulgaria, Yordanov, D. (Ed.). Vol. 6, BAS., Sofia, pp: 434-437.

Kusvuran, A., Y. Ralice and T. Saglamtimur, 2014. 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.

Kyriazopoulos, A.P., E.M. Abraham, Z.M. Parissi and A.S. Nastis, 2008. Herbage production and nutritive value of *Dactylis glomerata* L. and *Trifolium subterraneum* L. alone and in mixture. Options Mediterraneennes, 79: 211-214.

Ledgard, S.F. and K.W. Steele, 1992. Biological nitrogen fixation in mixed legume/grass pastures. Plant Soil, 141: 137-153.

Luscher, A., I. Mueller Harvey, J.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 terms of competition indices and monetary advantage index under acid lateritic soil of India. Am. J. Exp. Agric., 1: 1-6.

Mannetje, L.T., 2003. Methods for estimating botanical composition, species diversity and dry matter yields. Grassland Sci. Eur., 6: 311-323.

Marinov-Serafimov, Pl., A. Katova and I. Golubinova, 2015. Allelopathic activity of rhizosphere soil in some perennial grasses. Proc. Union Scient. Ruse Agrarian Vet. Sci., 7: 209-215, (In Bulgarian)..

Mihovski, T.S. and A. Kirilov, 2014. State of Ruminant Animal's Stockbreeding and the Respective Forage Base in Bulgaria. In: Aktualni Poznatky v Pestovani, Slechteni, Ochrane Rostlin a Zpracovani Produktu, Uroda 12/2014, Vedecka Priloha Casopisu, Badalikova, B. and J. Bartlova (Eds.). ZVT., Czech Republic, pp: 105-110.

Ostrem, L., B. Volden and A. Larsen, 2013. Morphology, dry matter yield and phenological characters at different maturity stages of × *Festulolium* compared with other grass species. Acta Agriculturae Scandinavica Sect. B-Soil Plant Sci., 63: 531-542.

Pecetti, L. and E. Piano, 1994. Observations on the rapidity of seed and burr growth in subterranean clover. J. Genet. Breeding, 48: 225-228.

Pecetti, L. and E. Piano, 1998. Leaf size variation in subterranean clover (*Trifolium subterraneum* L. sensu lato). Genet. Resour. Crop Evol., 45: 161-165.

Pecetti, L. and E. Piano, 2002. Variation of morphological and adaptive traits in subterranean clover populations from Sardinia (Italy). Genet. Resour. Crop Evolution, 49: 189-197.

Peeters, A., G. Parente and A. Le Gall, 2006. Temperate legumes: Key-species for sustainable temperate mixtures. Grassland Sci. Eur., 11: 205-220.

Peyraud, J.L., A. Le Gall and A. Luscher, 2009. Potential food production from forage legume-based-systems in Europe: An overview. Irish J. Agric. Food Res., 48: 115-135.

Piano, E., L. Pecetti and A.M. Carroni, 1996. Climatic adaptation in subterranean clover populations. Euphytica, 92: 39-44.

Porqueddu, C., G. Parente and M. Elsaesser, 2003. Potential of Grasslands. In: Optimal Forage Systems for Animal Production and the Environment, Kirilov, A., N. Todorov and I. Katerov (Eds.). Vol. 8, Grassland Science in Europe, Badajoz, pp: 11-20.

Pozdisek, J., B. Henriksen, A. Ponizil and A.K. Loes, 2011. Utilizing legume-cereal intercropping for increasing self-sufficiency on organic farms in feed for monogastric animals. Agron. Res., 9: 343-356.

Pypers, P., S. Verstraete, C.P. Thi and R. Merckx, 2005. Changes in mineral nitrogen, phosphorus availability and salt-extractable aluminium following the application of green manure residues in two weathered soils of South Vietnam. Soil Biol. Biochem., 37: 163-172.

Renne, I.J., B.G. Rios, J.S. Fehmi and B.F. Tracy, 2004. Low allelopathic potential of an invasive forage grass on native grassland plants: A cause for encouragement? Basic Applied Ecol., 5: 261-269.

SPSS., 2012. SPSS Version 20.0. SPSS Inc., Wacker Drive, Chicago, Ill.

Shaffer, J.A., G.A. Jung and U.R. Narem, 1994. Root and shoot characteristics of prairie grass compared to tall fescue and smooth brome grass during establishment. New Zealand J. Agric. Res., 37: 143-151.

Siegel, M.R. and L.P. Bush, 1997. Toxin Production in Grass/Endophyte Associations. In: The Mycota V: Plant Relationships, Carroll, G.C. and P. Tudzynski (Eds.). Springer, Berlin, Heidelberg, ISBN: 978-3-662-10372-2, pp: 185-207.

Smart, A.J., W.H. Schacht, J.F. Pedersen, D.J. Undersander and L.E. Moser, 1998. Prediction of leaf: Stem ratio in grasses using near infrared reflectance spectroscopy. J. Range Manage., 4: 447-449.

Vanlauwe, B., J. Wendt, K.E. Giller, M. Corbeels, B. Gerard and C. Nolte, 2014. A fourth principle is required to define conservation agriculture in sub-Saharan Africa: The appropriate use of fertilizer to enhance crop productivity. Field Crops Res., 155: 10-13.

Vasilev, E., 2000. Study of some regimes of using on lucerne in pure and mixture with orchardgrass stands for obtaining of forage and seeds production. Ph.D. Theses, Sofia University, Sofia.

Vasilev, E., V. Vasileva, T.Z. Mihovsky and G. Goranova, 2005. Assessment of legume based mixture swards constrained by the environmental conditions in Central North Bulgaria-COST Action 852. Proceedings of the 2nd COST 852 Workshop, November 10-12, 2005, Grado, Italy, pp: 177-180.

Vasilev, E., 2006. Productivity of subterranean clover (*Trifolium subterraneum* L.) in pasture mixtures with some perennial grasses for the conditions of Central North Bulgaria. Plant Sci., 43: 149-152, (In Bulgarian).

Vasilev, E., 2009. Chemical composition of subclovers forage (*Tr. subterraneum* L.) and crude protein yield in pasture mixtures with grasses. J. Mountain Agric. Balkans, 12: 329-341.

Vasileva, V., E. Vasilev and M. Athar, 2011. Nodulation and root establishment of two clover species grown in pasture mixture with wheatgrass. FUUAST J. Biol., 1: 1-4.

Vasileva, V. and E. Vasilev, 2012. Study on productivity of some legume crops in pure cultivation and mixtures. Agric. Conspectus Sci., 77: 91-94.

Vasileva, V., 2014. Productivity of dry aboveground and root mass in mixtures. J. Mountain Agric. Balkans Agric. Acad., 17: 956-969.

Vasileva, V., 2015a. Botanical composition of swards with subterranean clover (*Trifolium subterraneum* L.). Proc. Union Sci. Ruse, 7: 160-165.

Vasileva, V., 2015b. Morphological parameters and rations in some mixtures with subclover. Sci. Int., 3: 107-112.

Yakimova, Y. and H. Yancheva, 1986. Phytocenological and ecological characteristics of some annual clovers in Strandja region. Plant Sci., 23: 47-53, (In Bulgarian).