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The formation of the leaf surface of white melilot (*Melilotus albus*) depending on fertilization, seed mix and seeding rate

Kształtowanie się powierzchni liści nostrzyka białego (*Melilotus albus*) w zależności od współkomponentu w mieszance, ilości wysiewu nasion tego gatunku oraz dawki nawożenia

Summary. The study objective was to analyze the formation of the leaf surface of white melilot (*Melilotus albus*) cultivated in pure sowing and mixes with annual cereals, with varying seeding rates for this species and varying fertilization levels. White melilot was sown in pure sowing and mixes with maize, millet, Sudan grass, and sorghum, with four seeding rates (16, 18, 20, and 22 kg ha^{-1}). Four doses of fertilization with NPK were used in the investigations (0 – control treatment without fertilization, $N_{45}P_{45}K_{45}$, $N_{60}P_{60}K_{60}$, and $N_{60}P_{90}K_{90}$). The leaves' surface was assessed through scanning on the 30th, 40th, and 50th day after the germination of white melilot and in the period of its readiness for mowing (64th day). White melilot leaves were separated from the stems and placed in a transparent folder with a 25 cm calibration square, then scanned with a flatbed scanner in black-and-white mode. The obtained image was analyzed in Areas software, the built-in analytical tools of which were used to determine the area of the scanned leaves. After determining each plant's leaf surface, the mean for each variant of the experiment was calculated, and then, using the concentration of plants per 1 m^2, the mean *M. albus* leaf surface per hectare was obtained. The investigations demonstrated that the surface of white melilot leaves varied depending on the mix's component, the seeding rate for this species, and the fertilization doses. Depending on the seeding rate (16, 18, 20, and 22 kg ha^{-1}), one plant's mean leaf surface area was as follows: 0.014115, 0.013955, 0.013824, and 0.013654 m^2. The smallest *M. albus* leaf surface area per hectare was recorded in treatments without components (pure sowing) and with the highest seeding rate for this species' seeds. The significantly the largest leaf surface area was observed in treatments where *M. albus* was sown in 16 kg ha^{-1}, in a mix with Sudan grass and maize. Millet was the component with the strongest negative effect on this parameter, with each of the *M. albus* seeding rates used. Typically, the studied species' leaf surface area was successively decreasing by 2 to 11% as the seeding rate increased. When mineral fertilizers were introduced, the species under study's leaf surface area was increasing by 7 to 16% as the fertilization rate increased. Considering the mean values for the study period, the largest area of white melilot leaves (in the period of readiness for cutting) was recorded in a mixed treatment of white melilot with Sudan grass, i.e., 52.3 thousand m^2 ha^{-1} with fertilization of $N_{60}P_{90}K_{90}$ and seeding rate of 16 kg ha^{-1}.

Keywords: leaf surface area, *Melilotus albus*, pure sowing, mixed treatments, maize, millet, Sudan grass, sorghum, fertilizers, seeding rate

INTRODUCTION

Nowadays, legumes are poorly competitive in the agricultural products market and, therefore, areas allocated for them are insignificant in the structure of farmland. Basically, they are used to cover farmers' own needs with regard to food and fodder. However, a decrease in the amount of organic fertilizer used and the limited use of legumes in crop rotation result in a decrease of humus content and deterioration of soil properties, which leads to unsatisfactory conditions for plant cultivation. One of the manifestations of this condition is an increase in groundwater level and salinity of arable land [Brzezowska and Dreszczyk 2009].

Since it is impossible to improve the conditions of growth and development of plants immediately, it is necessary to use a comprehensive approach which includes the introduction of salt plants (with phytomeliorative properties) into the crop rotation. In these conditions, these plants can ensure stable, high and high-quality yields as well as the fullest use of natural, economic and energy resources. Alfalfa is a priority crop on irrigated land while white melilot on non-irrigated land [Wolf and Rohrs 2001, Luo et al. 2017].

White melilot culture is underestimated by producers due to the imperfect technology of cultivation, especially the technology of fodder preparation from this culture, as well as due to misconceptions about coumarin content in white melilot which only gives forage a bitter taste and peculiar odour. As far as its nutritional properties are concerned, white melilot almost equals other leguminous crops [Chorepsima et al. 2013, Sowa et al. 2018]. In addition, white melilot, similarly to other legumes, enriches the soil with humus and thus improves its structure. Thanks to its well-developed root system, this species is conducive to the improvement and preservation of soil fertility through the intake of phosphorus, potassium or calcium from the deeper layers of soil. Thanks to the symbiosis with rhizobia (which fix atmospheric nitrogen), white melilot contributes to the enrichment of soil with a form of nitrogen that is more assimilable to other plant species. White melilot itself can develop on nutrient-poor soils [Wolf and Rohrs 2001], and can be used for land reclamation thanks to the structure of the root system of this species [Evans and Thompson 2006, Al Sherif 2009, Zabala et al. 2018].

The plants' assimilation surface, identified as the surface of their leaves, is a key indicator of the plants' yielding capacity [Szmigiel and Oleksy 2004]. The optimum leaf surface area is an indicator of the effectiveness of complex or individual elements of the cultivation technology, which affect the process of yield formation, as evidenced by data reported by various researchers [Woźniak 2008, Oleksy et. al. 2009]. Leaf surface area is an integrated indicator of the plants' state in any phase of development. With an optimum assimilation surface, a significant increase in plant productivity is observed. For most crop species, the optimum leaf surface is four to five times larger than the land area, and can be even larger for highly productive cultivars [Oleksy et al. 2009]. A fairly large leaf surface is useful for two reasons: it promotes a better gas exchange and a more complete light absorption [Russell and Wilson 1994].

Having regard for the above, investigations were conducted to analyse the influence of the accompanying component in the mix, varying seeding rates and fertilization levels on the formation of the leaf surface area of white melilot (*Melilotus albus*) cultivated in pure sowing-and mixes with annual cereals.

MATERIALS AND METHODS

The research was carried out in the years 2015–2017 in a scientific laboratory of the Department of Fodder Production, Reclamation and Meteorology based in the Agronomic Research Station of the National University of Life and Environmental Sciences of Ukraine (Pshenichne). Soil of the experimental site – typical chernozem low humus coarse dust and light loam on the forest. Soil is characterized by a high content of total and mobile forms of nutrients. In the layer 0–20 cm contained: total nitrogen 0.29–0.31%, humus – 4.53%, phosphorus – 0.15–0.25%, potassium – 2,3–2,5%, pH in salt extract – 6.87 (in KCl). The density of soil in equilibrium – 1,16–1,25 g cm^{-3}, moisture of resistant wilting – 10.8%. Depth of groundwater – 2–4 m. Given the above figures, it can be argued that field experiments performed in soil conditions, typical for Forest-steppe zone.

The mean annual precipitation volume in the study period was 562 mm, including 354 mm in the growing season (63% of the annual volume). However, the distribution of precipitation was uneven throughout the year: 22.4% of the annual volume in the spring (126 mm); 36.3% in the summer (204 mm), and 18.9% in the autumn (106 mm). The mean daily ambient temperature in the study period was +10°C. The mean temperature of the warmest month (July) was 19.6°C, while in the case of the coldest month (January), it was –6.9°C. The frost-free period in the study area usually lasts 162 days. The mean depth of the snow cover ranged from 15–30 cm. Easterly winds prevailed in the spring while north-westerly winds in the summer. The mean wind speed was not more than 3.6 m s^{-1}. Thus the weather conditions were conducive to the development of the studied plant species in the study period.

The investigation was conducted on microplots with a sowing area of 50 m^2, and an accounting area of 25 m^2, in four repetitions. Three factors were taken into account: Factor A. grass mixes: white melilot (control, pure sowing), white melilot + maize (60 thousand seeds ha^{-1}, i.e. 20 kg ha^{-1}), white melilot + millet (2.25 million seeds ha^{-1}, i.e. 20 kg ha^{-1}), white melilot + Sudan grass (1.5 million seeds ha^{-1}, i.e. 15 kg ha^{-1}), white melilot + sorghum (0.375 million seeds ha^{-1}, i.e. 15 kg ha^{-1}) – the seeding rate for the accompanying components was 70% of the generally recommended one. Factor B. white melilot sowing rate kg ha^{-1}: 16 (8.8 million seeds ha^{-1}), 18 (9.9 million seeds ha^{-1}), 20 (11 million seeds ha^{-1}) and 22 (12.1 million seeds ha^{-1}). Factor C. fertilization: no fertilizer (control treatment), $N_{45}P_{45}K_{45}$, $N_{60}P_{60}K_{60}$ and $N_{60}P_{90}K_{90}$. Phosphorus-potassium fertilizers were applied in the autumn, while nitrogen in the spring before seeding.

The sowing was carried out in stages. First seeds were sown with a precision seed drill (row spacing: 12.5 cm, depth: 3 cm). Later on the same day, the seeds of a given mix component were sown with a spacing of 36 cm and depth of 5 cm, using a Great Plains seeder (the seed outlets in this seeder are 18 cm apart; for a row spacing of 36 cm, every other outlet was covered with special metal casing). Finally, the entire seeding area was levelled through gentle harrowing.

Photosynthetic activity was assessed by scanning the surface area of the leaves on the 30th, 40th and 50th day after the germination of white melilot and on the 64th day, at the time of its readiness for cutting. 10 plants were selected from each of the three most typical repetitions of each variant (if a plant had two or more shoots, all of them were cut) from each row (10 white melilot plants and 10 cereal plants). Then each plant was weighed, leaves were separated from the shoots and placed in separate packages, signed and numbered beforehand. In the next stage, the leaves of each selected plant were placed on a flatbed scanner (black-and-white mode with a resolution of 75 dpi). Then the files were saved (signed according to the variant) and the leaf surface area of each plant was determined using Areas software developed at Samara SAA and featuring built in analytical tools [Solomko at al. 2011]. When the leaf surface area of each plant was determined, the mean area of each experiment variant was calculated (total area for 10 selected plants multiplied by 3 repetitions divided by 30 = mean leaf surface area in 1 variant). The mean leaf surface area was multiplied by the concentration of plants per sq metre and the product was multiplied by 10 000 to obtain the mean leaf surface area per hectare.

The results obtained were processed statistically in SAS v.91 software, using single- or multi-factor variance analysis. Tukey's confidence intervals ($p \leq 0.05$) were used to verify the significance of the differences between the means. Mean values between which no statistically significant differences occurred are marked with the same letter.

RESULTS AND DISCUSSION

Physical and physiological processes which transform solar energy into organic matter in the atmosphere-leaf-plant-agrocenosis system have an important influence on the quantitative and qualitative indices of plant productivity [Glenn et al. 2008]. An appropriate growth rate of the surface of the assimilating organs is necessary to obtain high yields. Even with the same intensity of photosynthesis, yields will be higher in treatments where the plants have a larger leaf surface area. Therefore, it is important to form an optimum area for the maximum utilisation of solar radiation and moisture and nutrient reserves [Vozhehova 2014]. It has been established that important elements of cultivation technology such as fertilization, mixes and plant density influence the area of the assimilating surface [Kuraszkiewicz and Pałys 2002, Woźniak 2008, Oleksy et al. 2009]. With a greater number of leaves and, consequently, a larger leaf surface area, a plant can make better use of solar energy and accumulate more organic matter per unit of time and, consequently, provide a much higher yield [Jaśkiewicz 2007, Timoshkin et al. 2013]. Also in the present study, the photosynthetic activity of *Melilotus albus* was assessed based on the formation of the leaf surface area of this species depending on the accompanying component in the mix, *M. albus* seeding rate and fertilization rate.

When analyzing the obtained data, it was found that most often 1–2 shoots (1.3 shoot on average) occurred on one *M. albus* plant. On one square metre, depending on the seeding rate used: 16, 18, 20 and 22 kg ha^{-1} the mean number of specimens observed was: 329.4 (from 279 to 430); 367.65 (from 308 to 492); 402.85 (from 351 to 568) and 397.0 (from 313 to 550) specimens m^{-2}. Depending on the seeding rate and other factors, the mean leaf surface area of one plant was as follows: 0.014115 (from 0.009017 to 0.016763), 0.013955 (from 0.00887 to 0.016538), 0.013824 (from 0.008747 to 0.01641) and 0.013654 (from 0.008624 to 0.01625) m^2.

The present investigation confirmed the influence of the factors under study on the leaf surface area in *M. albus* (Tables 1–4). During the study period, the introduction of mineral fertilizers promoted the growth of the leaf surface of the plant cultures studied. On each of the assessment dates (30, 40 and 50 days after seedling emergence and on harvest day) and under conditions of all seeding rates (16, 18, 20 and 22 kg ha^{-1}), the leaf area of the plants both in pure and mixed treatments with different fertilizing variants was greater in comparison with the variant treatments without fertilizers and grew gradually, according to the increasing level of mineral fertilization. Other studies [Jaśkiewicz 2007, Olsen and Weiner 2007] also confirm that the application of fertilizers is one of the most important agrotechnical measures leading to an increase in the leaf surface area. If we compare the individual types of fertilizers, nitrogen fertilizers are particularly effective in increasing the leaf surface area.

Thus, on the 30th day after germination, with the introduction of mineral fertilizers at a dose of $N_{60}P_{90}K_{90}$ and white melilot seeding rate of 16 kg ha^{-1}, the maximum assimilation surface obtained was 12.1 thousand m^2 ha^{-1} in a mix with sorghum and 11.2 thousands m^2 ha^{-1} in a mix with maize (Tab. 1). The introduction of mineral fertilizers contributed to a 17 to 20.4% increase in the leaf surface area on the 30th day after germination.

The research results show that the species composition of the grass mixes and fertilizers influenced the growth of the leaf surface area also on the 40th day after germination. The maximum increase of this parameter in comparison with pure treatments was observed in the mixed treatment with maize (21.6 thousand m^2 ha^{-1})) and Sudan grass (20.7 thousand), while in pure sowing the level of the increase was 15.4 thousand m^2 ha^{-1} with the maximum mineral nutrition and seeding rate of 16 kg ha^{-1} (Tab. 1). The introduction of full mineral fertilization, in comparison with the treatment without fertilizers, led to an increase of the assimilation surface area by 10–15%.

On the 50th day after germination, for 10 days on average, the surface area increased by 36.2–39.8% and the largest increase occurred in mixed treatments with maize and Sudan grass – 34.1 and 33.0 thousand m^2 ha^{-1} respectively, with $N_{60}P_{90}K_{90}$ fertilization and white melilot seeding rate at 16 kg ha^{-1} (Tab. 1). The use of the maximum mineral fertilization contributed to an increase by 7.3–9.9%.

Table 1. The dynamics of leaf surface area formation (thous. m^2 ha^{-1}) in *Melilotus albus* cultivated in pure sowing and in mixes, with varied fertilization rates and the seeding rate of 16 kg ha^{-1} (average for 2015–2017)

Mix component	Fertilizers	Days after germination			
		30	40	50	64 (mowing)
White melilot	without fertilizer	7.8 [a]	13.9 [a]	22.8 [a]	34.2 [a]
	$N_{45}P_{45}K_{45}$	8.2 [bc]	14.6 [b]	23.9 [b]	36.7 [a]
	$N_{60}P_{60}K_{60}$	8.4 [c]	15.1 [c]	24.6 [c]	38.3 [b]
	$N_{60}P_{90}K_{90}$	8.7 [d]	15.4 [d]	24.9 [c]	39.9 [c]
White melilot + maize	without fertilizer	8.8 [d]	18.5 [hi]	30.8 [h]	45.6 [g]
	$N_{45}P_{45}K_{45}$	9.9 [f]	20.2 [k]	32.4 [k]	48.1 [ij]
	$N_{60}P_{60}K_{60}$	10.7 [h]	20.9 [ł]	33.4 [m]	49.1 [kł]
	$N_{60}P_{90}K_{90}$	11.2 [i]	21.6 [m]	34.1 [n]	50.1 [m]
White melilot + millet	without fertilizer	8.1 [b]	16.2 [e]	26.5 [d]	41.9 [d]
	$N_{45}P_{45}K_{45}$	8.8 [d]	17.3 [f]	27.8 [e]	43.9 [e]
	$N_{60}P_{60}K_{60}$	9.3 [e]	17.9 [g]	28.7 [f]	44.7 [f]
	$N_{60}P_{90}K_{90}$	9.8 [f]	18.4 [h]	29.1 [g]	45.7 [g]
White melilot + Sudan grass	without fertilizer	9.2 [e]	18.7 [l]	30.2 [g]	47.2 [h]
	$N_{45}P_{45}K_{45}$	9.9 [f]	19.9 [j]	31.4 [i]	49.7 [łm]
	$N_{60}P_{60}K_{60}$	10.6 [h]	20.6 [l]	32.2 [jk]	51.2 [m]
	$N_{60}P_{90}K_{90}$	11.1 [i]	20.9 [ł]	33.0 [ł]	52.3 [n]
White melilot + sorghum	without fertilizer	10.1 [g]	18.5 [hi]	30.2 [g]	45.8 [g]
	$N_{45}P_{45}K_{45}$	11.0 [i]	19.7 [j]	31.3 [i]	47.6 [hi]
	$N_{60}P_{60}K_{60}$	11.4 [j]	20.3 [k]	32.0 [j]	48.4 [ik]
	$N_{60}P_{90}K_{90}$	12.1 [k]	20.7 [lł]	32.5 [k]	49.2 [lł]

Identical letter designations in the column (for a set fertilization level and mix) denote the lack of a statistically significant difference between them

Table 2. The dynamics of leaf surface area formation (thous. m^2 ha^{-1}) in *Melilotus albus* cultivated in pure sowing and in mixes, with varied fertilization rates and the seeding rate of 18 kg ha^{-1} (average for 2015–2017)

Mix component	Fertilizers	Days after germination			
		30	40	50	64 (mowing)
White melilot	without fertilizer	7.5 [a]	13.5 [a]	22.5 [a]	33.5 [a]
	$N_{45}P_{45}K_{45}$	7.9 [b]	14.1 [b]	23.2 [b]	36.1 [b]
	$N_{60}P_{60}K_{60}$	8.2 [c]	14.6 [c]	24.1 [c]	37.6 [c]
	$N_{60}P_{90}K_{90}$	8.3 [cd]	15.1 [d]	24.4 [d]	39.4 [d]
White melilot + maize	without fertilizer	8.5 [d]	18.1 [h]	30.4 [j]	45.7 [i]
	$N_{45}P_{45}K_{45}$	9.5 [g]	19.7 [j]	32.1 [ł]	48.1 [kl]
	$N_{60}P_{60}K_{60}$	10.4 [ij]	20.3 [l]	33.0 [n]	49.0 [ł]
	$N_{60}P_{90}K_{90}$	11.0 [i]	21.2 [m]	33.6 [o]	50.3 [m]
White melilot + millet	without fertilizer	7.9 [b]	15.7 [e]	26.1 [e]	41.2 [e]
	$N_{45}P_{45}K_{45}$	8.5 [d]	17.0 [f]	27.2 [f]	43.2 [f]
	$N_{60}P_{60}K_{60}$	9.1 [f]	17.4 [g]	28.3 [g]	44.2 [g]
	$N_{60}P_{90}K_{90}$	9.4 [g]	18.1 [h]	29.0 [h]	45.0 [h]
White melilot + Sudan grass	without fertilizer	8.8 [e]	18.2 [h]	29.7 [i]	46.6 [j]
	$N_{45}P_{45}K_{45}$	9.5 [g]	19.3 [i]	31.1 [k]	49.2 [ł]
	$N_{60}P_{60}K_{60}$	10.2 [i]	20.1 [kl]	31.7 [l]	50.5 [m]
	$N_{60}P_{90}K_{90}$	10.7 [k]	20.4 [ł]	32.6 [m]	51.6 [n]
White melilot + sorghum	without fertilizer	9.8 [h]	18.1 [h]	29.7 [i]	45.1 [h]
	$N_{45}P_{45}K_{45}$	10.6 [jk]	19.2 [i]	31.0 [k]	46.8 [j]
	$N_{60}P_{60}K_{60}$	11.1 [l]	20.0 [k]	31.7 [l]	47.7 [k]
	$N_{60}P_{90}K_{90}$	11.5 [ł]	20.3 [l]	32.1 [ł]	48.5 [l]

Explanations as in Table 1

Table 3. The dynamics of leaf surface area formation (thous. m^2 ha^{-1}) in *Melilotus albus* cultivated in pure sowing and in mixes, with varied fertilization rates and the seeding rate of 20 kg ha^{-1} (average for 2015–2017)

Mix component	Fertilizers	Days after germination			
		30	40	50	64 (mowing)
White melilot	without fertilizer	7.4 [a]	13.1 [a]	22.2 [a]	33.1 [a]
	$N_{45}P_{45}K_{45}$	7.7 [b]	13.8 [b]	22.8 [b]	35.6 [b]
	$N_{60}P_{60}K_{60}$	8.0 [c]	14.6 [c]	23.7 [c]	37.2 [c]
	$N_{60}P_{90}K_{90}$	8.1 [c]	14.9 [d]	24.1 [d]	39.1 [d]
White melilot + maize	without fertilizer	8.2 [c]	17.8 [hi]	30.2 [j]	45.3 [i]
	$N_{45}P_{45}K_{45}$	9.1 [e]	19.2 [j]	31.6 [l]	47.7 [kl]
	$N_{60}P_{60}K_{60}$	10.1 [gh]	20.0 [ł]	32.2 [m]	48.4 [łm]
	$N_{60}P_{90}K_{90}$	10.7 [i]	20.8 [m]	33.1 [n]	50.1 [o]
White melilot + millet	without fertilizer	7.7 [b]	15.3 [e]	25.7 [e]	40.7 [e]
	$N_{45}P_{45}K_{45}$	8.2 [c]	16.7 [f]	27.0 [f]	42.6 [f]
	$N_{60}P_{60}K_{60}$	8.7 [d]	17.1 [g]	28.1 [g]	43.9 [g]
	$N_{60}P_{90}K_{90}$	9.1 [e]	17.7 [h]	28.5 [h]	44.7 [kl]
White melilot + Sudan grass	without fertilizer	8.5 [d]	18.0 [i]	29.3 [i]	46.1 [j]
	$N_{45}P_{45}K_{45}$	9.1 [e]	19.1 [j]	30.7 [k]	48.9 [mn]
	$N_{60}P_{60}K_{60}$	10.0 [g]	19.7 [l]	31.2 [l]	50.1 [n]
	$N_{60}P_{90}K_{90}$	10.3 [h]	20.1 [ł]	32.3 [m]	51.2 [o]
White melilot + sorghum	without fertilizer	9.4 [f]	17.7 [h]	29.2 [i]	44.6 [h]
	$N_{45}P_{45}K_{45}$	10.2 [gh]	19.0 [j]	30.6 [k]	46.2 [j]
	$N_{60}P_{60}K_{60}$	10.7 [i]	19.5 [k]	31.1 [l]	47.3 [k]
	$N_{60}P_{90}K_{90}$	11.1 [j]	20.1 [ł]	31.8 [ł]	48.1 [lk]

Explanations as in Table 1

Table 4. The dynamics of leaf surface area formation (thous. m^2 ha^{-1}) in *Melilotus albus* cultivated in pure sowing and in mixes, with varied fertilization rates and the seeding rate of 22 kg ha^{-1} (average for 2015–2017)

Mix component	Fertilizers	Days after germination			
		30	40	50	64 (mowing)
White melilot	without fertilizer	7.2 [a]	12.8 [a]	21.9 [a]	32.6 [a]
	$N_{45}P_{45}K_{45}$	7.6 [b]	13.4 [b]	22.5 [b]	35.1 [b]
	$N_{60}P_{60}K_{60}$	7.9 [c]	14.3 [c]	23.2 [c]	36.7 [c]
	$N_{60}P_{90}K_{90}$	8.0 [c]	14.5 [c]	24.0 [d]	38.3 [d]
White melilot + maize	without fertilizer	8.0 [c]	17.3 [f]	29.8 [j]	44.7 [i]
	$N_{45}P_{45}K_{45}$	8.8 [e]	19.2 [i]	31.2 [ł]	46.9 [j]
	$N_{60}P_{60}K_{60}$	9.7 [f]	19.8 [k]	31.7 [n]	48.1 [ł]
	$N_{60}P_{90}K_{90}$	10.4 [h]	20.4 [l]	32.7 [p]	49.3 [m]
White melilot + millet	without fertilizer	7.5 [b]	15.1 [d]	25.3 [e]	40.3 [e]
	$N_{45}P_{45}K_{45}$	8.0 [c]	16.3 [e]	26.6 [f]	42.2 [f]
	$N_{60}P_{60}K_{60}$	8.4 [d]	16.7 [e]	27.6 [g]	43.1 [g]
	$N_{60}P_{90}K_{90}$	8.9 [e]	17.3 [f]	28.1 [h]	44.1 [h]
White melilot + Sudan grass	without fertilizer	8.3 [d]	17.8 [g]	29.0 [i]	45.6 [j]
	$N_{45}P_{45}K_{45}$	9.0 [e]	18.7 [h]	30.2 [k]	48.1 [ł]
	$N_{60}P_{60}K_{60}$	9.7 [f]	19.3 [ij]	30.9 [l]	49.6 [m]
	$N_{60}P_{90}K_{90}$	10.1 [g]	19.6 [j]	32.0 [o]	50.7 [n]
White melilot + sorghum	without fertilizer	9.1 [e]	17.2 [f]	28.8 [i]	44.2 [h]
	$N_{45}P_{45}K_{45}$	10.0 [g]	18.7 [h]	30.2 [k]	46.0 [j]
	$N_{60}P_{60}K_{60}$	10.4 [h]	19.1 [i]	30.7 [l]	46.7 [k]
	$N_{60}P_{90}K_{90}$	10.8 [i]	19.8 [k]	31.3 [m]	47.5 [l]

Explanations as in Table 1

In treatments where the white melilot seeding rate was 16 kg ha^{-1}, the leaf surface area increased by 5.8–13.0 thousand m^2 ha^{-1} in mixed treatments in the period of mowing readiness. In comparison with the control treatment, fertilization increased the surface area by 7–16% (Tab. 1). On average, during the three years of research, the largest leaf surface area in the mowing period was recorded in the mixed treatment of white melilot with Sudan grass (52.3 thousand m^2 ha^{-1}), in conditions of the highest level of fertilization. A somewhat smaller leaf surface area was observed in the grass mixture of white melilot with maize: 50.1 thousands m^2 ha^{-1}. High indices were also observed in the case of mineral nutrition at the level of $N_{60}P_{90}K_{90}$, where, in a mixed treatment of white melilot with maize, the leaf surface area before mowing was 49.2 thousand m^2 ha^{-1}, and with millet it was 45.7 thousand m^2 ha^{-1}. A positive effect of fertilization in *Melilotus albus* crops was confirmed, among other studies, by Timoshkin et. al [2013] who investigated the photosynthetic activity of leguminous grasses with the use of microfertilizers and biostimulators in 2011–2012, on the Middle Volga black earth, and found that the leaf surface area of white melilot was 64.5 thousand m^2 ha^{-1} in the first year of life, and 94.3 thousand m^2 ha^{-1} in the second year, with the use of Micromak and Microel microfertilizers, and the net photosynthesis productivity was 2.34 and 4.67 g m^2 day^{-1}.

The effect of the fertilization levels used was also similar in treatments where the seeding rate of white melilot seeds was 18 kg ha^{-1} (Tab. 2), 20 kg ha^{-1} (Tab. 3) and 22 kg ha^{-1} (Tab. 4). However, with the increasing number of seeds sown per hectare, the leaf surface area of the species under study was decreasing. Even when the highest level of fertilization was used, increasing the seeding rate to 22 kg ha^{-1} caused a reduction of the white melilot leaf surface area by 6–11% on the 30th day of the measurements, by 5–7% on the 40th day, by 3–6% on the 50th day after the seedling emergence, and by 2–5% on the harvest day – in comparison to treatments with a seeding rate of 16 kg ha^{-1}.

Assessing the influence of components used in the mixes with *Melilotus albus* on the growth dynamics of its leaves depending on the measurement date (regardless of fertilization level and seeding rate), it was found that the surface area was significantly the smallest in pure sowing treatments on each of the measurement dates (Fig. 1). Similar observations were made by Szmigiel and Oleksy [2004] when studying the leaf surface area in cereal plants. One of the cultivars sown in mixes had a significantly greater leaf surface area than single-species treatments. Also in studies by Woźniak [2008], the leaf surface area of wheat, expressed as LAI, was the smallest in pure treatments. In his studies, Smirnova et al. [2013] also demonstrated a significantly higher index of photosynthetic activity of white melilot (measured by the surface of its leaves) in a treatment with barley than in a pure treatment. On the first measurement date (30 days after seedling emergence), *Melilotus albus* plants in a treatment with sorghum had significantly the highest area. No significant differences in the leaf area of white melilot were found only between treatments where maize and Sudan grass were the other components in the seed mix. On the second measurement date (40 days after the emergence date), significantly the largest leaves of the studied species were found in treatments with maize and, only slightly smaller, in treatments with Sudan grass. The measurement taken 50 days after the emergence date showed that significantly the largest surface of white melilot leaves occurred in treatments with maize as well. In that period, no significant differences were found only between leaves in white melilot + sudan grass and white melilot + sorghum treatments. In the period of readiness for mowing (harvesting day), the significantly largest leaf area was observed in mixed treatments of white melilot and Sudan grass. No significant correlations were found only in white melilot + maize and white melilot + sorghum treatments. Varied responses of white melilot to the accompanying species in the mix were also demonstrated by Kuraszkiewicz and Pałys [2002] who studied the influence of four cereal species that were sown together with white melilot, i.e. winter rye, winter triticale, spring barley and oats, on the volume of the aboveground mass (leaves) of white melilot. The largest biomass of this species was obtained in the mix with triticale while the smallest – in the mix with barley.

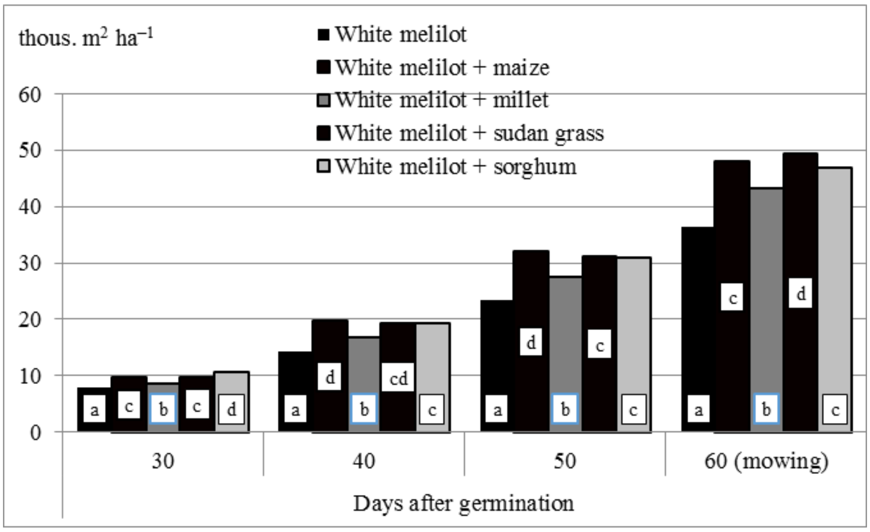

Fig. 1. Mean leaf surface area (thous. m^2 ha^{-1}) *Melilotus albus* depending on the mix and measurement date (regardless of fertilization level and seeding rate kg ha^{-1}, average for 2015–2017)

When assessing the photosynthetic activity of *Melilotus albus* based on the leaf surface area, depending on the mix and seeding rate, it was found that the lowest values were obtained in pure treatments with the highest seeding rate (22 kg ha^{-1}). Similar values were also obtained in pure treatments with a seeding rate of 20 and then 18 kg ha^{-1}. The significantly largest leaf area was observed in treatments where *Melilotus albus* was sown in the amount of 16 kg ha^{-1}, in mixes with Sudan grass and with maize. Similar (only slightly smaller) values were obtained in treatments where the seeding rate applied was 18 kg ha^{-1}, also in mixes with maize and then with Sudan grass (Fig. 2). Among all the components, millet showed the highest negative effect on the surface area of *Melilotus albus* leaves. Such a condition was observed for all seeding rates of the species under study (Fig. 2).

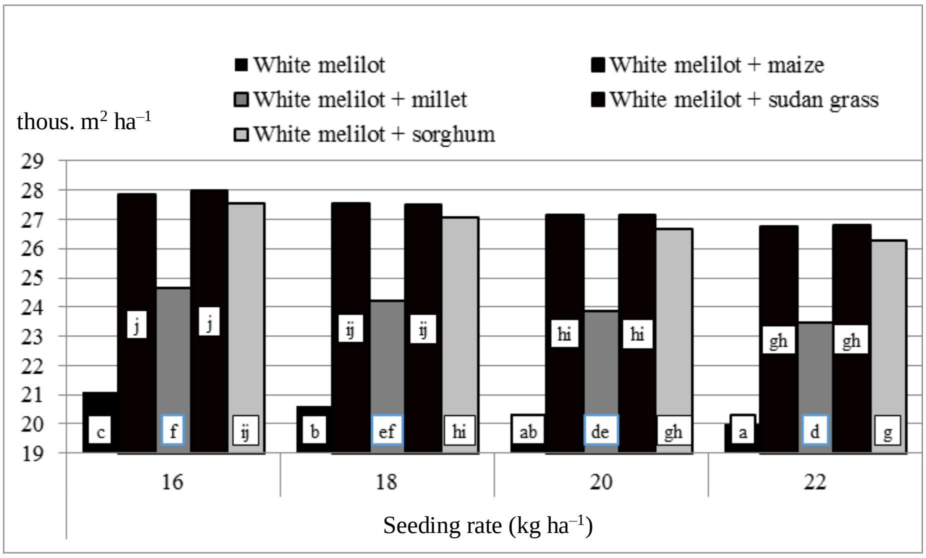

Fig. 2. Mean leaf surface area (thous. m^2 ha^{-1}) M*elilotus albus* depending on the mix and seeding rate kg ha^{-1} (regardless of fertilization level and measurement date, average for 2015–2017)

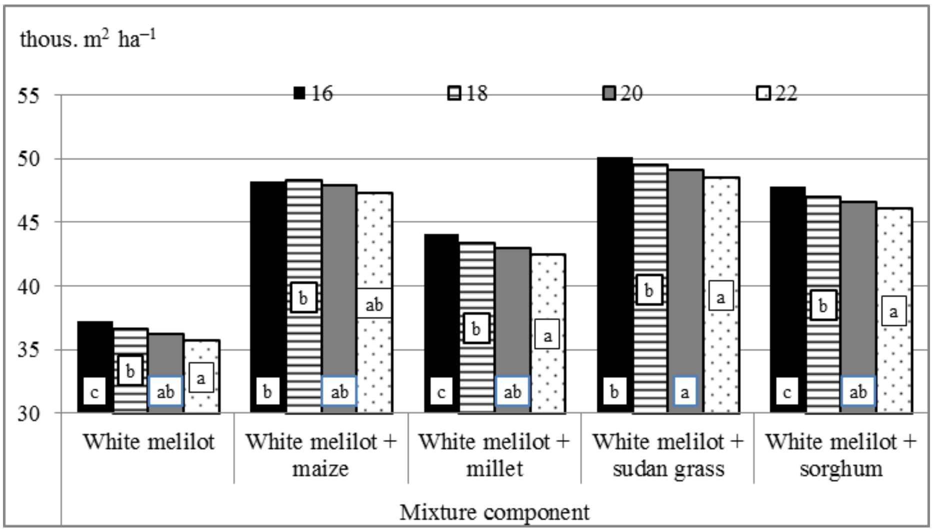

Fig. 3. Mean leaf surface area (thous. m^2 ha^{-1}) of white melilot 60 days after the sowing (harvesting day), in a pure treatment and in a mix with another species, with varying seeding rates kg ha^{-1} (regardless of fertilization level, average for 2015–2017)

In the studies, the number of seeds sown per hectare was found to have a significant influence on the surface area of *Melilotus albus* leaves. The highest values of this parameter were observed with a seeding rate of 16 kg ha^{-1}. According to literature data [Dąbrowska-Żądło 2017], the recommended seeding rates are circa 15–20 kg ha^{-1} in crops for green fodder. Regardless of the fertilization level and measurement date, the leaf surface area of the studied species was decreasing with the increasing seeding rate, with the exception of mixes of *Melilotus albus* with maize, where no significant differences were observed between the seeding rates used. The leaf surface area of *Melilotus albus* sown in a mix with Sudan grass in treatments with a seeding rate of 16 and 18 kg ha^{-1} did not vary significantly although these values were significantly higher than in treatments with a seeding rate of 20 and 22 kg ha^{-1} (Fig. 3).

CONCLUSIONS

1. In the experiments, it was established that the dynamics of leaf surface area varied depending on the studied factors.

2. Thus, the use of mineral fertilizers contributed to a 7–16% increase in the leaf surface area of white melilot, while an increase in the sowing rate reduced this index by 2–11%.

3. The surface area of white melilot leaves was the smallest in pure treatments, whereas millet was the component that had the strongest negative effect on this parameter.

4. Taking into account the average values in the research period, it can be concluded that the largest surface area of white melilot leaves (52.3 thousand m^2 ha^{-1}) was observed in mixed treatments with Sudan grass where white melilot was sown at a seeding rate of 16 kg ha^{-1} and the fertilization level applied was $N_{60}P_{90}K_{90}$.

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Streszczenie. Celem badań była analiza kształtowania się powierzchni liści nostrzyka białego (*Melilotus albus*) uprawianego w siewie czystym i w mieszankach z jednorocznymi zbożami, w warunkach zróżnicowanych ilości wysiewu tego gatunku oraz dawek nawożenia. Nostrzyk biały wysiano w siewie czystym oraz w mieszankach z kukurydzą, prosem zwyczajnym, trawą sudańską i sorgiem, stosując 4 normy wysiewu nasion (16, 18, 20 i 22 kg · ha^{-1}). W badaniach zastosowano 4 dawki nawożenia NPK (0 – kontrola bez nawożenia, $N_{45}P_{45}K_{45}$, $N_{60}P_{60}K_{60}$ i $N_{60}P_{90}K_{90}$). Powierzchnię liści oceniano metodą skanowania w 30., 40. i 50. dniu po wykiełkowaniu nostrzyka oraz w okresie jego gotowości do koszenia (w 64. dniu). Liście nostrzyka oddzielano od łodyg i umieszczano w przezroczystej teczce z naklejonym 25-centymetrowym kwadratem do kalibracji, następnie skanowano płaskim skanerem w trybie czarno-białym. Powstały obraz analizowano w programie Areas, w którym określono powierzchnię zeskanowanych liści za pomocą wbudowanych narzędzi analitycznych. Po określeniu powierzchni liści każdej rośliny obliczono średnią dla każdego wariantu doświadczenia, a następnie, wykorzystując zagęszczenie roślin na 1 m^2, wyliczono średnią powierzchnię liści *M. albus* na hektar. W badaniach wykazano, że powierzchnia liści nostrzyka białego była zróżnicowana w zależności od współkomponentu w mieszance, ilości wysiewu nasion tego gatunku oraz dawek nawożenia. Średnia powierzchnia liści jednej rośliny w zależności od ilości wysiewu (16, 18, 20 i 22 kg · ha^{-1}) wynosiła odpowiednio: 0,014115, 0,013955, 0,013824 i 0,013654 m^2. W przeliczeniu na hektar najmniejszą powierzchnię liści *Melilotus albus* notowano na obiektach bez udziału współkomponentów (siew czysty) przy najwyższej zastosowanej normie wysiewu nasion tego gatunku. Istotnie największą powierzchnię liści otrzymano na obiektach, gdzie *Melilotus albus* wysiano w ilości 16 kg · ha^{-1} w mieszance z trawą sudańską oraz z kukurydzą. Spośród współkomponentów największy ujemny wpływ na ten parametr wykazywało proso, w warunkach każdej z zastosowanej ilości wysiewu nasion *M. albus*. Z reguły wraz z rosnącą ilością wysiewu powierzchnia liści badanego gatunku zmniejszała się sukcesywnie od 2 do 11%. Natomiast wprowadzenie nawozów mineralnych przyczyniło się do zwiększenia powierzchni liści badanego gatunku (od 7 do 16%) wraz ze wzrostem ich dawek. Biorąc pod uwagę średnie wartości za okres badań, największą powierzchnię liści nostrzyka białego w okresie dojrzałości kośnej odnotowano w siewie mieszanym: nostrzyk biały + trawa sudańska, tj. 52,3 tys. m^2 · ha^{-1}, przy nawożeniu $N_{60}P_{90}K_{90}$ i ilości wysiewu 16 kg · ha^{-1}.

Słowa kluczowe: powierzchnia liści, *Melilotus albus*, siew czysty, siewy mieszane, kukurydza, proso, trawa sudańska, sorgo, nawożenie, gęstość siewu

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