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DRY MATTER AND PROTEIN YIELDS AND LEAF GREENNESS INDEX (SPAD) OF ALFALFA AND FESTULOLIUM DEPENDING ON CULTIVATION METHOD AND SOIL MOISTURE LEVEL

Summary

The aim of the study was to assess the influence of water deficiency in the soil and cultivation method on dry matter and protein yields as well relative chlorophyll content of alfalfa and festulolium leaves. The pot experiment was conducted in the years 2012-2014 in the greenhouse of IUNG-PIB in Puławy, in a completely randomized system, in 4 repetitions. The research included alfalfa and festulolium cultivated in pure stand and in a mixture, at two soil moisture levels: 70% field water capacity (optimal humidity) and 40% FWC (drought stress). The study showed that long-term drought stress significantly reduced the dry matter yield of alfalfa and festulolium, with the highest loss in pure alfalfa stand (by 39.8% on average), lower in the mixture (by 29.1%), and lowest in pure festulolium stand (by 13.6%). Regardless of the soil moisture conditions, the highest total protein yield was obtained from alfalfa grown in pure stand, and lower by only 12% from the mixture. The protein yield from festulolium cultivated in pure stand was, on average, lower by 68% in comparison with the yield of alfalfa. The greenness index of the alfalfa leaf was significantly higher than that of the hybrid festulolium, regardless of the soil moisture conditions. The water requirement in the soil and the method of cultivation did not significantly differentiate the relative content of chlorophyll in the leaves of the tested plant species.
***Key words**: legumes, grass, drought stress, yield, SPAD index, mixture, pure stand*

PLON SUCHEJ MASY I BIAŁKA ORAZ INDEKS ZIELONOŚCI LIŚCIA (SPAD) LUCERNY I FESTULOLIUM W ZALEŻNOŚCI OD SPOSOBU UPRAWY I POZIOMU WILGOTNOŚCI GLEBY

Streszczenie

Celem badań była ocena wpływu niedoboru wody w glebie oraz sposobu uprawy na plon suchej masy i białka oraz względną zawartość chlorofilu w liściach lucerny i festulolium. Doświadczenie wazonowe przeprowadzono w latach 2012-2014 w hali wegetacyjnej IUNG-PIB w Puławach, w układzie kompletnie zrandomizowanym, w 4 powtórzeniach. W badaniach uwzględniono lucernę siewną i festulolium uprawiane w siewach czystych i w mieszance, przy 2 poziomach wilgotności gleby: 70% polowej pojemności wodnej (wilgotność optymalna) i 40% ppw (stres suszy). W badaniach wykazano, że długotrwały stres suszy istotnie redukował plon suchej masy lucerny i festulolium, przy czym największą stratę wykazano w czystym zasiewie lucerny (średnio o 39.8%), mniejszą u mieszanki (o 29.1%), a w najmniejszą u mieszańca festulolium (o 13.6%). Niezależnie od warunków wilgotnościowych gleby, największy łączny plon białka ogólnego uzyskano z uprawy lucerny w siewie czystym, a tylko o 12% mniejszy z uprawy mieszanki. Plon białka z uprawy festulolium w siewie czystym był średnio o 68% mniejszy w porównaniu do plonu lucerny. Indeks zieloności liścia (SPAD) lucerny był istotnie większy w porównaniu do mieszańca festulolium, niezależnie od warunków wilgotnościowych gleby. Niedobór wody w glebie i sposób uprawy nie różnicowały istotnie względnej zawartości chlorofilu w liściach badanych gatunków roślin.
***Słowa kluczowe**: bobowate, trawy, stres suszy, plon, SPAD indeks, mieszanka, siew czysty*

1. Introduction

For many years, scientists have been devoting much time to droughts, especially those caused by rainfall shortages. Also, in Poland, there is a growing interest in this phenomenon, which results from the fact that, there are more and more frequent periodic water shortages covering large areas of the country and causing large losses in agricultural production [3, 9]. Spring droughts generally contribute to a decrease in spring cereal yields, first sward regrowth, and pasture productivity, while summer droughts adversely affect field forage yields and second sward regrowth, which results in difficulties in obtaining adequate quantities of high-quality animal feed [10]. Plants grown on light and very light soils of limited retention capacity are particularly sensitive to the effects of water stress.

The response of legume-grass mixtures to adverse environmental conditions is weaker than these species grown in pure stand due to the fact that mixture components have different habitat requirements, developmental rhythms, and root system structures. This makes them generally better to harvest and more durable [6]. In addition, feed made of mixtures is better balanced in terms of protein content and energy, while reduced nitrogen fertilization, reduces milk production costs. Their cultivation has a positive effect on the physical, chemical and biological properties of the soil, which makes the post-mixture stand very favorable for subsequent plants, especially cereals [2].

The majority of legumes have high water requirements. According to Rojek [12] alfalfa's demand for water is 400-600 mm, including 340-470 mm during the growing season. Alfalfa needs most water in the early stages of its development, when it still has a poorly developed root system and cannot draw water from the deeper layers of the soil. Grasses also have high water needs - from 0,5 to 3,0 $dm^3 \cdot m^{-2}$ per day [17]. Individual grass species exhibit varied water needs. Even within one species, varietal differences are often observed, resulting from specific morphological and biological properties. A water deficit may affect legumes and grasses either directly or consequentially. Short-term, temporary stress generally has no consequential impact, but as the deficit worsens, growth slows down for a long period of time, even after the drought has subsided [16].

Chlorophyll pigments are an important indicator of plant life processes indirectly affecting biomass production but are also an indicator of plant vitality and their resistance to stress conditions [7]. The chlorophyll content of leaves is a genetic trait associated with the species and the cultivar, but it also depends to a large extent on habitat and weather conditions, especially rainfall [13].

The aim of the research was to assess the influence of soil moisture level (optimal and water stress) and cultivation method (pure and mixed stand) on dry matter and protein yields as well as on relative chlorophyll content of alfalfa and festulolium leaves.

2. Material and methods

The pot experiment was carried out in the years 2012-2014 in the greenhouse of the Institute of Soil Science and Plant Cultivation - State Research Institute in Puławy, in a completely randomized design, in four repetitions. The study included alfalfa (*Medicago sativa* L.) variety Ulstar and festulolium (*Festulolium braunii* (K. Richt.) A. Camus) variety Sulino, grown in pure stand and in a mixture. The experiments were carried out in Mitscherlich pots filled with very light soil (weak loam sand) coming from the arable layer of the field (0-30 cm). The soil abundance in elements were as follows ($g \cdot kg^{-1}$ of soil): P - 24.8 (very high content), K - 14.2 (high content), and Mg - 2.2 (medium content). The soil pH measured in 1 mol $KCl \cdot dm^{-3}$ was 7.40. Two soil moisture levels were applied: 70% field water capacity (optimal humidity) and 40% FWC (drought stress). Soil moisture in stressful areas was reduced: in the sowing year - 2 months after sowing, and in the following two years - 2 weeks after the vegetation had started.

The appropriate soil moisture was maintained by watering the soil one or two times a day (depending on weather conditions) to a specific weight of a pot with soil. The experiment was established on 11 April 2012. Eight plants were grown in each pot (in a mixture of 4 pcs. of grass and 4 pcs. of legume). In all years of vegetation, phosphorus and magnesium were used once in spring in the following quantities: 1,0 g P and 0,5 g Mg per pot, and a dose of K of 1,5 g per pot in the form of solutions: NH_4NO_3, KH_2PO_4, K_2SO_4 and $MgSO_4$ x 7, divided into two parts (in spring and after harvesting of the second regrowth). Nitrogen fertilization was applied at a rate of 0.5 g N per pot for each grass regrowth, and half of this dose was applied for each mixture regrowth. Alfalfa in pure stand was not fertilized with nitrogen (with the exception of the starting dose). In all years of vegetation, micronutrient solution was also applied.

In the first year of the study, the plants were harvested three times, and in the following two years, four times during the growing season, in the stages of full budding/beginning of flowering of alfalfa. During each harvest, the plant yield and leaf greenness index (SPAD) were evaluated using Minolta's SPAD 502 chlorophyll meter. The result is given in the so-called SPAD units (Soil and Plant Analysis Development) in the range from 0 to 800, and its value is proportional to the chlorophyll content in the area of 6 mm^2 of leaf. The measurements were carried out at weekly intervals throughout the vegetation period.

The content measurement of total nitrogen in plant samples was performed by flow spectrophotometry in the certificated Main Chemical Analysis Laboratory (IUNG-PIB) in Puławy, on the basis of average treatment samples of dry matter of plants. Treatments with optimal (70% FWC) and limited (40% FWC) soil moisture, were taken into account. The total protein (CP) content was calculated from the formula $CP = N \times 6.25$, and the protein yield according to the formula: DMY (dry matter yield) × CP. The results were obtained statistically with the use of the variance analysis method at the significance level of $\alpha = 0.05$. When comparing the mean values, the significance of differences was evaluated with the use of the Tukey test.

3. Results and discussion

Soil moisture was an important factor affecting the dry matter yield of festulolium and alfalfa cultivated in pure stand and in mixture. In the first and second year of vegetation, under optimal humidity conditions, alfalfa cultivated in pure stand yielded the best, while significantly lower – mixture, and the lowest – festulolium in pure stand (Tables 1, 2). Both studied species responded to a lower soil moisture content with a significant decrease in dry matter yield. The smallest decrease in total yield was recorded in festulolium (by 13.6 and 16.8% respectively), higher in mixture (by 28.0 and 30.3% respectively), while the highest in alfalfa grown in pure stand (by 41.6 and 41.0% respectively for years). In the third year of vegetation, regardless of the soil moisture conditions, alfalfa cultivated in a mixture with grass yielded the best, while festulolium in pure stand yielded the lowest (Table 3). Long-time stress caused a significant decrease in dry matter yield of both species in comparison with optimally moisturized treatments. The highest total yield loss was recorded in pure alfalfa stand (on average by 36.9%), lower in the mixture (by 28.9%), while the lowest in pure festulolium stand (on average by 10.5%). In the first year of the research, regardless of the soil moisture level, the percentage of yield of individual regrowths in the total yield was very aligned (by 32.4% on average), while in the second and third years, the largest share in the annual yield was the first regrowth (38.7% on average), while the next three were respectively smaller (27.3, 17.8 and 16.2% on average).

The water requirement of plants increases with the growth of green matter, usually to the flowering stage, and then decreases during the maturity stage. Periods of the highest water requirements occur simultaneously with the greatest sensitivity to water shortages and are called critical periods in water management of plants [1]. Water shortage during this time causes the greatest yield losses of cultivated plants. Different species respond differently to stress, which is related to the different structure of their root systems, individual developmental rhythm, and different habitat requirements. The results of our study showed that the hybrid of festulolium was more resistant to long-term drought stress than alfalfa, whereas the method of cultivation also had a significant impact on yields. Alfalfa cultivated in a mixture with festulolium showed significantly lower reduction in dry matter yield under drought conditions (on average by 22.9% in 3 years) than in the case of cultivation in pure stand (39.8%). This shows that the mixtures are more suitable for growing in areas with limited precipitation. On the other hand, festulolium hybrid cultivated in pure stand was more resistant to stress, especially in the first and second year of vegetation (average yield loss 15.2%), than in the mixture with alfalfa. These results are confirmed by Głażewski [4], who showed a lower yield loss of alfalfa cultivated in a mixture with an orchard grass than in the case of pure stand. Also in a study by Küchenmeister et al. [8], alfalfa cultivated in a mixture with perennial ryegrass yielded better than alfalfa in pure stand. Olszewska [11] showed significantly higher yield-forming potential of the festulolium hybrid cultivated in the mixture with birdsfoot trefoil and white clover than in pure stand.

Table 1. Dry matter yield of alfalfa and festulolium depending on the cultivating method and the level of soil moisture in 2012 [g·pot^{-1}]

Tab. 1. Plon suchej masy lucerny i festulolium w zależności od sposobu uprawy i poziomu wilgotności gleby w 2012 r. [g·wazon^{-1}]

Treatment	I regrowth		II regrowth		III regrowth		Total	
	level of soil moisture (% FWC)							
	70	40	70	40	70	40	70	40
Alfalfa – PS*	17.6 ^{a}	11.7 ^{a}	22.0 ^{d}	11.6 ^{ab}	22.0 ^{c}	12.6 ^{a}	61.7 ^{d}	36.0 ^{ab}
Festulolium – PS	15.1 ^{a}	12.3 ^{a}	11.2 ^{a}	10.5 ^{a}	11.8 ^{a}	10.3 ^{a}	38.2 ^{b}	33.0 ^{a}
Mixture	17.7 ^{a}	13.4 ^{a}	17.4 ^{c}	14.1 ^{b}	19.1 ^{b}	11.4 ^{a}	54.2 ^{c}	39.0 ^{b}
Mean for treatment								
Alfalfa – PS	14.7 ^{a}		16.8 ^{b}		17.3 ^{c}		48.9 ^{b}	
Festulolium – PS	13.7 ^{a}		10.9 ^{a}		11.0 ^{a}		35.6 ^{a}	
Mixture	15.6 ^{a}		15.7 ^{b}		15.3 ^{b}		46.6 ^{b}	
Mean for level of soil moisture (% FWC)								
70	16.8 ^{b}		16.9 ^{b}		17.7 ^{b}		54.4 ^{b}	
40	12.5 ^{a}		12.1 ^{a}		11.4 ^{a}		36.0 ^{a}	

*PS – pure stand *Source: own study / Źródło: opracowanie własne*

Table 2. Dry matter yield of alfalfa and festulolium depending on the cultivating method and the level of soil moisture in 2013 [g·pot^{-1}]

Tab. 2. Plon suchej masy lucerny i festulolium w zależności od sposobu uprawy i poziomu wilgotności gleby w 2013 r. [g·wazon^{-1}]

Treatment	I regrowth		II regrowth		III regrowth		IV regrowth		Total	
	level of soil moisture (% FWC)									
	70	40	70	40	70	40	70	40	70	40
Alfalfa – PS*	58.3 ^{c}	41.1 ^{b}	46.4 ^{e}	18.8 ^{b}	22.0 ^{d}	13.1 ^{bc}	20.5 ^{c}	13.9 ^{a}	147.2 ^{e}	86.9 ^{bc}
Festulolium – PS	28.6 ^{a}	28.6 ^{a}	25.7 ^{c}	15.5 ^{a}	11.4 ^{ab}	10.4 ^{a}	17.8 ^{b}	15.0 ^{ab}	83.5 ^{b}	69.5 ^{a}
Mixture	54.6 ^{c}	41.1 ^{b}	33.7 ^{d}	23.5 ^{c}	23.8 ^{d}	14.6 ^{c}	24.2 ^{d}	15.7 ^{ab}	136.2 ^{d}	94.9 ^{c}
Mean for treatment										
Alfalfa – PS	49.7 ^{b}		32.6 ^{c}		17.6 ^{b}		17.2 ^{a}		117.1 ^{b}	
Festulolium – PS	28.6 ^{a}		20.6 ^{a}		10.9 ^{a}		16.4 ^{a}		76.5 ^{a}	
Mixture	47.9 ^{b}		28.6 ^{b}		19.2 ^{b}		19.9 ^{b}		115.5 ^{b}	
Mean for level of soil moisture (% FWC)										
70	47.2 ^{b}		35.3 ^{b}		19.1 ^{b}		20.8 ^{b}		122.3 ^{b}	
40	36.9 ^{a}		19.2 ^{a}		12.7 ^{a}		14.9 ^{a}		83.7 ^{a}	

*PS – pure stand *Source: own study / Źródło: opracowanie własne*

Table 3. Dry matter yield of alfalfa and festulolium depending on the cultivating method and the level of soil moisture in 2014 [g·pot^{-1}]

Tab. 3. Plon suchej masy lucerny i festulolium w zależności od sposobu uprawy i poziomu wilgotności gleby w 2014 r. [g·wazon^{-1}]

Treatment	I regrowth		II regrowth		III regrowth		IV regrowth		Total	
	level of soil moisture (% FWC)									
	70	40	70	40	70	40	70	40	70	40
Alfalfa – PS*	42.0 ^{c}	27.6 ^{a}	33.7 ^{d}	22.4 ^{b}	25.3 ^{b}	14.5 ^{a}	19.6 ^{c}	11.6 ^{ab}	120.7 ^{c}	76.2 ^{ab}
Festulolium – PS	25.8 ^{a}	24.8 ^{a}	19.7 ^{ab}	17.5 ^{a}	15.4 ^{a}	12.6 ^{a}	11.2 ^{ab}	9.7 ^{a}	72.1 ^{a}	64.5 ^{a}
Mixture	43.2 ^{c}	33.2 ^{b}	36.6 ^{d}	26.6 ^{c}	26.3 ^{b}	15.9 ^{a}	19.0 ^{c}	13.3 ^{b}	125.2 ^{c}	89.0 ^{b}
Mean for treatment										
Alfalfa – PS	34.8 ^{b}		28.1 ^{b}		19.9 ^{b}		15.6 ^{b}		98.5 ^{b}	
Festulolium – PS	25.3 ^{a}		18.6 ^{a}		14.0 ^{a}		10.4 ^{a}		68.3 ^{a}	
Mixture	38.2 ^{b}		31.6 ^{c}		21.1 ^{b}		16.2 ^{b}		107.1 ^{b}	
Mean for level of soil moisture (% FWC)										
70	37.0 ^{b}		30.0 ^{b}		22.4 ^{b}		16.6 ^{b}		106.0 ^{b}	
40	28.5 ^{a}		22.2 ^{a}		14.3 ^{a}		11.5 ^{a}		76.6 ^{a}	

*PS – pure stand *Source: own study / Źródło: opracowanie własne*

The total protein yield depended on the dry matter yield and the total protein content, the latter one being a species characteristic. In optimal moisture conditions, in the first and second year of research, the highest protein yield was obtained from the cultivation of alfalfa in pure stand, while in the third year - from the cultivation of alfalfa in pure and mixed sowing (Table 4). During the entire research period, the lowest yields were obtained from the festulolium hybrid. Under optimal moisture conditions, the total protein yield obtained from cultivation of the mixture for the 3-year study period was lower by 8.9% than from alfalfa grown in pure stand, and the total protein yield of festulolium was lower by as much as 58% in comparison to alfalfa. Water deficit in the soil had little effect on the protein content in the plant species studied. It slightly increased the content of this component in the dry matter of festulolium, but did not affect the amount of protein in alfalfa, both in pure sowing and in the mixture. The highest total protein yield under stress conditions was obtained from alfalfa cultivated in pure stand, followed by mixture cultivation (lower by 15.5%) and festulolium (lower by 79% lower). Seguin at al. [14] also showed a low impact of water scarcity on the total protein content of alfalfa. However, Küchenmeister et al. [8] reported a decrease in the total protein content of yellow alfalfa and black medick under severe drought stress.

Studies on the relative chlorophyll content of alfalfa leaves and festulolium have shown that the SPAD index of a legume was significantly higher than that of a grass, regardless of cultivation and moisture conditions. In all years of the study, the leaf greenness index of alfalfa and festulolium was higher under stress conditions than under optimal soil moisture conditions, with generally no statistically significant differences (Tables 5, 6, 7). In optimal moisture conditions, the festulolium hybrid grown in pure stand had a higher SPAD index than the mixtures, whereas under stress conditions, in the first and second year of the study, a higher SPAD index was recorded for the mixture. The cultivation regime of alfalfa did not result in a significant variation in the SPAD index.

Table 4. Total protein yield of alfalfa and festulolium depending on the cultivating method and the level of soil moisture [g·pot^{-1}]
Tab. 4. Plon białka ogólnego lucerny i festulolium w zależności od sposobu uprawy i poziomu wilgotności gleby [g·wazon^{-1}]

Treatment	I regrowth		II regrowth		III regrowth		IV regrowth		Total	
	level of soil moisture (% FWC)									
	70	40	70	40	70	40	70	40	70	40
2012										
Alfalfa – PS*	3.03	1.76	3.45	2.16	4.33	2.42	-	-	10.8	6.34
Festulolium – PS	1.43	1.08	1.52	1.66	1.96	1.78	-	-	5.04	4.59
Mixture	2.21	1.80	2.52	2.07	3.61	2.01	-	-	8.29	5.93
2013										
Alfalfa – PS*	11.9	7.73	10.4	3.87	5.02	3.09	3.63	3.09	30.6	18.51
Festulolium – PS	3.69	3.89	3.50	1.98	1.81	1.99	3.06	2.70	12.4	11.05
Mixture	10.4	7.40	6.34	4.35	5.26	2.98	4.62	3.17	27.0	18.32
2014										
Alfalfa – PS*	7.39	5.38	5.59	4.17	4.86	3.12	4.12	2.31	22.4	15.16
Festulolium – PS	2.63	2.21	2.30	2.08	1.97	1.97	1.85	1.88	9.23	9.03
Mixture	7.82	5.35	6.44	4.42	4.94	3.13	3.59	2.37	22.9	15.58

*PS – pure stand *Source: own study / Źródło: opracowanie własne*

Table 5. SPAD index in alfalfa and festulolium grown in pure stand and in mixure depending on the level of soil moisture in 2012
Tab. 5. Indeks SPAD u lucerny i festulolium uprawianych w siewie czystym i mieszanym w zależności od poziomu wilgotności gleby w 2012 r.

Treatment	I regrowth		II regrowth		III regrowth		Mean	
	level of soil moisture (% FWC)							
	70	40	70	40	70	40	70	40
Alfalfa – PS*	602 [a]	644 [a]	639 [a]	647 [a]	592 [a]	639 [a]	611 [a]	643 [a]
Festulolium – PS	581 [a]	409 [a]	437 [a]	455 [a]	393 [a]	353 [a]	470 [a]	406 [a]
Alfalfa – MX**	588 [a]	663 [a]	517 [a]	561 [a]	527 [a]	584 [a]	544 [a]	603 [a]
Festulolium – MX	415 [a]	415 [a]	428 [a]	485 [a]	456 [a]	465 [a]	433 [a]	455 [a]
Mean for treatment								
Alfalfa – PS	622 [b]		643 [b]		616 [c]		627 [b]	
Festulolium – PS	495 [a]		446 [a]		373 [a]		438 [a]	
Alfalfa – MX	625 [b]		539 [b]		555 [b]		573 [b]	
Festulolium – MX	415 [a]		456 [a]		461 [ab]		444 [a]	
Mean for level of soil moisture (% FWC)								
70	546 [a]		505 [a]		492 [a]		515 [a]	
40	532 [a]		537 [a]		510 [a]		527 [a]	

* PS – pure stand, **MX – species grown in mixture *Source: own study / Źródło: opracowanie własne*

Table 6. SPAD index in alfalfa and festulolium grown in pure stand and in mixure depending on the level of soil moisture in 2013

Tab. 6. Indeks SPAD u lucerny i festulolium uprawianych w siewie czystym i mieszanym w zależności od poziomu wilgotności gleby w 2013 r.

Treatment	I regrowth		II regrowth		III regrowth		IV regrowth		Mean	
	level of soil moisture (% FWC)									
	70	40	70	40	70	40	70	40	70	40
Alfalfa – PS*	708^{c}	678^{c}	632^{a}	705^{a}	572^{a}	646^{a}	518^{a}	549^{a}	608^{a}	644^{a}
Festulolium – PS	384^{a}	405^{a}	383^{a}	415^{a}	358^{a}	386^{a}	429^{a}	422^{a}	388^{a}	407^{a}
Alfalfa – MX**	679^{c}	714^{c}	576^{a}	653^{a}	599^{a}	651^{a}	572^{a}	554^{a}	606^{a}	643^{a}
Festulolium – MX	371^{a}	547^{b}	317^{a}	367^{a}	272^{a}	410^{a}	295^{a}	436^{a}	314^{a}	440^{a}
Mean for treatment										
Alfalfa – PS	693^{b}		668^{b}		609^{b}		533^{b}		626^{b}	
Festulolium – PS	394^{a}		399^{a}		372^{a}		425^{a}		398^{a}	
Alfalfa – MX	696^{b}		615^{b}		625^{b}		563^{b}		625^{b}	
Festulolium – MX	459^{a}		342^{a}		341^{a}		366^{a}		377^{a}	
Mean for level of soil moisture (% FWC)										
70	535^{a}		477^{a}		450^{a}		454^{a}		479^{a}	
40	586^{a}		535^{b}		523^{b}		490^{a}		534^{b}	

* PS – pure stand, **MX – species grown in mixture *Source: own study / Źródło: opracowanie własne*

Table 7. SPAD index in alfalfa and festulolium grown in pure stand and in mixure depending on the level of soil moisture in 2014

Tab. 7. Indeks SPAD u lucerny i festulolium uprawianych w siewie czystym i mieszanym w zależności od poziomu wilgotności gleby w 2014 r.

Treatment	I regrowth		II regrowth		III regrowth		IV regrowth		Mean	
	level of soil moisture (% FWC)									
	70	40	70	40	70	40	70	40	70	40
Alfalfa – PS*	687^{a}	728^{a}	670^{a}	667^{a}	627^{c}	626^{c}	604^{a}	611^{a}	647^{c}	658^{c}
Festulolium – PS	440^{a}	459^{a}	419^{a}	436^{a}	380^{b}	402^{b}	397^{a}	463^{a}	409^{b}	440^{b}
Alfalfa – MX**	672^{a}	707^{a}	691^{a}	687^{a}	671^{c}	644^{c}	623^{a}	700^{a}	664^{c}	685^{c}
Festulolium – MX	326^{a}	383^{a}	342^{a}	411^{a}	278^{a}	407^{a}	301^{a}	386^{a}	312^{a}	397^{b}
Mean for treatment										
Alfalfa – PS	707^{b}		668^{b}		627^{b}		608^{b}		652^{b}	
Festulolium – PS	450^{a}		427^{a}		391^{a}		430^{a}		424^{a}	
Alfalfa – MX	690^{b}		689^{b}		658^{b}		662^{b}		674^{b}	
Festulolium – MX	354^{a}		377^{a}		342^{a}		343^{a}		354^{a}	
Mean for level of soil moisture (% FWC)										
70	531^{a}		531^{a}		489^{a}		481^{a}		508^{a}	
40	569^{a}		550^{b}		520^{b}		540^{a}		545^{b}	

* PS – pure stand, **MX – species grown in mixture *Source: own study / Źródło: opracowanie własne*

Chlorophyll pigments play an important role in assessing the drought resistance of plants. They determine the life processes of plants as well as their chemical composition. They are also an indicator of the vitality of plants and their response to the changing environmental conditions. Gregorczyk and Raczyńska [5] demonstrated a strong correlation between SPAD index and chlorophyll contents calculated using the traditional laboratory method (0.947 to 0.973 for various plant species). An estimation of the chlorophyll content by means of a chlorophyllometer can therefore be considered reliable. The results of our own research showed a significantly higher leaf greenness index of legumes than of grasses, regardless of the moisture conditions, which indicates a higher vitality of alfalfa than festulolium. In addition, there was an increase in the SPAD index in soil drought conditions, which had been confirmed by our previous studies, showing an increase in the leaf greenness index in several fodder grass species under soil drought conditions [15]. These results were also confirmed by Olszewska [11], who recorded a higher content of chlorophyll in the leaves of festulolium, white clover, and birdsfoot trefoil in the conditions of rainfall deficit than in the years with optimal weather conditions. Also, in the studies of Rumasz-Rudnicka [13], Dutch ryegrass had a higher concentration of chlorophyll in leaves under water shortage in the soil than under crop irrigation.

4. Conclusions

1. Long-term stress caused by water deficiency in soil significantly reduced dry matter yield, the most in alfalfa (on average by 39.8% in 3 years), to a lesser extent in the mixtures (by 29.1%), and the least in festulolium hybrids (by 13.6%).
2. Regardless of the soil moisture conditions, the highest total protein yield was obtained from the cultivation of alfalfa in pure stand, slightly lower from the cultivation of the mixture (by 12% on average) and significantly lower from the cultivation of festulolium in pure stand (by 68% on average) in comparison with alfalfa.
3. Both under optimal moisture conditions and drought stress, the leaf greenness index was significantly higher in alfalfa than in festulolium hybrid. The water shortage in the soil as well as the method of cultivation did not signifi-

cantly differentiate the relative chlorophyll content in the leaves of the tested species.

4. In regions with temporary water shortages, an alfalfa/festulolium mixture is more suitable for cultivation than monoculture sowing, due to the lower yield loss and a favorable total protein yield under stress conditions.

5. References

[1] Chmura K., Chylińska E., Dmowski Z., Nowak L.: Rola czynnika wodnego w kształtowaniu plonu wybranych roślin polowych. Infrastruktura i Ekologia Terenów Wiejskich, PAN Kraków, 2009, 9, 33-44.

[2] Crews T.E., Peoples M.B.: Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutr. Cycl. Agroecosys., 2005, 72, 101-120.

[3] Doroszewski A., Jadczyszyn J., Kozyra J., Pudełko R., Stuczyński T., Mizak K., Łopatka A., Koza P., Górski T., Wróblewska E.: Podstawy systemu monitoringu suszy rolniczej. Woda Środowisko Obszary Wiejskie, 2012, 12, 2(38), 77-91.

[4] Głażewski S.: Wpływ stresu wodnego na plon lucerny i kupkówki pospolitej uprawianej w siewie czystym lub mieszanym. Mat. Konferencyjne - Ekofizjologiczne aspekty reakcji roślin na działanie abiotycznych czynników stresowych. (Red. S. Grzesiak, Z. Mieszalski), Wyd. PAN, Kraków, 1996, 241-245.

[5] Gregorczyk A., Raczyńska A.: Badania korelacji między metodą Arnona a pomiarami zawartości chlorofilu za pomocą chlorofilometru. Zesz. Nauk. AR Szczecin, 1997, 181, 119-123.

[6] Jelinowska A., Staniak M.: Wzajemne oddziaływanie roślin w siewach jednogatunkowych i mieszanych na przykładzie mieszanek lucerny z trawami. Post. Nauk Rol., 2007, 5, 37-49.

[7] Kozłowski S., Goliński P., Golińska B.: Barwniki chlorofilowe jako wskaźniki wartości użytkowej gatunków i odmian traw. Zesz. Probl. Post. Nauk Rol., 2001, 474, 215-223.

[8] Küchenmeister K., Küchenmeister F., Kayser M., Wrage-Mönnig N., Isselstein J.: Influence of drought stress on nutritive value of perennial forage legumes. Int. J. Plant Prod., 2013, 7(4), 693-710.

[9] Lipiec J., Doussan C., Nosalewicz A., Kondracka K.: Effect of drought and heat stresses on plant growth and yield: a review. Int. Agrophys., 2013, 27, 463-477.

[10] Łabędzki L.: Susze i powodzie – zagrożenia dla rolnictwa. W: Woda w krajobrazie rolniczym. (ed. Mioduszewski), Woda-Środowisko-Obszary Wiejskie. Rozpr. Nauk. i Monogr., 18, IMUZ Falenty, 2006, 29-43.

[11] Olszewska M.: Leaf greenness (SPAD) and yield of *Festulolium braunii* (K. Richt.) A. Camus grown in mixtures with legumes depending on multiple nitrogen rates. Pol. J. Natur. Sci., 2008, 23(2), 310-325.

[12] Rojek S.: Potrzeby wodne roślin motylkowatych. Frag. Agron., 1986, 2(10), 3-20.

[13] Rumasz-Rudnicka E.: Wpływ nawadniania i nawożenia azotem na asymilację i transpirację życicy westerwoldzkiej. Acta Agrophys., 2010, 15(2), 395-408.

[14] Seguin P., Mustafa A.F., Sheaffer C.C.: Effects of soil moisture deficit on forage quality, digestibility and protein fractionation of Kura clover. J. Agron. Crop Sci., 2002, 188, 260-266.

[15] Staniak M.: The impact of drought stress on the yields and food value of selected forage grasses. Acta Agrobot., 2016, 69(2), 1663.

[16] Starck Z.: Wpływ warunków stresowych na koordynację wytwarzania i dystrybucji fotoasymilatów. Post. Nauk Rol., 2010, 1, 9-26.

[17] Thomas H.: Diversity between and within temperate forage grass species in drought resistance. Water use and related physiological responses. Aspects App. Biol., 1994, 38, 47-55.

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