RESERVES OF *TRIFOLIUM PRATENSE* AND *TRIFOLIUM REPENS* AS MEDICINAL RAW MATERIALS IN DIFFERENT LANDSCAPE ZONES OF THE MIDDLE VOLGA REGION

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ABSTRACT

In this article, we analyze the variability of aboveground dry plant biomass and yield of aboveground phytomass in *Trifolium pratense* L. and *Trifolium repens* L. under different growing conditions. The studied species are valuable melliferous, fodder and medicinal plants, which accumulate ascorbic acid, carotenoids, higher fatty acids, glycosides, phenols, flavonoids and other substances. During the growing seasons 2018-2020, we studied 15 natural coenopopulations of *T. pratense* and *T. repens*, distributed in the southern taiga, subtaiga, broad-leaf and forest-steppe landscape subzones within the Republic of Tatarstan, Russia. The yield of aboveground dry phytomass in the coenopopulations of *T. pratense* and *T. repens* noticeably varied: 59-622 kg/ha and 20-210 kg/ha, respectively. The high yield of dry aboveground phytomass in *T. pratense* (434-622 kg/ha) and *T. repens* (178-210 kg/ha) is observed in the subzone of broad-leaf forests, which is characterized by light gray and gray forest soils, as well as the highest precipitation per year, 500-540 mm. At the same time, a significant positive correlation ($R=0.63$; $p<0.05$) was revealed between the dry aboveground plant biomass and its yield in *T. pratense,* and a high significant positive correlation was determined between these parameters in *T. repens* ($R=0.87$; $p<0.05$). The conditions of the broad-leaf forest subzone within the Republic of Tatarstan are the most favorable for the accumulation of aboveground phytomass in *T. pratense* and *T. repens*. Therefore, the territories within this zone can be recommended for the collection and procurement of herbal medicinal raw materials of these species.
Keywords: landscape subzones, *Trifolium pratense*, *Trifolium repens*, habitat conditions, biomass yield

INTRODUCTION

Plant species are characterized by different intensity of growth and development within their range, which reflects a certain degree of their plasticity [1]. When assessing the resources of wild-growing economically significant plants, an important aspect is the identification of the ecological and coenotic conditions that ensure high productivity of the aboveground phytomass [2-3].

Red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) are melliferous, forage and pasture plants. In addition, these species can be used as medicinal plant raw materials. Essential and fatty oils, organic acids, coumarins, resins, tannins, trifolin and isotrifolin glycosides, vitamins (ascorbic acid, carotene, tocopherol,

https://doi.org/10.5593/sgem2021/5.1/s20.052

thiamine), flavonoids and isoflavonoids (96 in total) are synthesized in the aboveground organs of *T. pratense* [4-6]. Due to its phytochemical composition, *T. pratense* can be used as a food additive or therapeutic agent, since water and ethyl acetate extracts of the leaves effectively protect tissues from oxidative stress [7] and have an antitumor effect [6]. *Trifolium repens* shoots accumulate vitamins (ascorbic acid and carotene), glycosides and flavonoids [6, 8], so it is used in traditional medicine as an expectorant and diaphoretic agent, and extracts have an anthelmintic effect.

The territory of the Republic of Tatarstan (RT), Russia, includes four landscape subzones: the southern taiga and subtaiga subzones as part of the boreal landscape zone, as well as broad-leaf and forest-steppe subzones as part of the subboreal northern semi-humid landscape zone [9]. Taking into account the diversity of natural landscapes, it is important to study the influence of natural and climatic conditions on the productivity of coenopopulations of economically valuable species. The aim of our research was to assess the reserves of raw plant materials in terms of the dry aboveground phytomass and the yield of aboveground phytomass in *Trifolium pratense* and *Trifolium repens* in the zonal perspective, as well as to recommend the areas that are the most favorable for collecting and obtaining medicinal raw materials of these species in the Republic of Tatarstan.

RESEARCH METHODS

Research objects: *T. pratense* and *T. repens*, perennial summer-green or summer-winter-green herbaceous polycarpic plants. *Trifolium pratense* belongs to hemicryptophytes, it is characterized by a tap-root system with a multi-headed caudex, on which semi-rosette erect or ascending shoots are formed [10]. *Trifolium repens* is a chamaephyte with elongated, creeping, sympodially growing shoots. *Trifolium pratense* has a Euro-West Asian distribution, and *T. repens* has a Euro-Asian distribution. Within the range, species are widely represented in meadow and forest edge communities, as well as in weedy areas and along roads [10-11].

During the growing seasons 2018-2020, 15 natural coenopopulations (CPs) of *T. pratense* and 15 natural CPs of *T. repens* were studied in the southern taiga, subtaiga, broad-leaf and forest-steppe landscape subzones on the territory of 11 administrative districts of the Republic of Tatarstan, Russia (Tables 1-2).

Table 1. Sampling points and sample sizes of *Trifolium pratense* L. in various landscape subzones on the territory of the Republic of Tatarstan

Landscape subzone	CP no	Collection year	Sampling point (district, locality)	Coordinates	Sample size, pc.
Southern taiga	1	2019	Vysokogorskij, s. Gar'	56°24′54′′N 49°11′05′′E	32
Subtaiga	2	2020	Arskij, p. Urnjak	56°23′ 75′′N 50°00′ 06′′E	52
	3	2020	Baltasinskij, s. Karelino	56°44′ 19′′N 50°25′ 95′′E	33
	4	2018	Zelenodol'skij, p. Oktjabr'skij	55°83′68′′N 48°78′53′′E	12

	5		Zelenodol'skij, p. Novonikolaevka	55°84´34´´N 48°73´90´´E	14
Broad-leaf	6	2018	Laishevskij, s. Nikol'skoe	55°53´60´´N 49°15´37´´E	43
	7		Laishevskij, s. Atabaevo	55°28´39´´N 49°35´53´´E	43
	8		Laishevskij, d. Orel	55°53´36´´N 49°12´40´´E	20
	9	2018	Verhneuslonskij, p. Vvedenskaja sloboda	55°77´01´´N 48°71´66´´E	17
	10	2019	Apastovskij, s. Bol'shie Kokuzy	55°31´08´´N 48°64´28´´E	20
	11	2019	Kamsko-Ust'inskij, s. Krasnovidovo	55°34´89´´N 49°06´10´´E	22
	12	2018	Tetjushskij, s. Tetjushi	54°96´45´´N 48°82´76´´E	25
	13		Tetjushskij, s. Dolgaja Poljana	55°05´61´´N 48°93´35´´E	11
	14		Tetjushskij, r. Kljara	55°10´10´´N 49°00´92´´E	18
Forest-steppe	15	2019	Spasskij, g. Bolgar	54°97´86´´N 49°04´93´´E	38

Table 2. Sampling points and sample sizes of *Trifolium repens* L. in various landscape subzones on the territory of the Republic of Tatarstan

Landscape zone	CP no	Collection year	Sampling point (district, locality)	Coordinates	Sample size, pc.
Subtaiga	1	2020	Arskij, p. Urnjak	56°23´ 75´´N 50°00´ 06´´E	86
	2		Baltasinskij, s. Karelino	56°44´ 19´´N 50°25´ 95´´E	49
	3	2018	Zelenodol'skij, p. Oktjabr'skij	55°83´68´´N 48°78´53´´E	67
	4		Zelenodol'skij, p. Oktjabr'skij	55°83´80´´N 48°76´97´´E	66
	5		Zelenodol'skij, p. Novonikolaevka	55°84´34´´N 48°73´90´´E	67
Broad-leaf	6	2018	Laishevskij, d. Orel	55°53´36´´N 49°12´40´´E	7
	7	2018	Laishevskij, s. Nikol'skoe	55°53´60´´N 49°15´37´´E	42
	8	2018	Verhneuslonskij, d. Grebeni	55°5805´´N 48°9693´´E	33
	9		Verhneuslonskij,	55°61´22´´N	23

https://doi.org/10.5593/sgem2021/5.1/s20.052

			s. Tashevka	48°95´09´´E	
	10		Verhneuslonskij, s. Shelanga	55°53´30´´N 48°97´98´´E	72
	11		Verhneuslonskij, p. Vvedenskaja sloboda	55°77´01´´N 48°71´66´´E	98
	12		Tetjushskij, s. Tetjushi	54°96´45´´N 48°82´76´´E	68
	13	2018	Tetjushskij, s. Dolgaja Poljana	55°05´61´´N 48°93´35´´E	63
	14		Tetjushskij, r. Kljara	55°10´10´´N 49°00´92´´E	86
Forest-steppe	15	2020	Aktanyshskij r-n, p. Aktanysh	55°71´09´´N 54°05´81´´E	64

In meadow and forest edge phytocoenoses, five plots of 1 m^2 were laid, on which all plants of the studied species were dug up for herbarization and further analysis of dry phytomass. In the process of morphological research, dry aboveground phytomass (W, g) and its yield (Y, kg/ha) were determined in dried plants. The yield in different CPs was determined by summing up the air-dry aboveground phytomass of plants collected from five-meter plots in terms of kg/ha. The plant (genet) in *T. pratense* with a taproot system and the shoot (ramet) in a vegetatively mobile plant *T. repens* were used as counting units for assessing the plant phytomass and its yield. The sample size was 12-52 and 7-98 plants in *T. pratense* and *T. repens* CPs, respectively (Tables 1-2). All data were statistically processed. The significance of differences according to the Duncan's test, as well as the values of the correlation coefficient (R) between the mean values, were calculated in the AGROS program.

RESEARCH RESULTS

On the territory of the Republic of Tatarstan, the southern taiga subzone occupies the smallest area (389.4 km^2), being located in the extreme north-west of the Predkam'e, while the forest-steppe subzone occupies the largest area (41454.4 km^2), covering the areas of the Zakam'e region and the extreme south of the Predvolzh'e. The areas of the subtaiga and broad-leaf subzones are 14344 and 11812 km^2, respectively (Table 3).

Table 3. Characteristics of soil and climatic conditions in various landscape subzones on the territory of the Republic of Tatarstan

Landscape subzone	Area in RT, km^2	Precipitati on per year, mm	Hydrothermal coefficient	Soils
Southern taiga	389	500-520	1.7-1.9	Sod-podzolic and light gray forest
Subtaiga	14344	480-520	1.6-1.9	Light gray forest and sod-podzolic
Broad-leaf	11812.6	500-540	1.6-1.8	Light gray and gray forest
Forest-steppe	41454.4	460-500	1.5-1.7	Leached, podzolized and typical chernozems

When moving from the southern taiga to the forest-steppe subzone, the annual amount of precipitation varies from 540 to 460 mm, while more than 500 mm of precipitation per year is typical for the southern taiga, subtaiga and broad-leaf subzones [9]. The highest values of the hydrothermal coefficient (up to 1.9) are observed in the southern taiga and subtaiga subzones, which indicates some waterlogged conditions for the moisture supply of plants. The values of the hydrothermal coefficient from 1.5 to 1.7 in the forest-steppe subzone indicate the insufficient moisture supply of the landscapes. On the territory of the Republic of Tatarstan, in the southern taiga and subtaiga subzones, sod-podzolic and light gray forest soils are common, while the broad-leaf subzone is characterized by light gray and gray forest soils, and the forest-steppe subzone includes leached, podzolized and typical chernozems [9].

In different landscape subzones of the Republic of Tatarstan, the dry aboveground biomass of a *T. pratense* plant was 0.57–8.69 g, and the yield of dry aboveground phytomass was 59–622 kg/ha (Table 4).

Table 4. Dry above-ground phytomass (W) and yield of above-ground phytomass (Y) of *Trifolium pratense* in different landscape subzones on the territory of the Republic of Tatarstan

Landscape subzone	CP no	W (mean), g*	W (error of the mean), g	Y, kg of dry weight/ha*
Southern taiga	1	3.64 abcde	0.52	341 de
Subtaiga	2	0.57 a	0.07	59 a
	3	1.52 a	0.13	109 abc
	4	6.02 efghi	0.70	181 abcd
	5	8.50 cdefgh	0.30	296 cde
Broad-leaf	6	2.61 abc	0.43	217 abcd
	7	2.41 abc	0.29	227 abcd
	8	4.80 bcdef	1.06	199 abcd
	9	5.73 ghi	1.16	251 abcde
	10	8.69 i	2.11	434 e
	11	5.99 hi	1.63	622 f
	12	3.37 abcde	0.46	169 abcd
	13	6.77 ghi	0.51	248 abcde
	14	5.25 defghi	0.89	236 abcd
Forest-steppe	15	3.29 abcde	0.50	280 bcde

*Note: values accompanied by the same letters differ insignificantly according to the Duncan's criterion.

We found a significant positive correlation between the dry plant biomass and the total yield of phytomass (R=0.63**).

Reliably low values of the total aboveground plant phytomass (0.57 and 1.52 g) and the yield of aboveground phytomass (59 and 109 kg/ha) were determined in CPs 2 and 3, which are part of the forest edge phytocoenoses in the subtaiga subzone. The highest aboveground plant phytomass (5.99 and 8.69 g) and yield (434 and 622 kg/ha) were noted for CPs 10 and 11, which belong to the fescue-forb phytocoenoses of the broad-leaf forest subzone. In the rest of the CPs, the yield of aboveground phytomass slightly

https://doi.org/10.5593/sgem2021/5.1/s20.052

differed, being in the range of 169-341 kg/ha. According to the research by N.I. Kasatkina [12], the average yield of *T. pratense* in Udmurtia (southern taiga subzone) is 145-157 kg/ha, which is 1.5-3 times lower than the yield in the broad-leaf subzone. On the contrary, in the mountains of Kabardino-Balkaria on leached chernozems, the yield of *T. pratense* can reach 2.4-3.5 t/ha [13], which is 3-5 times higher than on the territory of the Republic of Tatarstan.

As shown earlier, the plant density in the studied CPs varies from 3 to 17.2 pcs/m^2, and the highest values of dry phytomass yield are characteristic for CPs with a high density [14].

The dry phytomass of *T. repens* shoot was 0.12–0.87 g, and the yield of aboveground phytomass was 20–210 kg/ha (Table 5).

Table 5. Dry aboveground phytomass (W) and yield of aboveground phytomass (Y) of *Trifolium repens* in different landscape subzones on the territory of the Republic of Tatarstan

Landscape subzone	CP no	W (mean), g*	W (error of the mean), g	Y, kg of dry weight/ha*
Subtaiga	1	0.12 a	0.01	27.2 a
	2	0.13 a	0.01	20.7 a
	3	0.31 a	0.02	55.0 a
	4	0.25 a	0.01	52.3 a
	5	0.22 a	0.02	51.8 a
Broad-leaf	6	0.87 f	0.17	210 c
	7	0.19 a	0.02	20.8 a
	8	0.49 d	0.06	40.2 a
	9	0.23 a	0.01	30.0 a
	10	0.45 cd	0.03	77.2 a
	11	0.70 e	0.03	177.5 bc
	12	0.27 a	0.02	68.6 a
	13	0.15 a	0.01	33.3 a
	14	0.16 a	0.01	30.0 a
Forest-steppe	15	0.45 bcd	0.03	–

*Note: values accompanied by the same letters differ insignificantly according to the Duncan's criterion.

We found a high positive correlation between the shoot dry biomass and the total yield of dry aboveground phytomass (R=0.87 **).

Reliably high values of shoot dry phytomass (0.7 and 0.87 g), as well as the total aboveground phytomass (177.5 and 210 kg/ha) were found in CPs 6 and 11, which are common in the forest and grass-forb phytocoenoses in the broad-leaf subzone. In other CPs, the yield of aboveground phytomass varied within the range of 20.7-77.2 kg/ha and did not significantly differ among these CPs.

The density of shoots in the *T. repens* CPs is 15–48 pcs/m^2, with the highest density observed in the southern regions of the broad-leaf subzone, and the lowest density noted for central and northern regions of the broad-leaf subzone [15]. Our studies show that in the central and northern parts of the broad-leaf subzone, *T. repens* coenopopulations have a high productivity of the aboveground phytomass. Therefore, a decrease in the density of plagiotropic shoots leads to an increase in their phytomass.

The studies of the relationship between the accumulation of flavonoids (quercetin, kaempferol) and the morphological parameters of plants have shown that the high biomass productivity of white clover negatively correlates with the ability of plants to synthesize and accumulate flavonoids that increase their stress resistance [8].

CONCLUSION

On the territory of the Republic of Tatarstan, in the *Trifolium pratense* and *Trifolium repens* coenopopulations, a high variability of the values in the yield of dry aboveground biomass was revealed, depending on the soil and climatic conditions, which are characteristic for different landscape subzones. The minimum yield of dry aboveground phytomass in *Trifolium pratense* (59-109 kg/ha) and *Trifolium repens* (21 kg/ha) is observed in the subtaiga subzone, where the largest area is occupied by sod-podzolic and light gray forest soils, the average annual precipitation varies from 480 to 520 mm, and the hydrothermal coefficient is 1.6-1.9. The high productivity of dry aboveground phytomass in *Trifolium pratense* (434-622 kg/ha) and *Trifolium repens* (178-210 kg/ha) are characteristic for some coenopopulations within the broad-leaf subzone, where light gray and gray forest soils are widespread, the average annual precipitation is higher than in the subtaiga subzone, being 500-540 mm, and the hydrothermal coefficient is 1.6-1.8. We believe that the territories of the broad-leaf forest subzone within the Republic of Tatarstan are the most optimal in terms of the prevailing conditions for the accumulation of aboveground phytomass and they can be recommended for collecting medicinal raw materials of *Trifolium pratense* and *Trifolium repens*.

ACKNOWLEDGMENTS

This work was carried out with the financial support of the Russian Foundation for Basic Research and the Government of the Republic of Tatarstan in the framework of the scientific project No. 18-44-160015.

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