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The sources of nitrogen for yellow lupine and spring triticale in their intercropping

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Abstract: The aim of the study was to evaluate the amount of nitrogen taken up from air, mineral fertilizers and soil by yellow lupine (*Lupinus luteus* L.) and spring triticale (*Triticosecale* Wittm. ex A. Camus) in their intercropping cultivation. The factor examined in the experiment was percentage of yellow lupine seeds and spring triticale grain in sown mixtures: 100/0, 75/25, 50/50, 25/75 and 0/100, respectively. Yellow lupine yield was reduced by spring triticale when intercropped. In the yield of both plants the percentage share of spring triticale grain was larger and that of yellow lupine seed was smaller than in the sown mixture. Land equivalent ratio informs that yield advantage under intercropping of lupine and triticale was minor. The total protein yield was the highest in lupine cultivation when sown pure, and decreased with increasing share of triticale in their intercropping. The mean percentage nitrogen share from N_2 fixation, fertilizer and soil reserves in yellow lupine mass was: 65.2, 8.8 and 26.0%, respectively, while in the mass of spring triticale it was 10.1, 23.5 and 68.8%, respectively.

Keywords: nitrogen fixation; isotope ^{15}N; legumes; cereals; seed rate

The benefits of growing leguminous plants in monocultures or intercropping systems should be considered both in terms of obtaining seeds with high protein content, but also in agrotechnical terms – leaving a favourable position in crop rotation for successive plants (Wysokiński et al. 2014, Gałęzewski et al. 2017, Szymańska et al. 2017). Nitrogen accumulated in their post-harvest residues is usually introduced into the soil and can be used by successive plants – often cereals (Wysokiński et al. 2014). These two plant species are also grown in mixed sowing (Eskandari et al. 2009, Mousavi and Eskandari 2011, Kaci et al. 2018). The predominance of this plant production system in comparison with pure cropping may result from the interaction between components in intercrops and the difference in competition for the use of environmental resources (Mahapatra 2011, Duchene et al. 2017, Franco et al. 2018), reducing damage caused by pests, diseases and weeds (Banik et al. 2006). Legumes live in symbiosis with diazotrophic bacteria reducing molecular nitrogen to forms available to the host plant (Liu et al. 2011, Księżak et al. 2018). When growing plants from the Leguminosae family with cereals, it is important to determine the amount of atmospheric nitrogen fixation that can be made available to the cereal component by excretion from the legume component (Fujita et al. 1992, Corre-Hellou et al. 2006, Hauggaard-Nielsen et al. 2009). This information can be used to precisely determine the nitrogen fertilization of mixed crops of these two species, taking into account their different share in the field.

Interesting components of legume-cereal mixtures may include yellow lupine and spring triticale, because when compared to other plants, they have low soil requirements and can be grown in poor soil (Podleśny and Podleśna 2016, Gałęzewski et al. 2017, Księżak et al. 2018). In addition, yellow lupine seeds contain high amounts of protein (even more than 40%), which is the reason they are of great interest to fodder producers (Lošák 2007).

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Bearing in mind the foregoing considerations, the aim of the study was to determine the uptake of nitrogen from air, mineral fertilizers and soil reserves by yellow lupine and spring triticale in their intercropping cultivation system with their different share in the sown mixtures of seeds and grain, calculated by isotopic dilution method and with ^{15}N used in the experiment.

MATERIAL AND METHODS

Field experiment was carried out in 2015–2017 in eastern Poland, on a farm in Golaszyn, near the Siedlce city (51°97'N, 22°35'E). This experiment was conducted on a slightly acidic soil with the granulometric composition of loamy sand (Table 1). Plots with an area of 1 m^2 were marked out in a growing crop of yellow lupine and spring triticale mixed-intercropping in a traditional soil cultivation system. The experiment had a split-plot arrangement with three replications and Mister and Milewo cultivars of lupine and triticale were cultivated, respectively. The factor examined in the experiment was the percentage share of sprouting yellow lupine seeds and spring triticale grain in sown mixtures as follows: 100/0 – only lupine 100 seeds per m^2; 75/25 – 75 lupine seeds and 125 triticale grain per m^2; 50/50 – 50 lupine seeds and 250 triticale grain per m^2; 25/75 – 25 lupine seeds and 375 triticale grain per m^2; 0/100 – only triticale 500 seeds per m^2. The preceding crop in each year of study included long-term cereal monoculture. Mineral nitrogen was introduced into the soil at a dose of 30 kg N/ha in the form of $(NH_4)_2SO_4$ with ^{15}N excess of 5%. P and K were applied at doses of 40 kg/ha (triple superphosphate) and 90 kg/ha (KCl). Seed sowing was carried out in the 1st decade of April in the amounts specified above. The counted amount of seeds and grains was mixed and sown by hand in 6 rows on 1 m^2. Weeds were removed manually. At full maturity growth stage the plants were harvested manually from all 1 m^2 plots by digging them up from soil using a spade, to the depth of 0.25 m. The plants grown in mixed intercropping were separated into lupine and triticale. Both plants species from all treatments were counted and afterwards they were separated into the roots, crop residues and seeds/grains, respectively. In each obtained plant sample, the following values were determined: dry matter yield (DM in 105°C), nitrogen content (Kjeldahl method) and ^{15}N excess (NOI-6e emission spectrometer, Carl Zeiss, Jena, Germany).

Table 1. Selected properties of sandy soil

Parameter	Unit	2015	2016	2017
$pH_{1\ mol\ KCl/L}$	–	6.1	6.0	6.0
C_{tot}	(g/kg)	12.6	13.1	12.5
N_{tot}	(g/kg)	1.10	1.09	1.15
P_{Egner}	(mg/kg)	45.2	47.0	45.9
K_{Egner}	(mg/kg)	83.4	86.3	89.4

The amount of nitrogen taken up by yellow lupine and spring triticale from the air (by triticale from lupine), fertilizer and soil reserves were calculated according to the formulas provided by Kalembasa (1995).

In addition, the land equivalent ratio (LER) was calculated according to the formulas given by Willey (1979).

The results of the experiments were analysed by ANOVA. The significance of sources of variation was checked with the Fisher-Snedecor test, and the mean values were separated with the Tukey's test at the significance level of $P \leq 0.05$. For these calculations, the Statistica 12 PL (StatSoft, Tulsa, USA) was used.

The growing seasons in 2015–2017 were average for the growth, development and yielding of yellow lupine and spring triticale (Figure 1). The total amount of precipitation was satisfactory to meet the plants' needs, however it was not properly distributed in particular months. The least favourable year was 2016 in which, besides a significant water deficit during the intensive growth of the tested plants (May–June), slightly higher temperatures than those in 2015 and 2017 were additionally noted during the growing season.

RESULTS AND DISCUSSION

Yellow lupine and spring triticale mass and protein yield. Table 2 indicates that the harvested spring triticale crop in pure sowing (0/100) was similar to the total of lupine seeds and triticale grain harvest in intercropping at proportions of 25/75 and 50/50. Increasing the share of lupine and reducing the amount of triticale in their intercropping to the ratio of 75/25 caused reduction in the amount of harvested seeds and grains. The yield of lupine seeds cultivated in pure sowing (100/0) was lower compared to the sum of the seed and grain masses in all variants of its intercropping with triticale. The

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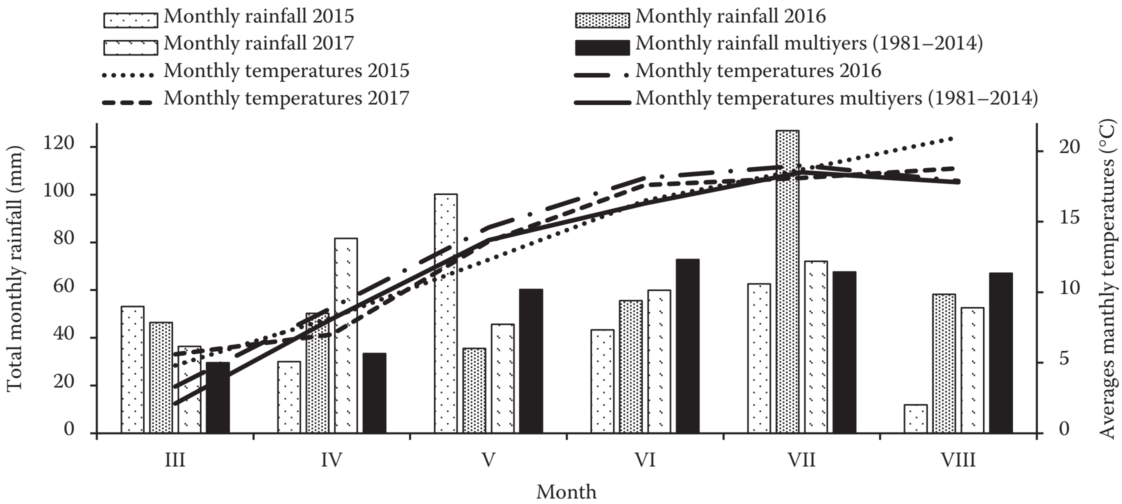

Figure 1. Rainfall and air temperatures in Siedlce, Institute of Meteorology and Water Management, National Research Institute, Warszawa

variation in the amount of total mass of both studied plants was subject to such dependences as in the case of seeds and grains. In comparison with them, the variation in the content and yield of protein were the opposite (Table 3). The smallest content and yield of protein were obtained in the triticale grain yield in pure sowing. While increasing the amount of lupine seeds in the sown mixture (from 25% to 75%), until its cultivation in pure sowing, there was a significant increase in the protein content and yield in the harvest. The calculated LER coefficient shows that in case of growing lupine and triticale at a 75/25 ratio, there was practically no yield advantage obtained compared to their cultivation in pure sowing (Table 4), as compared to the cultivation of lupine and triticale at 50/50 and 25/75 variants, where the obtained benefits from intercropping were minor. Many authors reported the limiting effect of cereals on legumes yield in intercropping with cereals and the predominance of the grain component (Gałęzewski 2010, Podleśny and Podleśna 2016, Księżak et al. 2018). The lower yields of yellow lupine mixtures with various spring cereals obtained as a result of increasing the share of lupine seeds in the sown mixture were reported by Księżak et al. (2018). In addition, they obtained higher content of total protein in the grain of cereals grown in mixtures with lupine. According to Podleśny and Podleśna (2016), as well as Księżak et al. (2018), decreasing yellow lupine share and increasing triticale share in the sown mixture causes reduction in the number of pods per plant, in the number of seeds per pod and per plant, as well as the weight of thousand lupine seeds weight. Gałęzewski et al. (2017), however,

Table 2. Number of plants and dry mass yield of yellow lupine and spring triticale

Treatment		Lupine					Triticale					Sum	
		number of plants m^2	mass (t/ha)				number of plants m^2	mass (t/ha)				seed and grain	total mass
			root	crop residue	seed	total		root	crop residue	grain	total		
Percentage seeds/grain	100/0	76.9	0.66^d	3.63^d	1.94^d	6.23^d	–	–	–	–	–	1.94^a	6.23^a
	75/25	53.5	0.41^c	2.31^c	1.19^c	3.91^c	105.9	0.49^a	1.45^a	1.12^a	3.06^a	2.31^b	6.97^b
	50/50	33.7	0.30^b	1.69^b	0.87^b	2.86^b	185.2	0.67^b	2.26^b	1.80^b	4.73^b	2.67^c	7.59^c
	25/75	17.3	0.15^a	0.74^a	0.43^a	1.32^a	244.4	0.97^c	3.06^c	2.35^c	6.38^c	2.78^c	7.70^c
	0/100	–	–	–	–	–	361.7	1.23^d	3.60^d	2.87^d	7.70^d	2.87^c	7.70^c
Year	2015	49.6	0.42^b	2.23^a	1.24^b	3.89^b	237.9	0.94^b	2.78^b	2.23^b	5.95^b	2.78^b	7.87^b
	2016	43.8	0.36^a	2.05^a	1.07^{ab}	3.48^{ab}	217.4	0.80^a	2.55^{ab}	1.98^a	5.33^a	2.44^a	7.04^a
	2017	42.7	0.35^a	2.00^a	1.01^a	3.37^a	217.6	0.78^a	2.46^a	1.89^a	5.13^a	2.33^a	6.81^a

^{a,b,c,d}Averages with different letters in the columns are significantly different ($P \leq 0.05$)

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Table 3. The content and the yield of protein in yellow lupine seeds and spring triticale grain

Treatment		Protein content in seeds and grain (%)			Protein yield (kg/ha)		
		lupine	triticale	means in mixture	lupine	triticale	sum for mixture
Percentage seeds/grain	100/0	39.8^{a}	–	39.8^{e}	772.7^{c}	–	772.7^{d}
	75/25	38.4^{a}	14.0^{c}	26.6^{d}	457.2^{b}	156.4^{a}	613.6^{c}
	50/50	39.3^{a}	13.4^{bc}	21.9^{c}	342.0^{b}	241.2^{b}	583.2^{bc}
	25/75	39.2^{a}	12.7^{ab}	16.8^{b}	169.3^{a}	298.5^{c}	467.8^{ab}
	0/100	–	12.5^{a}	12.5^{a}	–	358.2^{d}	358.2^{a}
Year	2015	38.8^{a}	13.0^{a}	23.3^{a}	483.1^{a}	286.8^{b}	615.1^{b}
	2016	40.0^{a}	13.6^{b}	24.2^{a}	429.2^{a}	263.9^{ab}	554.4^{ab}
	2017	38.7^{a}	12.9^{a}	23.1^{a}	393.6^{a}	240.7^{a}	507.4^{a}

^{a,b,c,d}Averages with different letters in the columns are significantly different ($P \leq 0.05$)

indicate a smaller negative effect of spring triticale on yellow lupine in their strip intercropping, compared to the effect of oat. This negative impact was limited only to the first immediately adjacent row. At the same time, the authors state that the neighbourhood of yellow lupine was beneficial for spring triticale. This beneficial effect of lupine on spring triticale was also noted in this study. Increasing the yield of this cereal in intercropping with lupine at variants of 25/75, 50/50 and 75/25 exceeds 8.9, 25.0 and 55.5% share of this plant in the sown seed and grain mixture, respectively.

The highest yield of seeds and grain, the total mass of both plants (Table 2), protein yield (Table 3) and LER coefficient (Table 4) were obtained in the first year of experiment (2015). The amount of precipitation was the smallest in April and the highest in May in this year in comparison to the same months in the other years of the study.

Nitrogen sources for yellow lupine and spring triticale (Table 5). The largest total amount of nitrogen was taken up by yellow lupine (215.3 kg/ha), the smallest by spring triticale (93.8 kg/ha), both grown in pure sowing. The greater the share of triticale and the smaller the share of lupine in the sown mixture, the smaller the total amount of nitrogen – sum for both – taken up by test plants. The percentage of nitrogen taken up by lupine from the atmosphere in total uptake ranged from 63.0% to 67.5%. In quantitative terms, most nitrogen from the atmosphere was taken up by lupine grown in pure sowing (135.4 kg/ha). As a result of decreasing the lupine share while increasing the amount of triticale in the sown mixture, the amount of nitrogen taken up by lupine from this source in the area of 1 hectare decreased. In comparison to sole cropping of lupine and combinations of 75/25, 50/50 and 25/75 this plant took up less nitrogen from the air, by 40.4, 55.2 and 79.1%, respectively. The percentage of nitrogen from the fixation process by lupine in the total mass of triticale cultivated in treatments 75/25, 50/50 and 25/75 was 15.2, 9.7 and 5.5%, respectively. The amount of nitrogen taken up by cereal from this source was similar to all combinations: 75/25, 50/50 and 25/75 – 6.52, 6.16 and 4.38 kg/ha, respectively. The small amount of nitrogen from nitrogen fixation by lupine in triticale and its small variation in all intercropping combinations resulted in the variability of the sum of nitrogen taken by both test plants from the atmosphere within these treatments subjected to the same dependencies as in the case of lupine. In total, both plants growing in combinations 75/25, 50/50 and 25/75 took up 87.2, 71.1 and 34.7 kg N/ha from air. For the listed treatments, the share of nitrogen from this source in the total uptake by both plants was 50.9, 44.3 and 27.8%, respectively.

Other studies have shown a significant direct transfer of fixed N_2 to the associated non-legume species (Stern 1993, Elgersma et al. 2000). Increase

Table 4. Land equivalent ratio for seeds, grain and total mass of yellow lupine and spring triticale

Treatment		Sum seeds and grain	Total mass both plants
Percentage seeds/grain	75/25	1.00	1.02
	50/50	1.07	1.07
	25/75	1.04	1.04
Year	2015	1.15	1.14
	2016	1.00	1.01
	2017	0.96	0.98

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Table 5. Nitrogen (N) uptake from different sources by yellow lupine and spring triticale (kg N/ha)

Treatment		Lupine				Triticale				Sum of both plants	
		roots	crop residues	seeds	sum	roots	crop residues	grain	sum	seeds and grain	total mass
Total uptake											
Percentage seeds/grain	100/0	13.8^{c}	77.9^{d}	123.6^{c}	215.3^{d}	–	–	–	–	123.6^{d}	215.3^{d}
	75/25	7.9^{b}	47.4^{c}	73.1^{b}	128.4^{c}	4.1^{a}	13.9^{a}	25.0^{a}	43.0^{a}	98.1^{c}	171.4^{c}
	50/50	6.3^{b}	35.5^{b}	54.7^{b}	96.4^{b}	5.5^{a}	20.2^{b}	38.6^{b}	64.3^{b}	93.3^{bc}	160.7^{c}
	25/75	3.0^{a}	15.0^{a}	27.1^{a}	45.1^{a}	7.5^{b}	24.7^{bc}	47.8^{c}	78.0^{c}	74.9^{ab}	125.1^{b}
	0/100	–	–	–	–	8.9^{b}	27.6^{c}	57.3^{d}	93.8^{d}	57.3^{a}	93.8^{a}
Year	2015	8.5^{b}	45.6^{a}	77.3^{a}	131.4^{b}	7.1^{a}	22.5^{a}	45.7^{b}	75.4^{b}	98.4^{b}	165.4^{b}
	2016	7.6^{ab}	44.9^{a}	68.7^{a}	121.2^{ab}	6.5^{a}	22.3^{a}	42.2^{ab}	71.0^{ab}	88.7^{ab}	153.8^{ab}
	2017	7.1^{a}	41.1^{a}	63.0^{a}	111.2^{a}	5.9^{a}	20.0^{a}	38.5^{a}	64.4^{a}	81.2^{a}	140.4^{a}
Uptake from air (by triticale from N fixation by lupine)											
Percentage seeds/grain	100/0	8.0^{d}	47.5^{c}	79.9^{c}	135.4^{d}	–	–	–	–	79.9^{c}	135.4^{d}
	75/25	4.6^{c}	29.2^{b}	46.9^{b}	80.7^{c}	0.5^{a}	2.1^{a}	3.9^{a}	6.5^{a}	50.8^{b}	87.2^{c}
	50/50	3.9^{b}	23.5^{b}	37.6^{b}	65.0^{b}	0.4^{a}	1.8^{a}	3.9^{a}	6.2^{a}	41.5^{b}	71.2^{b}
	25/75	1.9^{a}	10.0^{a}	18.4^{a}	30.3^{a}	0.3^{a}	1.4^{a}	2.7^{a}	4.4^{a}	21.1^{a}	34.7^{a}
Year	2015	5.0^{a}	28.5^{a}	50.3^{a}	83.7^{b}	0.4^{a}	1.7^{a}	3.9^{b}	6.0^{ab}	53.2^{b}	88.2^{b}
	2016	4.5^{a}	28.9^{a}	45.9^{a}	79.2^{ab}	0.3^{a}	2.6^{a}	4.7^{b}	7.6^{b}	49.4^{ab}	84.9^{b}
	2017	4.3^{a}	25.2^{a}	41.0^{a}	70.5^{a}	0.5^{a}	1.0^{a}	1.8^{a}	3.4^{a}	42.4^{a}	73.1^{a}
Uptake from fertilizer											
Percentage seeds/grain	100/0	1.5^{d}	7.5^{d}	11.2^{d}	20.2^{d}	–	–	–	–	11.2^{a}	20.2^{a}
	75/25	0.9^{c}	4.5^{c}	6.7^{c}	12.1^{c}	1.0^{a}	2.9^{a}	5.4^{a}	9.3^{a}	12.1^{ab}	21.4^{a}
	50/50	0.6^{b}	2.9^{b}	4.4^{b}	7.9^{b}	1.3^{a}	4.6^{b}	8.9^{b}	14.8^{b}	13.3^{ab}	22.7^{a}
	25/75	0.3^{a}	1.2^{a}	2.2^{a}	3.7^{a}	1.9^{b}	5.8^{c}	11.5^{c}	19.2^{c}	13.7^{ab}	22.9^{a}
	0/100	–	–	–	–	2.4^{c}	6.8^{c}	14.7^{d}	23.9^{d}	14.7^{b}	23.9^{a}
Year	2015	1.0^{b}	4.5^{a}	7.4^{b}	12.9^{b}	1.9^{b}	5.6^{b}	11.7^{b}	19.3^{b}	15.3^{b}	25.8^{b}
	2016	0.8^{a}	4.0^{a}	5.9^{ab}	10.7^{a}	1.6^{a}	5.1^{ab}	10.0^{a}	16.6^{a}	12.7^{a}	21.8^{a}
	2017	0.7^{a}	3.7^{a}	5.2^{a}	9.6^{a}	1.4^{a}	4.4^{a}	8.7^{a}	14.6^{a}	11.1^{a}	19.3^{a}
Uptake from soil											
Percentage seeds/grain	100/0	4.3^{c}	22.9^{d}	32.5^{d}	59.7^{d}	–	–	–	–	32.5^{a}	59.7^{a}
	75/25	2.4^{b}	13.7^{c}	19.5^{c}	35.6^{c}	2.7^{a}	8.9^{a}	15.7^{a}	27.3^{a}	35.2^{ab}	62.9^{a}
	50/50	1.8^{b}	8.9^{b}	12.8^{b}	23.5^{b}	3.7^{a}	13.8^{b}	25.8^{b}	43.3^{b}	38.6^{abc}	66.8^{a}
	25/75	0.9^{a}	3.7^{a}	6.4^{a}	11.0^{a}	5.3^{b}	17.6^{c}	33.5^{c}	56.4^{c}	39.9^{bc}	67.4^{a}
	0/100	–	–	–	–	6.6^{c}	20.7^{c}	42.7^{d}	70.0^{d}	42.7^{c}	70.0^{a}
Year	2015	2.5^{a}	12.6^{a}	19.6^{a}	34.7^{a}	4.9^{a}	15.6^{a}	31.0^{a}	51.6^{a}	40.5^{a}	69.0^{a}
	2016	2.4^{a}	12.0^{a}	16.9^{a}	31.3^{a}	4.7^{a}	15.3^{a}	28.7^{a}	48.7^{a}	36.5^{a}	64.0^{a}
	2017	2.1^{a}	12.2^{a}	16.8^{a}	31.0^{a}	4.0^{a}	14.8^{a}	28.4^{a}	47.3^{a}	36.2^{a}	62.7^{a}

^{a,b,c,d}Averages with different letters in the columns are significantly different ($P \leq 0.05$)

of nitrogen availability to the associated crop was probably an effect of mineralisation of decomposing legume roots in the soil (Evans et al. 2001, Torma et al. 2018). Competition between cereals and legumes for nitrogen may stimulate fixation activity in legumes (Fujita et al. 1990, Hardarson and Atkins 2003). The cereal component effectively drains the soil of nitrogen, forcing the legume to fix more N_2.

In total, in the roots and post-harvest residues of yellow lupine and spring triticale, which are most

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often left in the field after harvesting (seeds and grains), the following amounts of nitrogen from nitrogen fixation were introduced into the soil (kg/ha) in treatments 100/0, 75/25, 50/50 and 25/75: 55.44, 36.37, 29.67 and 13.56, respectively. Wysokiński (2013) and Wysokiński et al. (2014) achieved the use of nitrogen fixation, introduced into the soil with post-harvest residues of yellow lupine at an average level of 51.4%. Taking into account the value of this coefficient, it can be predicted that the succeeding plants will absorb: 28.50, 18.70, 15.25 and 6.97 kg of nitrogen derived from the nitrogen fixation process, respectively.

The amount of nitrogen taken up by lupine and triticale from soil reserves in variants 100/0, 75/25 and 50/50 was less than from atmosphere, while in variant 25/75 its dependence was inversed. In general, the total amount of nitrogen taken up from the soil by both test plants cultivated in monocropping and in intercropping in all combinations did not differ significantly. The percentage of nitrogen taken up by both plants from soil reserves in total uptake in combinations 100/0, 75/25, 50/50, 25/75 and 0/100 was: 27.6, 36.7, 41.6, 53.9 and 74.6%, respectively. In the foregoing studied combinations, the percentage of nitrogen taken up from fertilizer in total uptake was: 9.4, 12.5, 14.1, 18.4 and 25.4%, respectively. In terms of quantity, the amount of nitrogen taken up from the fertilizer in monocropping of lupine and triticale and in their intercropping in all combinations, was not significantly different ranging from 20.2 to 23.9 kg per hectare.

The highest uptake of nitrogen derived from the atmosphere and mineral fertilizer were obtained in plants cultivated in 2015. The amount of nitrogen taken up from soil reserves was not diversified significantly in the investigation years.

In conclusion, in total yield of intercropping, the percentage share of the spring triticale seed was higher and the share of yellow lupine seed was lower than in the sowing mixture. To achieve high protein yield, the share of yellow lupine in the sown mixture should be at least 50%.

In conditions of intercropping cultivation and low nitrogen fertilization (30 kg/ha), irrespective of percentage share in sown seed and grain mixture, the main source of this macronutrient was atmosphere for lupine (65.2% in total uptake of N), and soil reserves for triticale (68.8%). This cereal intercropped with lupine absorbed a small amount of fixed nitrogen excreted by the legume component (max 6.5 kg/ha).

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