

LEAF GREENNESS AND YIELD OF GRASS MIXTURES CONTAINING *MEDICAGO MEDIA* PERS. WITH VARIED PROPORTIONS OF MIXTURE COMPONENTS

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ABSTRACT

The study was carried out in 2011-2013. The first experimental factor was the grass mixture: festulolium + alfalfa (*Fb/Mm*) and orchard grass + alfalfa (*Dg/Mm*). The second experimental factor was the percentage of alfalfa seeds in the mixture: 30%, 50%, 70%. The influence of the percentage of *Medicago media* Pers. in mixtures with *Festulolium braunii* (K. Richt) A. Camus and *Dactylis glomerata* L. on the leaf greenness index of grasses and the yield of mixtures, and the effect of relative chlorophyll content on dry matter yield were determined. The leaves of pure-sown festulolium contained significantly less chlorophyll than the leaves of orchard grass. When alfalfa was introduced to the mixture, leaf greenness increased in both tested grass species, and the highest SPAD values were noted in treatments with 70% alfalfa seeds. The average yield for a three-year period was significantly higher in grass mixtures than in pure-sown grasses. The mixture with orchard grass produced higher yield than the mixture with festulolium. Variations in the proportion of alfalfa in mixtures led to significant differences in yield. The lowest yield was noted in treatments with 50% alfalfa seeds, whereas mixtures containing 30% and 70% alfalfa seeds were characterized by similar yield values. A strong positive correlation was observed between leaf greenness and dry matter yield.

Key words: *Dactylis glomerata*, *Festulolium braunii*, *Medicago media*, mixtures, leaf greenness (SPAD), yield.

INTRODUCTION

Chlorophyll pigments are organic compounds that play a key role in photosynthesis. Chlorophyll absorbs light, and chemical reactions between organic compounds generate chemical energy. The chlorophyll content of leaves affects radiation absorbance, which influences the rate of photosynthesis and biomass production. Leaf greenness is an indicator of the nitrogen nutritional status of plants (Agnusdei et al., 2010; Gáborčík, 2003; Madakadze et al., 1999). It is also a predictor of yield because the presence of chlorophyll in leaf blades and dry matter yield are positively correlated (Radkowski, 2013; Rumasz-Rudnicka, 2010; Olszewska, 2005; Gáborčík, 1996). Chlorophyll levels are a reliable indicator of plant vitality and resistance to environmental stressors. Chlorophyll concentrations in leaves are determined by various factors, including genetic characteristics of species and varieties, climate and soil conditions, availability of

nutrients and water, and the plant's development stage (Radkowski, 2013; Radkowski and Radkowska, 2013; Zielewicz and Kozłowski, 2011; Rumasz-Rudnicka, 2010; Jodelka and Sosnowski, 2010; Michałek and Sawicka, 2005; Olszewska, 2005, 2006, 2008c; Olszewska et al., 2008a; 2008b). Competition between species in grass mixtures also influences chlorophyll levels. A previous study conducted by Olszewska (2008) demonstrated that pure-sown grasses were characterized by lower SPAD values than grasses grown with legumes, and significantly higher SPAD values were observed in grasses grown with white clover than with *Lotus corniculatus*.

The objective of this study was to determine the effect of the percentage of *Medicago media* Pers. in mixtures with *Festulolium braunii* (K. Richt) A. Camus and *Dactylis glomerata* L. on the leaf greenness index of grasses and the yield of mixtures, and to evaluate the influence of relative chlorophyll content on dry matter yield.

MATERIAL AND METHODS

The study was carried out in 2011-2013. A field experiment was conducted in the Research Station in Băldy, on mineral soil of quality class IVa and very good rye complex. A two-factor experiment in a split-plot design with four replications was established in the spring of 2010. The first experimental factor was the grass mixture: festulolium + alfalfa (Fb/Mm) and orchard grass + alfalfa (Dg/Mm). The second experimental factor was the percentage of alfalfa seeds in the mixture: 30%, 50% and 70%. Plot size was 10 m². Before sowing, all plots were fertilized with nitrogen, phosphorus and potassium at 30 kg N, 80 kg P₂O₅ and 60 kg K₂O ha⁻¹, respectively. Seeds were broadcast, and cover crops were not used. Control treatments were pure-sown festulolium (100%) and orchard grass (100%). In the years of full utilization, pure-sown festulolium and orchard grass were fertilized with nitrogen at 180 N·ha⁻¹, and grass mixtures were fertilized with nitrogen at 90 kg N·ha⁻¹. Nitrogen rates were divided into three equal parts and applied in early spring, after the first and second harvest. All plots were fertilized with phosphorus at 80 kg P₂O₅·ha⁻¹ (one spring application) and potassium at 120 kg K₂O·ha⁻¹ (in spring and after the first harvest). Leaf greenness was measured in the youngest, fully developed shoot leaf selected randomly from each treatment. Measurements were performed with the use of the Minolta SPAD-502 chlorophyll meter. SPAD values are proportional to leaf chlorophyll content

(Chapman and Baretto, 1997; Samborski and Rozbicki, 2002). They are calculated based on the quantity of light passing through the leaf at two wavelengths that are differently absorbed by chlorophyll (650 and 940 nm). The quotient of absorbance at two wavelengths is expressed in SPAD values. Two measurements were performed in each regrowth in five replications. Dry matter yield and the coefficient of correlation between leaf greenness and yield were determined. The significance of the coefficient of correlation was evaluated based on critical values of Spearman's rank correlation coefficient at $\alpha=0.05$ and $\alpha=0.01$. The results were processed in the Statistica application. The significance of differences was verified by Tukey's test at $\alpha=0.05$.

Weather conditions in experimental years are presented in Figure 1.

The growing season in 2011 was characterized by favorable weather, and average monthly temperatures were similar to the multi-annual average. Excluding April and October, total precipitation exceeded the long-term average, and record-high precipitation levels in July exceeded the multi-annual average nearly four-fold.

The second year of the study was characterized by moderate average temperatures, high precipitation in April, June and July, and moderate water deficiency in August and September. In 2013, weather conditions were less supportive of plant growth due to a cold spell in early spring and considerable precipitation shortage in June, August and October.

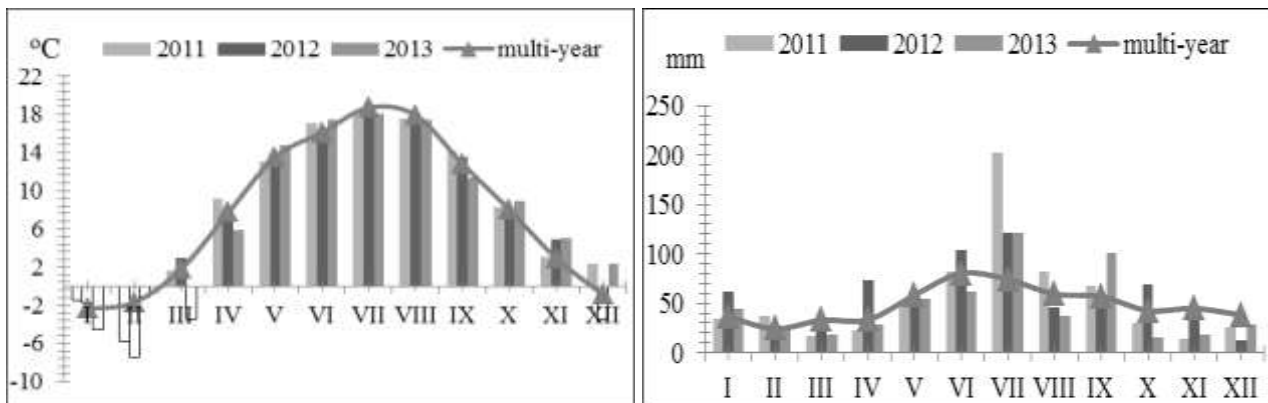


Figure 1. Mean air temperature and rainfalls in the period 2011-2013

RESULTS AND DISCUSSION

The botanical composition of sward changed considerably across experimental years. The share of mixture components indicates that alfalfa has high competitive advantage over festulolium and, to a lesser extent, over orchard grass (Figure 2). Its percentage in the sward increased steadily in successive years of the experiment. In the first year of full utilization, the share of alfalfa plants in treatments with 30% alfalfa seeds was estimated at 38% in the

mixture with festulolium and 40% in the mixture with orchard grass, in treatments with 50% alfalfa seeds – at 39% and 40%, and in treatments with 70% alfalfa seeds – at 48% and 52%, respectively. In the second and third year of the experiment, alfalfa was the dominant species over festulolium, but it was less competitive with regard to orchard grass. Similar results were reported by Gawel (2008) in whose study festulolium was a less persistent component than orchard grass in mixtures containing alfalfa.

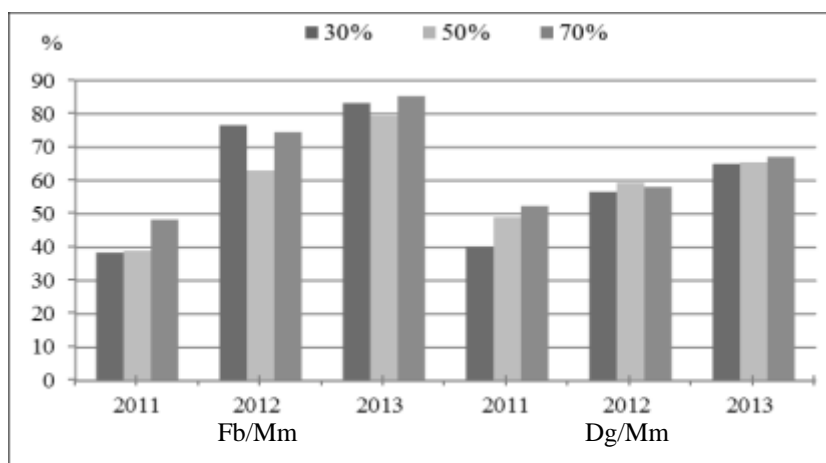


Figure 2. Mean share of alfalfa in mixtures in the period 2011-2013

Variations were noted in leaf greenness of the tested species across experimental years. In 2011, SPAD values were determined in the range of 34.63-47.60 (Table 1). The leaves of pure-sown festulolium contained more chlorophyll than the leaves of orchard grass. Average SPAD values were determined at 43.80 in festulolium and 39.28 in orchard grass. A significant decrease in SPAD values was noted in festulolium grown in mixture with alfalfa, and the lowest values were reported in treatments with 50% share of seeds of both species. The reverse was observed in treatments containing orchard grass, where the introduction of alfalfa increased SPAD values in the former species. Similarly to the Fb/Mm mixture, the lowest leaf greenness was reported in treatments where both species were sown in equal proportions. It should be stressed, however, that the actual share of alfalfa plants in sward

composition differed significantly from the percentage of alfalfa seeds in the mixture, and the differences noted in the analyzed treatments reached only several percent. During the growing season, the highest chlorophyll concentrations in grass leaves were noted in the second regrowth.

In the second year of the study, relative chlorophyll levels were lower in the range of 27.46 to 44.33 (Table 2). Higher SPAD values were noted in orchard grass leaves. Grass-alfalfa mixtures contained more chlorophyll than pure-grown grasses. In treatments with a higher percentage of alfalfa, chlorophyll concentrations were significantly higher in the leaves of both grass species. The highest SPAD values were reported in the third regrowth during a period of deficient precipitation, which, according to Michałek and Sawicka (2005) and Olszewska (2008), promotes the accumulation of chlorophyll in leaves.

ROMANIAN AGRICULTURAL RESEARCH

Table 1. Leaf greenness index (SPAD) of grasses in 2011

Object	Percentage seeds in the mixture	1 st regrowth	2 nd regrowth	3 rd regrowth	Mean
Festulolium (<i>Fb</i>)	100	42,13 *de	46,48 cd	42,80 de	43,80 c
Festulolium + alfalfa (<i>Fb/Mm</i>)	70	37,88 b	37,38 a	39,39 b	38,21 b
	50	36,44 ab	34,78 a	37,64 a	36,28 a
	30	40,14 cd	47,60 d	40,74 bc	42,83 c
Orchard grass (<i>Dg</i>)	100	36,71 ab	41,33 b	39,79 b	39,28 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)	70	38,56 bc	47,40 d	42,34 cd	42,77 c
	50	34,63 a	43,78 bc	40,96 bc	39,79 b
	30	43,18 e	42,03b	44,14 e	43,11 c
Mean for objects					
Festulolium (<i>Fb</i>)		42,13 c	46,48 b	42,80 b	43,80 c
Festulolium + alfalfa (<i>Fb/Mm</i>)		38,15 ab	39,92 a	39,25 a	39,11 a
Orchard grass (<i>Dg</i>)		36,72 a	41,33 a	39,79 a	39,28 a
Orchard grass + alfalfa (<i>Dg/Mm</i>)		38,79 b	44,40 b	42,48 b	41,89 b
Mean for percentage seeds in the mixture					
	100	39,42 b	43,90 bc	41,29 b	41,54 c
	70	38,22 b	42,39 b	40,86 b	40,49 b
	50	35,53 a	39,28 a	39,30 a	38,04 a
	30	41,66 c	44,81 c	42,44 c	42,97 d

* a, b, c, d, e – homogeneous group.

Table 2. Leaf greenness index (SPAD) of grasses in 2012

Object	Percentage seeds in the mixture	1 st regrowth	2 nd regrowth	3 rd regrowth	Mean
Festulolium (<i>Fb</i>)	100	34,13 a	27,46 a	35,78 a	32,45 a
Festulolium + alfalfa (<i>Fb/Mm</i>)	70	39,91 bc	36,89 bc	40,78 cd	39,19 cd
	50	41,48 c	39,76 cd	39,05 bc	40,10 cd
	30	39,49 bc	43,38 d	39,08 bc	40,65 d
Orchard grass (<i>Dg</i>)	100	35,89 ab	34,21 b	38,40 b	36,17 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)	70	37,24abc	34,50 b	42,93 de	38,22 bc
	50	38,71abc	35,63 bc	41,38 d	38,57 cd
	30	39,15 bc	36,45 bc	44,33 e	39,98 cd
Mean for objects					
Festulolium (<i>Fb</i>)		34,13 a	27,46 a	35,78 a	32,45 a
Festulolium + alfalfa (<i>Fb/Mm</i>)		40,29 c	40,01 c	39,63 b	39,98 c
Orchard grass (<i>Dg</i>)		35,89 ab	34,21 b	38,40 b	36,17 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)		38,37 bc	35,53 b	42,88 c	38,92 c
Mean for percentage seeds in the mixture					
	100	35,01 a	30,84 a	37,08 a	34,31 a
	70	38,58 b	35,69 b	41,85 c	38,71 b
	50	40,09 b	37,69 bc	40,21 b	39,33 bc
	30	39,32 b	39,91 c	41,70 c	40,31 c

In the third year of the study, relative chlorophyll concentrations were lower in festulolium than in orchard grass. Average SPAD values reached 34.43% in festulolium

and 41.94% in orchard grass (Table 3). Significant differences in chlorophyll levels were observed between grass species by Zielewicz and Kozłowski (2011), Jodełka and

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Sosnowski (2010) and Olszewska (2005, 2006 2008). In our study, the presence of alfalfa increased the chlorophyll content of festulolium leaves, but had no significant influence on chlorophyll levels in orchard grass leaves. In the last year of the experiment, the highest chlorophyll

concentrations were determined in the first regrowth. Significant variations in grass chlorophyll levels during the growing season were also determined by Zielewicz and Kozłowski (2001) who attributed those differences to species-specific vitality parameters.

Table 3. Leaf greenness index (SPAD) of grasses in 2013

Object	Percentage seeds in the mixture	1 st regrowth	2 nd regrowth	3 rd regrowth	Mean
Festulolium (<i>Fb</i>)	100	36,29 a	31,61 a	35,38 a	34,43 a
Festulolium + alfalfa (<i>Fb/Mm</i>)	70	41,11 ab	44,26 e	39,50 b	41,63 b
	50	41,58 b	35,15 b	34,20 a	36,98 a
	30	43,51 b	38,54bcd	41,90 bc	41,32 b
Orchard grass (<i>Dg</i>)	100	43,41 a	40,56 d	41,80 bc	41,93 b
	70	42,70 a	36,78 bc	41,75 bc	40,41 b
	50	42,29 a	40,18 cd	41,55 bc	41,34 b
	30	44,17 a	38,53bcd	44,63 c	42,44 b
Mean for objects					
Festulolium (<i>Fb</i>)		36,29 a	31,61 a	35,38 a	34,43 a
Festulolium + alfalfa (<i>Fb/Mm</i>)		42,07 b	39,32 b	38,53 b	39,97 b
Orchard grass (<i>Dg</i>)		43,41 b	40,56 b	41,80 c	41,93 c
Orchard grass + alfalfa (<i>Dg/Mm</i>)		43,05 b	38,49 b	42,64 c	41,40 c
Mean for percentage seeds in the mixture					
	100	39,85 a	36,09 a	38,59 ab	38,18 a
	70	41,91 ab	40,52 c	40,63 b	41,02 b
	50	41,93 ab	37,66 ab	37,88 a	39,16 a
	30	43,84 b	38,53bc	43,26 c	41,88 b

The results of the three-year experiment indicate that the leaves of pure-sown festulolium contained significantly less chlorophyll than the leaves of orchard grass. The average SPAD values for the three-year period reached 36.89 in festulolium and 39.12 in orchard grass (Table 4). Differences in chlorophyll accumulation were observed between species. Higher relative chlorophyll concentrations were noted in festulolium in the first year and in orchard grass in the third year of the study.

The incorporation of alfalfa into the mixture increased leaf greenness in both analyzed grass species, and the highest SPAD values were noted in treatments with 70% alfalfa seeds. Higher chlorophyll concentrations in grasses grown in mixtures with legumes were also observed in previous

studies by Olszewska (2008) and Grygierzec (2012). The above can probably be attributed to higher levels of available nitrogen due to the fixation of atmospheric nitrogen by legume plants. Nitrogen symbiotically fixed by legumes is more available to plants than nitrogen supplied by fertilizers (Mallarino et al., 1990). Chlorophyll concentrations in grass leaves are significantly influenced not only by the percentage of legume plants in the mixture, but also by the species composition of the mixture. In a previous study by Olszewska (2008), leaf greenness values were significantly higher in grasses grown with white clover than with *Lotus corniculatus*. According to Ta and Faris (1987), alfalfa and red clover are more likely to increase the concentrations of plant-available nitrogen in grass mixtures than *Lotus corniculatus*.

The yield of the examined grass mixtures varied across experimental years. The highest dry matter yield of 10.74-12.34 t per ha was reported in 2011 (Table 5). Yield decreased gradually in successive years of the study, and the most pronounced drop was reported in

festulolium whose yield decreased by 32% in the second year and by 59% in the third year of the experiment. The decrease in the yield of orchard grass was less profound, and it was estimated at 21% and 34% in successive years of full utilization.

Table 4. Leaf greenness index (SPAD) of grasses in the years 2011-2013

Object	Percentage seeds in the mixture	2011	2012	2013	2011-2013
Festulolium (<i>Fb</i>)	100	43,80 c	32,45 a	34,43 a	36,89 a
Festulolium + alfalfa (<i>Fb/Mm</i>)	70	38,21 b	39,19 cd	41,63 b	39,68 c
	50	36,28 a	40,10 cd	36,98 a	37,78 ab
	30	42,83 c	40,65 d	41,32 b	41,60 d
Orchard grass (<i>Dg</i>)	100	39,28 b	36,17 b	41,93 b	39,12 bc
Orchard grass + alfalfa (<i>Dg/Mm</i>)	70	42,77 c	38,22 bc	40,41 b	40,47 cd
	50	39,79 b	38,57 cd	41,34 b	39,90 c
	30	43,11 c	39,98 cd	42,44 b	41,84 d
Mean for objects					
Festulolium (<i>Fb</i>)		43,80 c	32,45 a	34,43 a	36,89 a
Festulolium + alfalfa (<i>Fb/Mm</i>)		39,11 a	39,98 c	39,97 b	39,69 b
Orchard grass (<i>Dg</i>)		39,28 a	36,17 b	41,93 c	39,12 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)		41,89 b	38,92 c	41,40 c	40,74 c
Mean for percentage seeds in the mixture					
	100	41,54 c	34,31 a	38,18 a	38,01 a
	70	40,49 b	38,71 b	41,02 b	40,07 c
	50	38,04 a	39,33 bc	39,16 a	38,84 b
	30	42,97 d	40,31 c	41,88 b	41,72 d

Table 5. Dry matter yield ($t \cdot ha^{-1}$)

Object	Percentage seeds in the mixture	2011	2012	2013	2011-2013
Festulolium (<i>Fb</i>)	100	10,74 a	7,35 a	4,43 a	7,48 a
Festulolium + alfalfa (<i>Fb/Mm</i>)	70	11,62 ab	10,80 c	10,30 cd	10,90 cd
	50	11,15 a	10,78 c	9,50 c	10,50 c
	30	10,91 a	11,58 cd	10,30 cd	10,95 cd
Orchard grass (<i>Dg</i>)	100	11,56 ab	9,18 b	7,63 b	9,48 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)	70	12,34 b	11,65 cd	10,88 cd	11,60 e
	50	12,21 b	11,30 c	9,88 cd	11,10 d
	30	12,33 b	12,45 d	11,18 d	12,00 e
Mean for objects					
Festulolium (<i>Fb</i>)		10,74 a	7,35 a	4,43 a	7,48 a
Festulolium + alfalfa (<i>Fb/Mm</i>)		11,23 a	11,05 c	10,03 c	10,78 c
Orchard grass (<i>Dg</i>)		11,56 a	9,18 b	7,63 b	9,48 b
Orchard grass + alfalfa (<i>Dg/Mm</i>)		12,29 b	11,80 d	10,64 c	11,57 d
Mean for percentage seeds in the mixture					
	100	11,15 a	8,26 a	6,03 a	8,48 a
	70	11,98 c	11,23 b	10,59 c	11,25 bc
	50	11,68 ab	11,04 b	9,69 b	10,80 b
	30	11,62 ab	12,01 c	10,74 c	11,48 c

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Festulium was characterized by significantly lower average yields than orchard grass throughout the entire experiment. The introduction of alfalfa to the grass mixture improved yield. In treatments containing alfalfa, average dry matter yield was 44% higher in mixtures with festulium and 22% higher in mixtures with orchard grass. Variations in the share of alfalfa in grass mixtures led to significant differences in yield. The lowest yield was noted in treatments with 50% alfalfa seeds, whereas mixtures containing 30% and 70% alfalfa

seeds were characterized by similar yield values. The above can be attributed to the fact that the actual share of alfalfa plants was much higher than the percentage of alfalfa seeds in the grass mixture, and it was very similar in the analyzed treatments.

A strong positive correlation was observed between leaf greenness and dry matter yield (Figure 3). The value of the coefficient of correlation reached 0.76, and it was highly statistically significant. Therefore, leaf greenness can be a reliable predictor of plant yield.

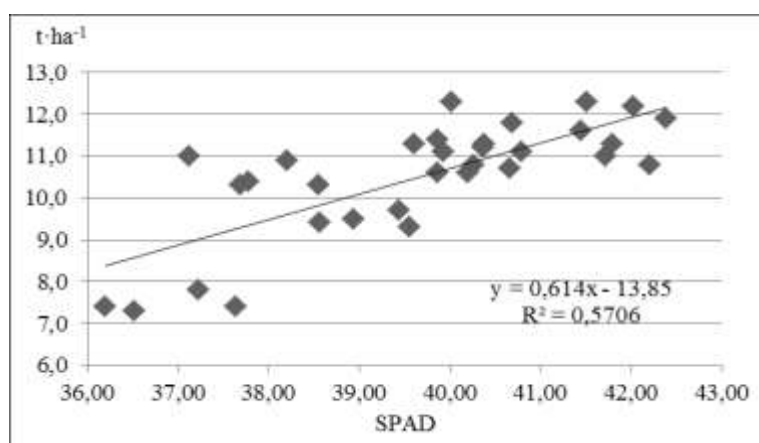


Figure 3. Correlation between leaf greenness index and dry matter yield

CONCLUSIONS

The results of this study indicate that the leaves of pure-sown festulium contained significantly less chlorophyll than the leaves of orchard grass. The addition of alfalfa to the mixture increased leaf greenness in both analyzed grass species, and the highest SPAD values were noted in treatments with 70% alfalfa seeds. Average yield values were significantly higher in grass mixtures than in pure-sown grasses throughout the entire experiment. The mixture with orchard grass produced higher yields than the mixture with festulium. Variations in the proportion of alfalfa in mixtures led to significant differences in yield. The lowest yield was noted in treatments with 50% alfalfa seeds, whereas mixtures containing 30% and 70% alfalfa seeds were characterized by similar yield values. A strong positive correlation was observed between leaf greenness and dry

matter yield. Thus, leaf greenness can be a reliable predictor of plant yield.

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