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STUDY OF MONOCULTURE AND MIXED GRASS AND LEGUME FORAGE CROPS UNDER MOUNTAIN CONDITIONS

SUMMARY

The aim of the study was to determine the productivity, growth and development of perennial grass forage crop species (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown in sole crops and binary mixtures with *Trifolium pratense* L., under mountain conditions.

It was found that in the sole grasses, the crops of *Phleum pratense* L. were proven to have the highest yield of fresh (2279.2 kg/da) and dry mass (929.3 kg/da). The excess in the values of the indicators was up to 23.8% and 29.0%, respectively. The lowest productivity was recorded in monoculture crops of *Lolium perenne* L.

In the two-component mixtures, the grasses of *Phleum pratense* L. with *Trifolium pratense* L. (2168.7 kg/da) and *Dactylis glomerata* L. with *Trifolium pratense* L. (722.2 kg/da) significantly exceeded the yield of fresh and dry mass compared to the mixture of *Lolium perenne* L. with *Trifolium pratense* L. by 21.2% and 26.5%, respectively.

In the conditions of the Middle Balkan Mountains, the mixture with the most optimal evenness in the height of the grass and legume component is the binary mixture of *Festuca rubra* L. + *Trifolium pratense* L. With the greatest difference in the mean values of the trait is the mixture *Phleum pratense* L. + *Trifolium pratense* L. On average over the period, the stem height of the forage grasses species in the stand-alone mixtures varied from 32.7 cm to 99.2 cm, and in the two-component mixtures from 35.4 cm to 101.9 cm.

Keywords: yield of fresh and dry mass, grass-legume mixtures

INTRODUCTION

In the mountain and foothill conditions of the Middle Balkan Mountains (Bulgaria), the composition of plant associations in natural and sown grasslands is closely related to the biological characteristic of species, their plasticity and stability (Georgieva *et al.*, 2018).

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Representatives of the family *Poaceae* have a dominant influence in the formation of the grasslands type. They are a reliable source of biomass for animals. They are distinguished by high resistance to adverse environmental conditions and undemanding to soil and climatic factors (Landi *et al.,* 2017; Indu *et al.,* 2023; Kociecka *et al.,* 2023). They grow well both in low-lying areas where soils are nutrient-rich and in high-altitude areas on poor and poorly productive land (Kostov and Pavlov, 1999; Valdez and Dumansi, 2020; Kurhak *et al*., 2021). The valuable forage qualities of meadow grasses (longevity, high productivity with relatively low cost, high carbohydrate and energy content) and the specific features related to the growth and development of the species significantly influence their selection (Lüscher *et al*., 2014).

In the context of sustainable agriculture, the selection of perennial grasses and legumes of high quality and nutritional value has a significant impact in determining the productivity and quality of forage mass, as well as in creating a stable forage base for ruminant livestock production. Their ability to combine is an important factor in maintaining dynamic stability in the herbage stand and securing forage throughout the grazing season (Rolando *et al*., 2018; Vasileva and Enchev, 2018; Vanek *et al.,* 2020).

Compared to monoculture crops, mixtures of perennial grass and legume are also distinguished by higher productivity, ecological plasticity and durability (Helgadottir *et al.,* 2018; Meza *et al.,* 2022). The optimal association of certain species of perennial forage grasses points out the advantages of mixed grasses regarding better utilization of natural resources, individual plant growth and development (Dhakal and Islam, 2018; Tahir *et al.,* 2022).

Considering the upland soil and climatic conditions, successful establishment of sown grasses require legumes to have good ecological adaptation. *Trifolium pratense* L. has been identified as such a species in a number of studies for the area (Naydenova *et al.,* 2010; Naydenova and Bozhanska, 2014; Mihovsky and Naydenova, 2017; Petkova *et al.,* 2023). This species is also a valuable component in the composition of natural and sown grasslands, with grazing and hay use in many areas of Europe (Herrmann *et al.,* 2008; Drobna, 2009). It shows good adaptability to soil type, is highly productive and is successfully incorporated into mixed grass stands. Its above-ground mass is tender and readily accepted by animals (Nedělník *et al.,* 2016).

The aim of the study was to determine the productivity, growth and development of monoculture crops of meadow grasses and their mixtures with *Trifolium pratense* L., under the conditions of the Middle Balkan Mountains.

MATERIAL AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria), on light gray, pseudopodzolic soils with pH=4.2-5.5 (Penkov, 1988). The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown as monoculture (100%) and in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

The experimental variants included:

Monoculture grass stands

1. *Festuca rubra* L. (FR)

2. *Lolium perenne* L. (LP)

3. *Dactylis glomerata* L. (DG)

4. *Phleum pratense* L. (PP)

Two-component mixtures

5. *Festuca rubra* L. + *Trifolium pratense* L. (FR+TrP)

6. *Lolium perenne* L. + *Trifolium pratense* L. (LP+TrP)

7. *Phleum pratense* L. + *Trifolium pratense* L. (PP+TrP)

8. *Dactylis glomerata* L. + *Trifolium pratense* L. (DG+TrP)

Sowing was done manually, scattered and the areas were rolled to ensure better contact of the seeds with the soil. The sowing rates of the studied forage species were calculated based on 100% seed germination. The plot size was 5 m^2, laid out in 4 replications. Once a year (the last ten days of March - 20-30.03.) mineral fertilizing was applied. The monoculture crops were treated with N_{12} and the mixed grasslands were with a combined fertilizer of N_{12} and P_8.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

The following indicators were reported and analysed:

- Meteorological observations - annual and vegetation mean temperatures (ºC) and rainfall totals (mm);

- Fresh and dry mass yield (kg/da) - determined by mowing, weighing grass in replicates for each harvest plot with subsequent drying of samples to constant weight at 105ºC and recalculated for 1da;

- Height (cm) - recorded by mowing at the time of harvest of the grass, along both diagonals of each plot, plants were measured at 4 points from the soil surface to the top of the tallest stems and averages calculated from the data;

Climate characteristics in the experimental area

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion (Sabev and Stanev, 1963). The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average of the annual temperatures is 10/11°C (Ninov, 1997). The distribution of precipitation is uneven with a maximum in summer (309 mm) and minimum (168 mm) in winter. Spring is relatively cool and well-supplied with rainfall.

During the experimental period, the average annual air temperatures were 0.4ºC to 2.0ºC higher compared to those of the multiannual period (10.6ºC) (Table 1).

The temperature values in March (3.2-7.8ºC) were suitable for germination and development of the studied forage species. The average temperature during the vegetation (March-October) was from 14.6ºC (2021) to 16.3ºC (2023) with an average for the experimental period of 15.3ºC and an average for a multiannual period of 14.8ºC.

The characteristic of weather during the experiment shows the variation of climate factors, which specifically affect the development, productivity, and quality of forage species.

Table 1. Average monthly air temperature (ºC) for the period 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average	Average for III-X
2020	0.4	4.4	7.1	9.4	14.7	17.8	20.4	21.1	17.8	12.7	5.2	3.8	11.2	***15.1***
2021	1.6	3.7	3.6	8.3	15.4	18.9	22.7	22.7	16.2	8.7	7.5	2.6	11.0	***14.6***
2022	0.8	3.8	3.2	10.3	15.9	19.8	22.1	21.8	16.3	12.0	8.3	4.4	11.6	***15.2***
2023	5.3	3.2	7.8	9.9	14.2	19.0	23.2	22.3	18.8	15.4	8.1	3.9	12.6	***16.3***
2020-2023	***2.0***	***3.8***	***5.4***	***9.5***	***15.1***	***18.9***	***22.1***	***22.0***	***17.3***	***12.2***	***7.3***	***3.7***	***11.6***	***15.3***
1990-2019	***-0.5***	***2.3***	***5.5***	***10.5***	***15.2***	***18.8***	***20.8***	***20.7***	***15.7***	***10.9***	***6.1***	***1.8***	***10.6***	***14.8***

The highest annual precipitation amount (712.9 mm) were reported in 2023 compared to the other experimental years, but also compared to the average annual norm for the period 1990-2019 (789.7 mm), the values are 76.8 mm lower (Table 2). The data indicate that the annual precipitation amount during the experimental period is lower by 33.3-167.6 mm compared to those for a multiannual period. Droughts in Bulgaria are observed in all seasons, which affects the physiological processes during the different phenophases of the individual development of forage species.

The lowest annual precipitation amount (545.3 mm) and vegetation precipitation amount (March-October - 379.9 mm) were registered in the third experimental year (2022) when the main components in the monoculture and mixed grasslands reached optimal development and increased their participation in the grassland.

Table 2. Monthly and annual precipitation amount (mm) for 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual amount	Amount for III-X
2020	15.4	66.2	53.4	24.4	63.8	129	75.4	56.4	33.6	114.2	20.4	27.4	***679.6***	***550.2***
2021	82.8	25.6	47.7	57.0	82.8	64.8	12.4	56.2	11.8	72.8	23.6	68.6	***606.1***	***405.5***
2022	21.8	55.4	14.4	95.8	28.8	78.9	35.4	64.6	58.8	3.2	61.6	26.6	***545.3***	***379.9***
2023	12.4	27.8	25.8	82.6	174.5	132.4	27.6	50.2	28.4	1.8	81.2	68.2	***712.9***	***523.3***
2020-2023	***33.1***	***43.8***	***35.3***	***65.0***	***87.5***	***101.3***	***37.7***	***56.9***	***33.2***	***48.0***	***46.7***	***47.7***	***636.0***	***464.7***
1990-2019	***41.6***	***40.6***	***56.7***	***66.9***	***98.2***	***111.8***	***98.0***	***66.7***	***69.5***	***58.0***	***37.5***	***44.4***	***789.7***	***625.6***

The sum of the annual and vegetation precipitation is lower by 153.7 mm and 160.9 mm, respectively, compared to the multiannual period. Air temperature and precipitation are factors impacting the composition, density, and resistance of the studied plant species. In the experimental years, spring moisture offered optimal conditions for the formation of the first regrowth, both in monoculture and mixed grasslands.

Analysis Toolpak for Microsoft Excel 2010 and *Statgraphics Plus v.2.1* software were used for statistical data processing.

RESULTS AND DISCUSSION

Yield of fresh and dry mass from individual stands of grass forage crops and their binary mixtures with *Trifolium pratense* L.

Given the global warming climate and to minimize the negative environmental impact on forage productivity and quality, it is challenging to establish forage crop species with high adaptive potential in areas with variable and uneven rainfall distribution (Churkova, 2013; Huang *et al.*, 2017; Ferner *et al.*, 2018; Churkova and Churkova, 2021).

Over the four-year test period, monoculture crops of grass forages recorded demonstrably higher productivity compared to the corresponding mixtures (Figures 1 and 2).

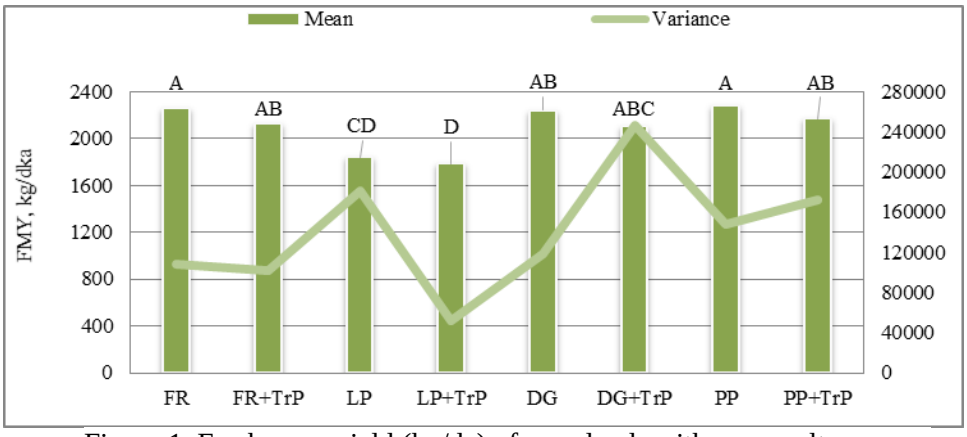

Figure 1. Fresh mass yield (kg/da) of grasslands with monoculture grass species and in mixtures with *Trifolium pratense* L. (average for the period 2020-2023)

The highest yields of fresh (2279.2 kg/da) and dry mass (929.3 kg/da) were recorded for the sole crop of *Phleum pratense* L., followed by the variants of *Dactylis glomerata* L. (2241.7 kg/da - fresh mass and 873.9 kg/da - dry mass). For the experimental period, the presence of the forage crop in the two grass stands was over 90%, which is an indication of good resistance and adaptability of the plants on the pseudopodzolic soils in the experimental area. The fresh mass yield in the monoculture crops of *Phleum pratense* L. and *Dactylis glomerata* L. was higher by 110.5 kg/da and 140.0 kg/da, respectively, compared to their two-component mixtures. There was a significant difference in dry mass yield between the two types of grasses. The values of the index in the mixtures of *Phleum pratense* L. with *Trifolium pratense* L. and *Dactylis glomerata* L. with *Trifolium pratense* L. were lower by 31.3% and 17.4%, respectively ($P<0.05$), compared to the monoculture grasslands of the respective grass species.

For the study period, the pure crops of *Lolium perenne* L. had a proven higher dry mass productivity compared to the mixture of this species with *Trifolium pratense* L. The excess in the values of the indicator was 20.7%. In the years of study, *Lolium perenne* L. dominated the mixed grass stand and was the main component forming the harvested forage yield (Bozhanska *et al.*, 2024).

Analysis of the data indicated that the grassland had the lowest yield (< 20%) of fresh (1789.6 kg/da) and dry mass (517.1 kg/da) compared to the average values of the parameters in the binary mixtures included in the study.

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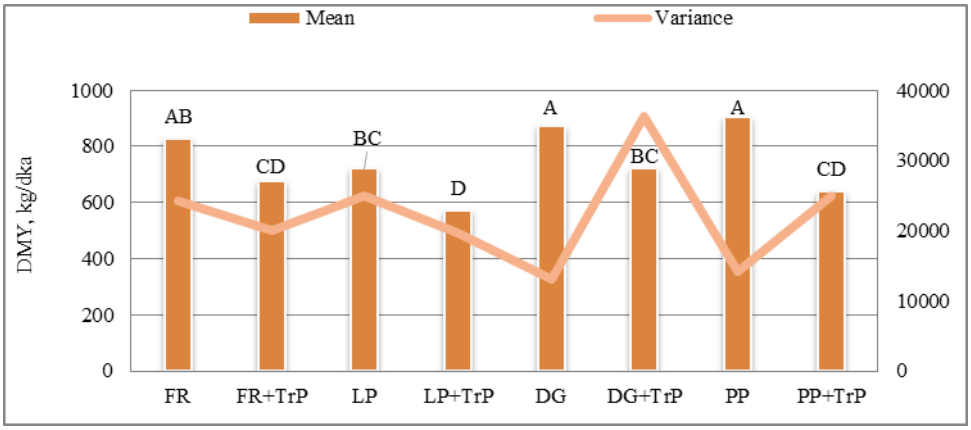

Figure 2. Dry mass yield (kg/da) of grasslands with monoculture grass species and in mixtures with *Trifolium pratense* L. (average for the period 2020-2023)

The monoculture grasslands with *Festuca rubra* L. had a higher fresh (2258.3 kg/da) and dry (825.0 kg/da) mass yield compared to the corresponding mixture. A proven excess in the amount of dry mass by 22.0% ($P < 0.05$) was found.

On average over the period, monoculture crops of *Lolium perenne* L. were shown to have the lowest fresh mass (by 17.8% to 19.2%) and dry mass (by 17.6% to 22.5%) productivity compared to sole stands of *Festuca rubra* L., *Dactylis glomerata* L. and *Phleum pratense* L.

In the two-component mixtures, the excess in fresh mass yield was 17.7-21.2% ($P < 0.05$) compared to the grasslands of *Lolium perenne* L. and *Trifolium pratense* L. It was found that in the conditions of the Middle Balkan Mountains, the mixture *Dactylis glomerata* L. + *Trifolium pratense* L. exceeded the dry mass yield in the grasslands of *Lolium perenne* L. + *Trifolium pratense* L. by 26.4% ($P < 0.05$).

Height of grass and legume component grown in sole and mixed stands

The height of the components in the grass stand provides information related to the sole and combined use of perennial grass and legume forage grasslands, assesses their compatibility, stability and productivity under the prevailing unfavourable soil and climatic conditions in upland areas.

Height of grass species

The average plant height in single crops of *Festuca rubra* L. varies from 38.1 cm to 80.2 cm (Figures 3-6). The values are 2.7 to 14.3 cm higher than in the corresponding two-component mixture. Grass crop growth in both types of grasslands (monoculture and mixed) was highest in the third growing season (2022), when *Festuca rubra* L. also reached maximum productivity. In the fourth growing season, with a more significant difference in grass height, mixed grasslands with *Trifolium pratense* L.

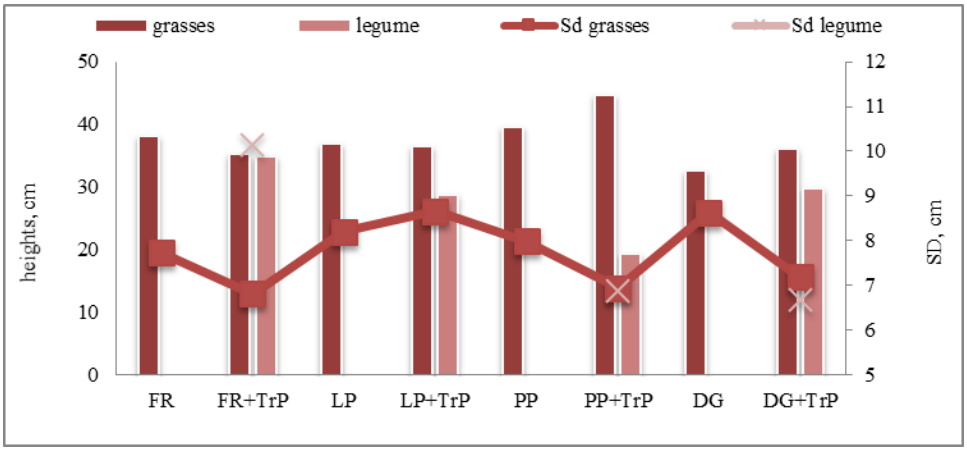

Figure 3. Heights (cm) of grass species and legume component in monoculture and mixed grasslands (first experimental year)

Lolium perenne L. is a valuable forage crop with good development in both upland and lowland conditions (Petkova *et al.*, 2021).

In the year of sowing, only the stems of *Lolium perenne* L. had almost identical values for the plant height trait in mixed and monoculture crops. The data indicate a minimal difference (36.6-36.9 cm) in the height of the grass in the two types of grasslands (single and mixed).

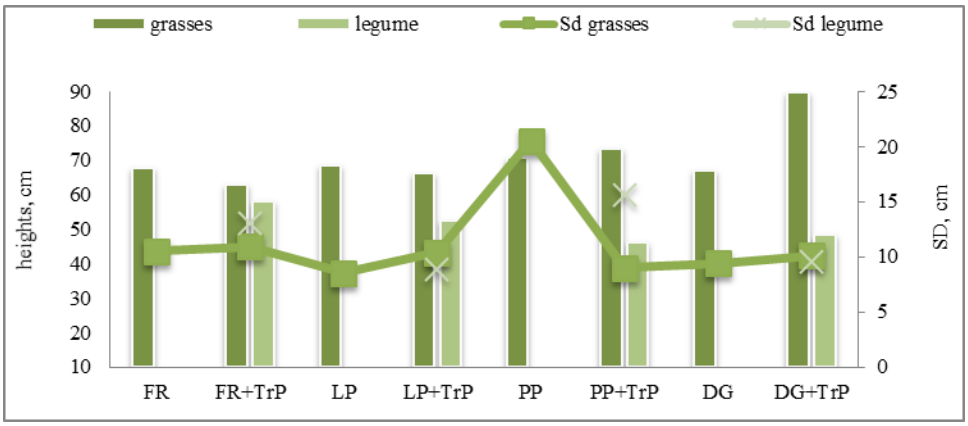

Figure 4. Heights (cm) of grass species and legume component in sole and mixed grasslands (second experimental year)

The association of *Lolium perenne* L. with *Trifolium pratense* L. as well as the increase in the age of the grasslands resulted in a more significant difference in stem height in the grass. In the monoculture grasslands, the mean value of the indicator ranged from 68.4 to 84.8 cm, and in the corresponding mixture from 66.1 to 81.5 cm. According to Katova and Vulchinkov, (2019), perennial ryegrass plants vary strongly in habit and height.

The height of *Phleum pratense* L. grasslands was found to be positively correlated with fresh biomass productivity (r=0.78**) (Janković *et al.*, 2018). According to the results obtained, plants of the species *Phleum pratense* L. recorded lower values in stem height in single grasslands, compared to mixed ones, in the period from the first to the third vegetation. In contrast, in 2023 (the fourth growing season, the year with the highest annual rainfall), *Phleum pratense* L. plants in the monoculture crop were 6.6 cm taller than those in the corresponding mixture (73.0 cm), suggesting higher fresh biomass productivity.

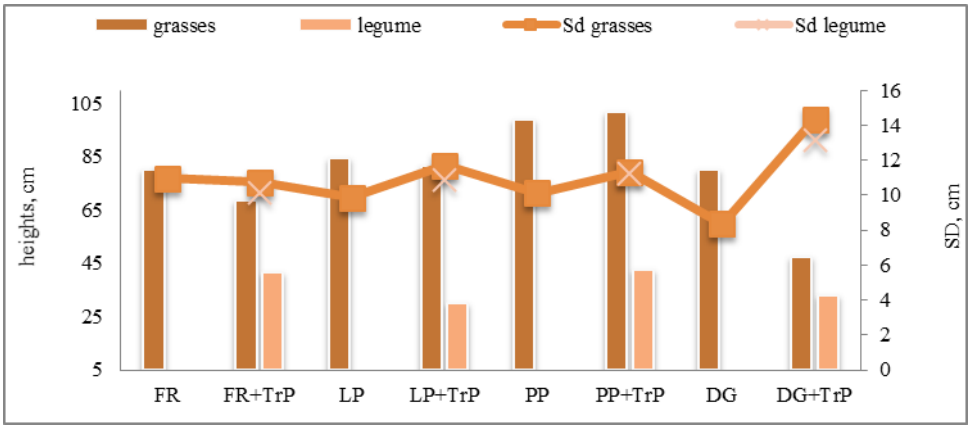

Figure 5. Heights (cm) of grass species and legume component in sole and mixed grasslands (third experimental year)

Dactylis glomerata L. is a grass forage crop with a strong competitive ability reflected by the values of the biometric indicator plant height (Bozhanska and Churkova, 2019). Data from the analysis indicate that plant height in monoculture cultivation ranged from 32.7 to 80.2 cm, and in mixed grasslands from 36.2 to 89.7 cm. In the first and second growing seasons, the plants of *Dactylis glomerata* L. recorded 3.5 to 22.7 cm lower values in stem height when grown alone compared with the two-component mixtures.

Dactylis glomerata L. is a perennial grass species, drought-resistant and with good productivity when grown in pure or mixed crops (Zhouri *et al.*, 2019). Interestingly in this case, in the year with the lowest rainfall amounts (2022), the difference in plant growth rate between the two grassland types is significant. The stems of *Dactylis glomerata* L. in the stand-alone grasslands were 32.9 cm taller than those in the corresponding two-component mixture (47.3 cm).

In the conditions of the Middle Balkan Mountains, grass species (the exception is *Dactylis glomerata* L. grown in a two-component mixture) in both types of grasslands are characterized by the highest rate of regrowth and emergence in the third growing season (2022 – is with the lowest amount of precipitation), which is a good indicator of their drought tolerance.

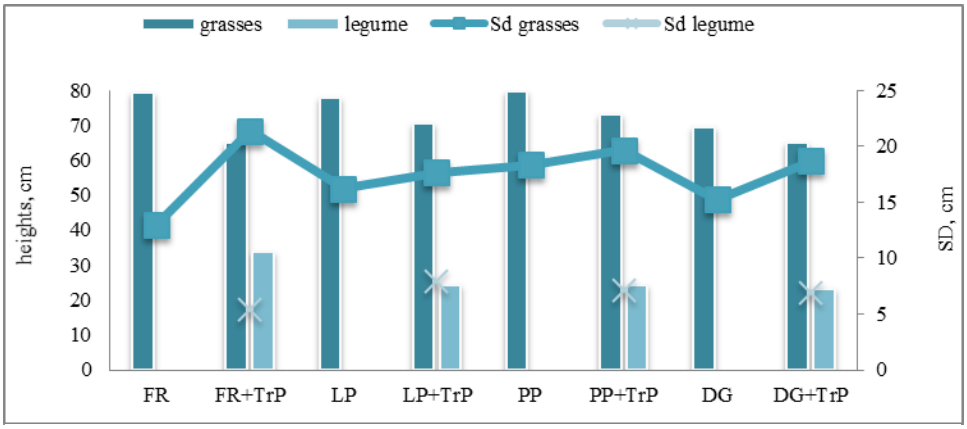

Figure 6. Heights (cm) of grass species and legume component in sole and mixed grasslands (fourth experimental year)

The average stalk height of forage grass species grown in sole grasslands and binary mixtures is:

- *Festuca rubra* L. – 66.3 cm and 58.0 cm;
- *Lolium perenne* L. – 66.9 cm and 63.7 cm;
- *Phleum pratense* L. – 72.2 cm and 73.2 cm;
- *Dactylis glomerata* L. – 62.3 cm and 59.6 cm.

Height of legume component

The good moisture supply during the first year of the experiment helped the growth rate of *Trifolium pratense* L., which is a mesophytic species.

The data show that the average height of *Trifolium pratense* L. in the binary mixtures ranged from 19.5 cm (*Phleum pratense* L. + *Trifolium pratense* L.) to 34.9 cm (*Festuca rubra* L. + *Trifolium pratense* L.). In the mixtures with *Lolium perenne* L. and *Dactylis glomerata* L. the values were 28.7 cm and 29.8 cm, respectively. In mixed cultivation of two or more species there are aspects of both intraspecific and interspecific competition (Razec and Razec, 2006). In the first growing season, the difference in the growth rate of the legume component depending on the grass species with which it is associated is significant. As an explanation for what was observed, the rapid rate of outgrowth and the greater competitive ability of grass can be pointed out, which suppresses the development of *Trifolium pratense* L., characterized by a slower rate of outgrowth in the year of sowing.

As a representative of the second cenotic group, *Trifolium pratense* L. demonstrates high competitive ability and the length of generative stems in the second vegetation reach the highest values in all variants. The trend characterising the minimum and maximum value of the legume component in the mixtures with *Phleum pratense* L. (46.3 cm) and *Festuca rubra* L. (57.9 cm) was also maintained. For the same period, the association of *Trifolium pratense* L. with *Lolium perenne* L. and *Dactylis glomerata* L. resulted in the formation of grassland where the mean height of the legume component was 52.4 cm and 48.3 cm, respectively.

The biological characteristics of the individual species as well as their interrelationships in mixed crops influence their longevity and purpose. In the conditions of the Middle Balkan Mountains, with increasing age of the stand, there is a delay in the development and growth process of *Trifolium pratense* L. In the third growing season, the length of generative stems in the legume component was above 30.0 cm in the mixtures with *Dactylis glomerata* L. (32.8 cm) and *Lolium perenne* L. (30.2 cm) and above 40.0 cm in the mixtures with *Phleum pratense* L. (42.9 cm) and *Festuca rubra* L. (41.8 cm).

In the fourth growing season, the legume crop recorded lower values for the plant height trait. The average height of *Trifolium pratense* in the two-component mixtures with *Festuca rubra* L. and *Phleum pratense* L. was 33.7 cm and 23.0 cm, respectively. Mixed grasslands of *Lolium perenne* L. (24.1 cm) and *Phleum pratense* L. (24.4 cm) had minimal difference in the values of the indicator.

In the conditions of the Middle Balkan Mountains, the mixture with the best uniformity in the height of the grass and legume component was the binary mixture of *Festuca rubra* L. + *Trifolium pratense* L. With the greatest difference in the mean values of the trait was the mixture of *Phleum pratense* L. + *Trifolium pratense* L.

Significance of factorial influence on productivity and height of sole and mixed grass and legume forage grasslands

The factors - environmental conditions, age and type of grassland had a significantly influenced ($P < 0.01$–$P < 0.001$) the values of the studied traits (Table 3).

Year and age (η^2 ranged from 14.7 to 67.6%) and type of herbage (η^2 ranged from 2.4 to 40.3%) significantly influenced forage crop productivity and height.

Table 3. Degree (η^2) and significance (P) of factorial influences on yield and height of monoculture forage grass grasslands and their mixtures with *Trifolium pratense* L.

Sources of variation / Signs	Year and age of grassland		Type of grasslands		Interaction	
	η^2 (%)	P	η^2 (%)	P	η^2 (%)	P
FMY-monocul. grasslands	14.7	P <0.01	21.3	P <0.001	29.9	ns
FMY- mixed grasslands	28.0	P <0.001	19.3	P <0.01	15.5	ns
DMY- monocul. grasslands	21.1	P <0.01	21.8	P <0.01	19.0	ns
DMY- mixed grasslands	61.2	P <0.01	11.8	P <0.01	3.9	ns
Hight – monocul. grasslands	67.6	P <0.001	2.4	P <0.001	1.8	P <0.001
Hight - mixed grasslands	21.7	P <0.001	40.3	P <0.001	14.6	P <0.001

The interaction between the factors under study accounted for a significant proportion of the factorial variance in the traits plant height in the sole (η^2=1.8%) and mixed grasslands (η^2=14.6%). According to the results obtained, the factorial interaction had insignificant influence on the traits of fresh and dry mass yield for both types of grasslands.

CONCLUSIONS

In the conditions of the Middle Balkan Mountains, the independent stands of *Phleum pratense* L. have been shown to have the highest yield of fresh (2279.2 kg/da) and dry matter (929.3 kg/da). The excess in the values of the indicators was up to 23.8% and 29.0%, respectively, compared to the other monoculture grass crops studied. In the two-component mixtures, the grassland of *Phleum pratense* L. with *Trifolium pratense* L. (2168.7 kg/da) and *Dactylis glomerata* L. with *Trifolium pratense* L. (722.2 kg/da) had the highest fresh and dry mass yields.

On average over the period, the stem height of the forage grass species in single grasses ranged from 32.7 cm to 99.2 cm, and in their two-component mixtures from 35.4 cm to 101.9 cm. The two-component mixture of *Festuca rubra* L. with *Trifolium pratense* L. had the most optimal evenness in the height of the wheat and bean components, as an indication of good compatibility under upland conditions.

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