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EFFECTS OF DIFFERENT PROPORTIONS OF *Medicago media* PERS. IN MIXTURES WITH *Dactylis glomerata* L. ON THE YIELD OF ABOVEGROUND BIOMASS, PROTEIN YIELD AND RELATIVE CHLOROPHYLL CONTENT IN ORCHARD GRASS LEAVES

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Abstract. The aim of the study was to determine the effects of different proportions of *Medicago media* Pers. in mixtures with *Dactylis glomerata* L. on the yield of aboveground biomass, protein yield and relative chlorophyll content in orchard grass leaves. The study was carried out in 2011-2013. A strict field experiment was conducted in the Educational and Research Station of the University of Warmia and Mazury in Olsztyn, Poland (53°72' N; 20°42' E), on mineral soil of quality class IVa and very good rye complex. Pure-sown orchard grass and orchard grass in mixtures with hybrid alfalfa were investigated. The experimental factor was a varied proportion (30%, 50%, 70%) of alfalfa seeds in the mixtures. Results demonstrated that the proportion of alfalfa in mixtures increased gradually in subsequent years, but its share in the aboveground biomass was disproportionate to the percentage of sown seeds. Alfalfa introduced as a component of the mixtures increased the relative chlorophyll content in orchard grass leaves, but no significant differences were found between plots with 30% and 70% proportions of alfalfa. The mixtures gave significantly higher yields when compared to pure-sown orchard grass. Depending on the proportion of alfalfa in the mixtures, the yield was about 18 to 27% higher and more reliable in the study years. The introduction of alfalfa to the mixtures caused a significant 56% increase in protein yield per area unit as compared to pure-sown orchard grass. A highly significant correlation coefficient shows that the leaf greenness index can be regarded as an important prediction rate of plant yield.

Key words: *Dactylis glomerata*, dry matter yield, *Medicago media*, leaf greenness (SPAD), protein yield

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INTRODUCTION

Alfalfa (lucerne) is a plant rich in protein, and grown in mixtures with grasses is a valuable roughage, intended mainly for feeding high-yield dairy cattle. In the feeding of ruminants it has been indicated for several years that a shortage of energy in feed is a greater problem than protein shortage [Andrzejewska *et al*. 2013]. Alfalfa protein decomposes rapidly in the rumen, and is lost if there is an energy shortage for its assimilation [Broderick 1995]. Therefore, in order to limit protein loss alfalfa should be grown in mixtures with grasses. The admixture of grasses increases sugar content in the biomass and the produced feed is better balanced in terms of energy and protein content. The chemical composition of biomass obtained from mixtures is more diversified, and thus has a higher nutritional value. Moreover, mixtures offer higher and more reliable crop yield than plants grown in monocultures [Jelinowska and Staniak 2007]. However, the economic value of mixtures with alfalfa depends on the proportion of components, frequency of use, the competitiveness of grass species and on the order regrowth of grasses in growing season [Ćwintal and Warda 2001, Gaweł 2008]. The selection of species and varieties of these plants is also important and primarily dependent on the habitat conditions. According to Casler and Walgenbach [1990], a tall fescue and medium-late maturing variety of orchard grass are good components of mixtures with alfalfa, during the periodic water scarcity. Under the polish climatic conditions, it is recommended to crop alfalfa with a medium-late maturing variety of orchard grass. Early varieties of orchard grass are characterized by high aggressiveness and during the Spring they enter into generative phase very quickly while alfalfa grows much slower as a thermophilic plant [Jelinowska and Staniak 2007]. Determining the applicable proportion of components in the mixture is very complicated as it depends on natural and agronomic factors. Frequently, changes in the proportion of alfalfa in the mixture do not cause any significant differences in yields [Zannone and *et al*. 1986, Charles and Lehmann 1989, Ćwintal 2000].

The research was conducted with the following assumptions, that the greater share of alfalfa in mixture will have a positive impact on yielding sward and the protein yield, while measurements of the relative content of chlorophyll in the leaves will help determine how alfalfa participation in mixture affects the nutritional status of nitrogen of orchard grass. The aim of research was to determine the effects of different proportions of *Medicago media* Pers. in mixtures with *Dactylis glomerata* L. on the yield of aboveground biomass, protein yield and relative chlorophyll content in orchard grass leaves.

MATERIAL AND METHODS

The study was carried out in 2011-2013. A field experiment was conducted in the Educational and Research Station of the University of Warmia and Mazury in Olsztyn, Poland ($53^{o}72'$ N; $20^{o}42'$ E) on mineral soil of quality class IVa and very good rye complex. The arable soil layer was characterized by a very low content of phosphorus (2.0 mg P in 100 g soil) and moderate potassium (12.4 K in 100 g soil) and magnesium (5.0 mg Mg in 100 g soil) content. Soil pH was neutral (pH_{KCl} – 7.2). The experiment, in a split--plot design with four replicates, was established in the spring of 2010. Pure-sown orchard grass cv. Berta and orchard grass in mixtures with hybrid alfalfa cv.

Radius were investigated. The experimental factor was varied proportions (30%, 50%, 70%) of alfalfa seeds in the mixtures. Plot size was 10 m^2. Before sowing, all plots were fertilized with nitrogen, phosphorus and potassium at 30 kg N, 35 kg P and 50 kg $K \cdot ha^{-1}$, respectively. Broadcast seeding was used without cover crops. In the harvest years, orchard grass was fertilized with nitrogen at 180 kg $N \cdot ha^{1}$, and grass mixtures were fertilized with 90 kg $N \cdot ha^{-1}$. Nitrogen rates were divided into three equal parts and applied in early spring, after the first and second harvests. All plots were fertilized with phosphorus at 35 kg $P \cdot ha^{-1}$ (one spring application) and potassium at 100 kg $K \cdot ha^{-1}$ (in spring and after the first harvest). Leaf greenness (relative chlorophyll content) of orchard grass was measured in the youngest, fully developed shoot leaf selected randomly from each treatment. Measurements were taken with a 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 taken in each regrowth in five replicates. The first measurement was conducted in the first decade of May, while in the following regrowth it was 3-4 weeks after cutting the sward. Second measurement was taken the day before harvest regrowth. Effective participation of alfalfa in mixtures was established on the basis of botanical and gravimetric analysis of yield. Dry matter yield, protein yield (protein content of the plant material × dry matter yield/100) and the coefficients of correlation between leaf greenness and dry matter yield was calculated. The significance of correlation was evaluated based on critical values of Spearman's rank correlation coefficient at $\alpha = 0.05$ and $\alpha = 0.01$. The results of the research were statistically developed by one-way analysis of variance ANOVA using STATISTICA software. The significance of differences was verified by Tukey's test at $\alpha = 0.05$.

Weather conditions in the study years are presented in Figure 1. The growing season in 2011 was characterized by favourable weather, and average monthly temperatures were similar to the multi-annual average. Except for 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.

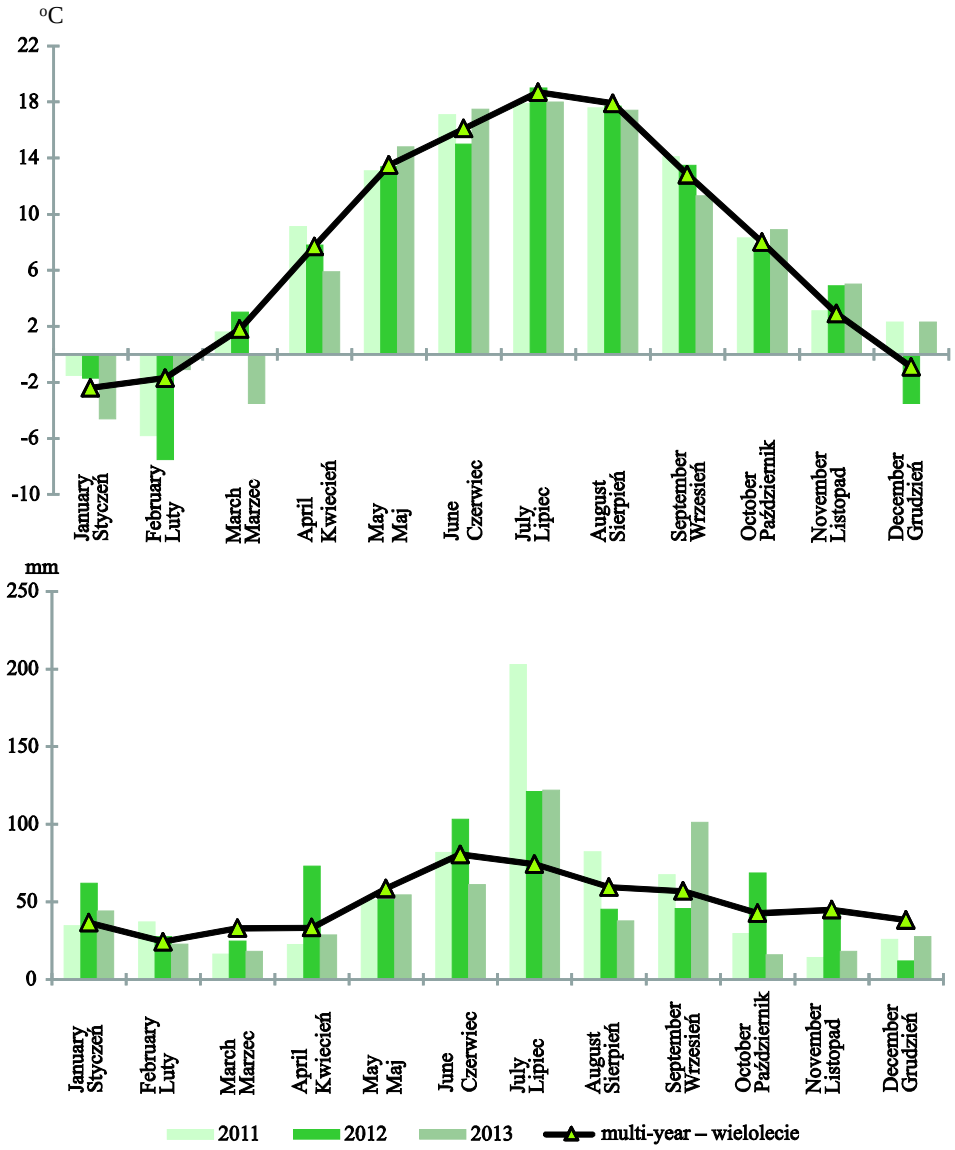

Fig. 1. Mean air temperature and rainfalls in the years 2011-2013
Rys. 1. Średnia temperatura powietrza i opady w latach 2011-2013

RESULTS AND DISCUSSION

The botanical composition of the sward demonstrated that the proportion of alfalfa in mixtures increased gradually in subsequent study years (Fig. 2). In the first year of harvest, the share of alfalfa plants in treatments with 30% alfalfa seeds was estimated at about 40%, in treatments with 50% and 70% alfalfa seeds – at 50% and 52%,

respectively. In the second year of the experiment, the share of alfalfa was higher, but differences between plots were insignificant. In the third year of the experiment the share of alfalfa in sward was about 65-67%. There was only about a 2% difference between plots with 30% and 70% shares of alfalfa seeds. These findings support the opinion that the percentage of sown seeds determines the initial number of plants per area unit, which is highly variable after seedling emergence and disproportionate to the increasing number of sown seeds [Ćwintal 2000]. Many authors have emphasized that the number of germinating seeds is disproportionate to the number of sown seeds and constitutes a larger percentage at lower than higher percentages of sown seeds [Jamriska 1992, 1993, Ćwintal 2000]. Zannone *et al*. [1986] and Charles and Lehmann [1989] attributed this to variable habitat conditions and competition between plants. Furthermore, according to Jelinowska and Staniak [2007], the production of deep and extensive root system by alfalfa in the initial growth period, causes a slower growth aboveground parts than in case of grass. It supports competitiveness of grass. In the following regrowth, aboveground mass is produced by alfalfa much faster, due to possibility of using spare substances accumulated in collar-root.

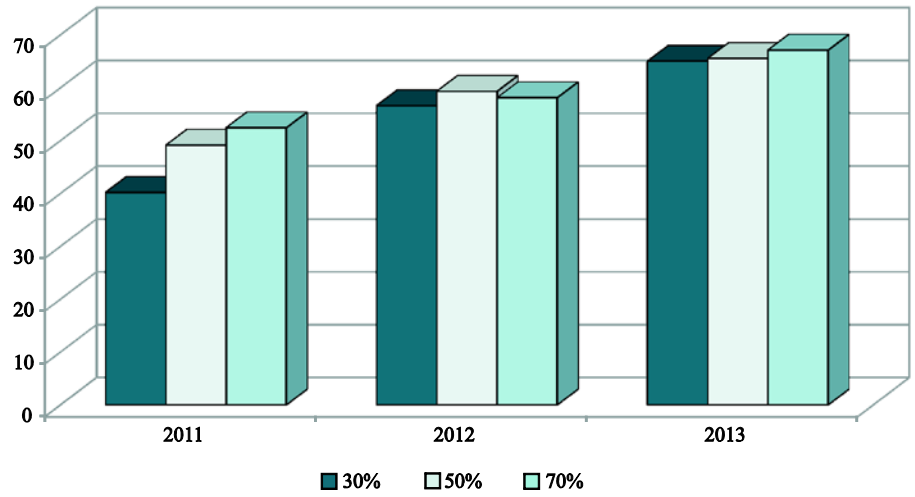

Fig. 2. Mean share of alfalfa in mixture in the years 2011-2013
Rys. 2. Średni udział lucerny w mieszankach w latach 2011-2013

The relative chlorophyll content in orchard grass leaves varied in individual study years (Table 1). The lowest SPAD values were recorded in the second year of harvest, characterised by a high level of precipitation. Weather conditions significantly affect the chlorophyll level in grass leaves. Under conditions of water shortage, higher chlorophyll content is found in plant leaves, and this results from the decrease in plant cell size and tissue density, and a subsequent increase in the concentration of low- and high-molecular weight compounds, including chlorophyll [Jones *et al*. 1980]. Increased chlorophyll content in grass leaves caused by water shortage in soil was also reported by Staniak [2013], Rumasz-Rudnicka [2010] and Olszewska [2006a, 2006b, 2008]. The analysis of mean SPAD values demonstrated the lowest chlorophyll content in the leaves of pure-sown orchard grass (39.12). Significantly higher values were found in the leaves of *Dactylis glomerata* grown in a mixture with *Medicago media*. However, no

significant differences between plots with 30% and 70% proportions of alfalfa were recorded. This could be explained by the fact that the actual proportion of alfalfa in the botanical composition of the sward differed significantly from the percentage of seeds in the mixture, and on individual plots these differences were in the range of only a few percent. Higher chlorophyll levels in grasses grown in mixtures with legumes were also found in previous studies by Olszewska [2008] and Grygierzec [2012]. This can probably be attributed to higher levels of available nitrogen due to the fixation of atmospheric nitrogen by legumes. Nitrogen symbiotically fixed by legumes is more available to plants than nitrogen supplied in mineral fertilizers [Mallarino et al. 1990]. Chlorophyll levels in grass leaves are significantly determined 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*. Mallarino *et al*. [1990] reported that nitrogen transfer from legumes to grasses can range from 22% of the total N uptake for orchard grass grown with alfalfa to as much as 58% for grasses grown with red clover.

Table 1. Leaf greenness index (SPAD) of orchard grass
Tabela 1. Indeks zieloności liści (SPAD) kupkówki pospolitej

Treatment – Obiekt	Years of study – Lata badań			Mean Średnia
	2011	2012	2013	
Orchard grass 100% Kupkówka pospolita 100%	39.28* a	36.17 a	41.93 ab	39.12 a
Orchard grass 70% + alfalfa 30% Kupkówka pospolita 70% + lucerna 30%	42.77 b	38.22 b	40.41 a	40.47 b
Orchard grass 50% + alfalfa 50% Kupkówka pospolita 50% + lucerna 50%	39.79 a	38.57 b	41.34 ab	39.90 ab
Orchard grass 30% + alfalfa 70% Kupkówka pospolita 30% + lucerna 70%	43.11 b	39.98 b	42.44 b	41.84 b

* the values in the columns marked with the same letters constitute a homogeneous group – wartości w kolumnach oznaczone tymi samymi literami tworzą grupy jednorodne

Yields of sward varied in individual study years (Table 2). The highest yield of dry matter was obtained in 2011, and was in the range of 11.56-12.34 $Mg \cdot ha^{-1}$. No significant differences in yields between individual experimental plots were found in 2011. The yield decreased in the subsequent years of the experiment, and was most pronounced in pure-sown *Dactylis glomerata*. The decrease in the yield of this species was about 21% in the second year, and about 34% in the third year of harvest. Reduction in the yield of mixtures was significantly lower, and accounted for 6 to 7.5% in the second year and 9 to 19% in the third year of the study. The higher and more reliable yield of mixed crops in comparison with pure-sown species results from better utilization of space, different growth and development cycles of component species, as well as the complementary use of habitat resources. Our three-year-long study indicated significantly higher mean yields of sward formed by mixed species. The yield was about 18 to 27% higher, depending on the proportion of alfalfa in the mixture. The lowest yield was found for plots with 50% of alfalfa in the mixture, while yields for

plots with 30 and 70% proportions of alfalfa seeds were similar. Other researchers explain the lack of significant differences in alfalfa yields despite different percentage of seeds in the mixture by the self-regulation of canopy density, which is due to the compensating ability of the species, and is reflected in a comparable number of shoots per area unit in a considerably different number of plants [Ćwintal 2000].

Table 2. Dry matter yield, Mg·ha^{-1}
Tabela 2. Plon suchej masy, Mg·ha^{-1}

Treatment – Obiekt	Years of study – Lata badań			Total Suma
	2011	2012	2013	
Orchard grass 100% Kupkówka pospolita 100%	11.56* a	9.18 a	7.63 a	28.36 a
Orchard grass 70% + alfalfa 30% Kupkówka pospolita 70% + lucerna 30%	12.34 a	11.65 bc	10.83 b	34.87 bc
Orchard grass 50% + alfalfa 50% Kupkówka pospolita 50%+ lucerna 50%	12.21 a	11.30 b	9.88 b	33.38 b
Orchard grass 30% + alfalfa 70% Kupkówka pospolita 30% + lucerna 70%	12.33 a	12.45 c	11.18 b	35.95 c

* the value in the columns marked with the same letters constitute a homogeneous group – wartości w kolumnach oznaczone tymi samymi literami tworzą grupy jednorodne

Protein yield is a function of dry matter yield and total protein content in plants. In the experiment the lowest total protein yield (3.33 Mg·ha^{-1}) for the 3-year study period was obtained from pure-sown *Dactylis glomerata*. The introduction of alfalfa to the mixture significantly increased protein yield per area unit. There were no significant differences between yields from plots where mixtures with 30% and 50% proportions of alfalfa were sown, but a significantly higher protein yield was obtained from sward with a 70% proportion of alfalfa seeds (total 5.18 Mg·ha^{-1} for a 3-year study). As compared to pure-sown orchard grass the protein yield increased by 56% (Table 3). Contrasting results were reported by Ciepiela *et al*. [2008], who found that pure-sown orchard grass provided a higher protein yield in comparison to that when grown in a mixture with hybrid alfalfa. However, the study by Ciepiela *et al*. [2008] was carried out under conditions of severe shortage of precipitation.

Table 3. Total protein yield, Mg·ha^{-1}
Tabela 3. Łączny plon białka, Mg·ha^{-1}

Treatment – Obiekt	Years of study – Lata badań			Total Suma
	2011	2012	2013	
Orchard grass 100% Kupkówka pospolita 100%	1.32* a	1.04 a	0.98 a	3.33 a
Orchard grass 70% + alfalfa 30% Kupkówka pospolita 70% + lucerna 30%	1.58 b	1.63 b	1.58 b	4.79 b
Orchard grass 50% + alfalfa 50% Kupkówka pospolita 50% + lucerna 50%	1.87 c	1.55 b	1.39 b	4.81 b
Orchard grass 30% + alfalfa 70% Kupkówka pospolita 30% + lucerna 70%	1.69 b	1.83 c	1.67 c	5.18 c

* the value in the columns marked with the same letters constitute a homogeneous group – wartości w kolumnach oznaczone tymi samymi literami tworzą grupy jednorodne

Our study demonstrated a close positive correlation between the leaf greenness and dry matter yield (Fig. 3). The calculated correlation coefficients for this parameter was highly significant and came to 0.75. Therefore, leaf greenness can be a reliable predictor of plant yield and protein yield. The positive correlation between chlorophyll levels in grass leaves and dry matter yield was also confirmed by Radkowski [2013], Rumasz-Rudnicka [2010] and Gáborčík [1996, 2003].

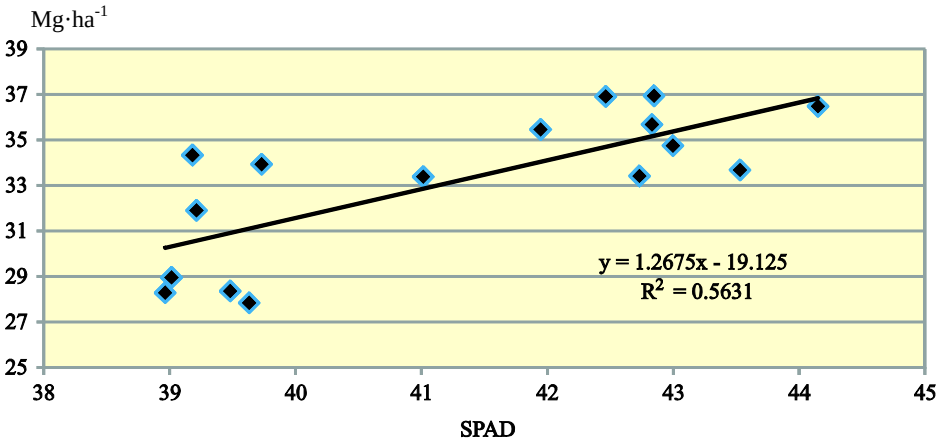

Fig. 3. Correlation between leaf greenness index and dry matter yield
Rys. 3. Korelacja między indeksem zieloności liści a plonem suchej masy

CONCLUSIONS

1. Proportion of alfalfa in mixtures increased gradually in subsequent years, but its share in the aboveground biomass was disproportionate to the percentage of sown seeds.
2. Alfalfa introduced as a component of the mixtures increased the relative chlorophyll content in orchard grass leaves, but no significant differences were found between plots with 30% and 70% proportions of alfalfa.
3. The mixtures gave significantly higher yields when compared to pure-sown orchard grass. Depending on the proportion of alfalfa in the mixtures, the yield was about 18 to 27% higher and more reliable in the study years.
4. The introduction of alfalfa to the mixtures caused a significant 56% increase in protein yield per area unit as compared to pure-sown orchard grass.
5. The high values of the correlation coefficients demonstrate that the leaf greenness index is an important predictor of plant yield.

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WPŁYWU ZRÓŻNICOWANEGO UDZIAŁU *Medicago media* PERS. W MIESZANCE Z *Dactylis glomerata* L. NA PLON BIOMASY NADZIEMNEJ, PLON BIAŁKA ORAZ WZGLĘDNĄ ZAWARTOŚĆ CHLOROFILU W LIŚCIACH KUPKÓWKI POSPOLITEJ

Streszczenie. Celem badań było określenie wpływu zróżnicowanego udziału *Medicago media* Pers. w mieszance z *Dactylis glomerata* L. na plon biomasy nadziemnej, plon białka oraz względną zawartość chlorofilu w liściach kupkówki pospolitej. Badania przeprowadzono w latach 2011-2013. Doświadczenie polowe zlokalizowano w Stacji Dydaktyczno-Badawczej Uniwersytetu Warmińsko-Mazurskiego w Olsztynie (53°72' N; 20°42' E), na glebie mineralnej, klasy bonitacyjnej IVa, kompleksu żytniego bardzo dobrego. Badaniami objęto kupkówkę pospolitą uprawianą w siewie czystym oraz w mieszance z lucerną mieszańcową. Czynnikiem doświadczalnym był zróżnicowany udział nasion lucerny w mieszance: 30, 50, 70%. Uzyskane wyniki wykazały, że udział lucerny w mieszankach systematycznie wzrastał w kolejnych latach badań, przy czym jej udział w biomasie nadziemnej był nieproporcjonalny do ilości wysianych nasion. Wprowadzenie lucerny jako komponentu mieszanki spowodowało wzrost względnej zawartości chlorofilu w liściach kupkówki pospolitej, jednak między obiektami z 30% i 70% udziałem lucerny nie stwierdzono istotnych różnic. Mieszanki plonowały istotnie wyżej w stosunku do siewu czystego kupkówki pospolitej. W zależności od udziału lucerny w mieszance plonowanie było o ok. 18 do 27% wyższe i wierniejsze w latach badań. Wprowadzenie lucerny do mieszanki istotnie zwiększyło plon białka z jednostki powierzchni. W porównaniu z siewem czystym kupkówki pospolitej plon białka wzrósł o ok. 56%. Wysoko istotny współczynnik korelacji dowodzi, że indeks zieloności liści można uznać za ważny wskaźnik prognozowania wielkości plonowania roślin.

Słowa kluczowe: *Dactylis glomerata*, indeks zieloności liści (SPAD), *Medicago media*, plon białka, plon suchej masy

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