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QUANTITATIVE ASSESSMENT OF THE PROCESS OF BIOLOGICAL NITROGEN REDUCTION BY YELLOW LUPINE (*Lupinus luteus* L.)*

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Abstract. This study aimed at estimating the amount of nitrogen derived by yellow lupine from the atmosphere, mineral fertilizer and soil at different nitrogen fertilization rates and developmental phases of this test plant. Yellow lupine was cultivated in three fertilization variants: without nitrogen fertilization and after application of 30 and 150 kg $N \cdot ha^{-1}$. Harvest was performed in flowering and full maturity. In the study ^{15}N nitrogen isotope was introduced into soil in the form of ammonium sulfate, and the method of isotopic dilution was used. The biomass yield of yellow lupine and the nitrogen content in its individual parts were not significantly dependent on the nitrogen rate. The amount of yellow lupine biomass harvested in full maturity was more than two times larger than in flowering. Nitrogen content in lupine harvested in flowering was higher than in full maturity. Differentiated fertilization with nitrogen, irrespective of the harvest time of lupine, did not have a significant effect on the total amount of taken up nitrogen, which was about two times higher in full maturity than in flowering. The amount of nitrogen derived from biological reduction in the biomass of yellow lupine harvested in flowering was smaller after application of the higher rate of this element, whereas in full maturity this relationship was opposite. The amount of nitrogen derived from the fertilizer increased along with increasing rate. Percentage of nitrogen derived from biological reduction in the biomass of yellow lupine was similar in flowering (53.4 %) and full maturity (51.6 %). Proportion of nitrogen derived from the fertilizer was larger in the 1st time of lupine harvest than in the 2nd time, whereas in the case of nitrogen derived from soil this relationship was opposite. Differentiated fertilization with nitrogen did not significantly affect the percentage of nitrogen derived from biological reduction in yellow lupine. Yellow lupine fertilized with the higher rate of nitrogen contained a higher percentage of this element derived from the fertilizer and lower from soil reserves than after application of the lower rate.

Key words: biologically reduced N_2, nitrogen, yellow lupine

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INTRODUCTION

Plant fertilization with nitrogen is always an effective yield-formation practice, in comparison with the application of other mineral nutrients. The proper level of fertilizing plants with this macroelement, at a rational utilization of mineral, natural and organic fertilizers, ensures obtaining yields desirable from the quantitative and qualitative point of view, and minimizing a negative effect on the environment [Gangbazo *et al*. 1995, Meng *et al*. 2005]. Apart from the use of natural and organic fertilizers, the most energy-efficient and effective form of nitrogen application, allowing a considerable reduction in the amount of this element introduced into soil in the form of mineral fertilizers, is the introduction of Fabaceae plants, living in symbiosis with bacteria of the genus *Rhizobium,* to crop rotation [Jasińska and Kotecki 2001, Hardason and Atkins 2003]. The ability of Fabaceae plants to fix atmospheric nitrogen in the process of biological reduction allows reduction of outlays related to nitrogen fertilization of both this group of plants and sequential crops [Kocoń 1999, Święcicki *et al*. 2007a]. Biological reduction of nitrogen is the most economical method for enriching soil in this nutrient for plants and an important factor in sustainable and organic agriculture [Peoples *et al*. 1995, Hardason and Atkins 2003].

When promoting the development of sustainable agriculture and consideration of the natural environment, the problems concerning an increase in nitrogen content in soils, particularly in mineral forms or easily subjected to mineralization, as well as a decrease in dispersing this biogenic element in the environment, are more frequently discussed [Gangbazo *et al*. 1995]. The amount of nitrogen from the process of biological reduction by Fabaceae plants is determined by many biological and anthropogenic factors, and its amount remaining in soil is dependent on the way of management of the obtained biomass. Particularly large amounts of this element are introduced into soil in the case of growing Fabaceae plants intended completely for green manure. Growing Fabaceae plants, nitrogen deriving from biological reduction should be taken into account in the balance of fertilization, to avoid an excessive accumulation of this element in soil and cultivated sequential crops.

The aim of this study was to estimate the amount of nitrogen biologically reduced by yellow lupine (*Lupinus luteus* L.) in the phase of flowering and full maturity of this plant.

MATERIALS AND METHODS

The field growing experiment was established in 2009 on land owned by University of Natural Sciences and Humanities in Siedlce (52°10' N; 22°17' E). In the experiment the traditional yellow lupine cultivar – Parys – was cultivated in 3 fertilization treatments:

a) control treatment, without nitrogen fertilization,
b) nitrogen at a rate of 30 kg $N \cdot ha^{-1}$ (i.e. 3 g $N \cdot m^{-2}$),
c) nitrogen at a rate of 150 kg $N \cdot ha^{-1}$ (i.e. 15 g $N \cdot m^{-2}$).

The second studied factor in the experiment was the developmental phase where yellow lupine was harvested:

a) full flowering (65 BBCH, I time),
b) full maturity (90 BBCH, II time).

To determine the amount of nitrogen deriving from the process of biological reduction by yellow lupine, spring triticale of the cultivar Milewo was cultivated parallel in identical treatments, as a plant with a similar length of the growth period and not showing the ability to grow and develop in symbiosis with nodule bacteria.

The experiments were conducted in three replications on plots with an area of 1 m^2, which were randomly marked in the stand of cultivated plants. Mineral nitrogen was introduced into soil before the sowing of yellow lupine and spring triticale in the form of ammonium sulfate after 10% enrichment with the isotope ^{15}N. Phosphorus and potassium fertilization rates were established based on the amounts of assimilable forms of those elements in soil determined with the Egner-Rhiem method. Potassium was introduced pre-sowing to all treatments in a dose corresponding to introduction of 100 kg $K \cdot ha^{-1}$ (i.e. 10 g $K \cdot m^{-2}$) to soil in the form of potash salt. Due to a very high content of phosphorus in forms assimilable for plants fertilization with this nutrient was not performed. Seeds of yellow lupine and spring triticale before sowing were dressed with the dressing Funaben T and additionally – only in lupine seeds – the bacteria *Rhizobium lupini* were applied. The sowing of lupine seeds in the amount corresponding to 150 $kg \cdot ha^{-1}$ and triticale in the amount of 220 $kg \cdot ha^{-1}$ was carried out in the beginning of April, after previous traditional soil preparation (plowing, cultivating, harrowing). The next day after sowing yellow lupine, spraying of soil with the herbicide Stomp 330 EC was applied in a dose corresponding to 4 $dm^3 \cdot ha^{-1}$. In the phase of the beginning of lupine budding spraying with Amistar 250 SC was applied in the amount of 1.0 $dm^3 \cdot ha^{-1}$ against anthracnose, which was then replied after 10 days. In spring triticale cultivation Chwastox Turbo 340 SL was applied against weeds in the phase of the beginning of tillering in a single dose of 2 $dm^3 \cdot ha^{-1}$. Harvesting whole test plants was performed in the flowering phase and in the phase of the full maturity of lupine and triticale. Plants harvested in the phase of flowering were divided into roots, stems, leaves and flowers (in triticale – flowering ears), whereas in the full maturity phase, into roots, stems, leaves, stropped pods and seeds in yellow lupine and roots stems, leaves, chaff and grain in spring triticale.

The experiment was conducted in soil with the granulometric composition of heavy loamy sand, classified as the very good rye complex, of soil quality class IVa, with a slightly acid reaction. The total content of carbon and nitrogen in this soil amounted to 23.2 and 1.78 $g \cdot kg^{-1}$, respectively.

Yellow lupine is a plant whose growth and development is largely dependent on the weather conditions [Bieniaszewski *et al*. 2000, Podleśny and Podleśna 2012]. Total precipitation in individual months and average monthly air temperatures during the growth period of cultivated test plants are presented in Table 1. It shows that the course of the weather conditions during the growth period was rather favorable for the growth, development and yield of yellow lupine.

Laboratory analyses were carried out on samples collected separately from each plot of yellow lupine and spring triticale. In each sample of biomass of the tested plants were determined the following:

- dry matter content (D.M.) – with the gravimetric method,
- total nitrogen content with the Kjeldahl method [Kalembasa *et al*. 1989],
- isotope enrichment with ^{15}N on the emission spectrometer NOI-6e.

Table 1. Rainfall and air temperatures during the test crop (data from Hydro-Meteorological Station in Siedlce, gave by IMiGW PIB in Warsaw)
Tabela 1. Opady i temperatura powietrza w okresie wegetacji (dane ze Stacji Hydrologiczno-Meteorologicznej w Siedlcach, podane przez IMiGW PIB w Warszawie)

Month Miesiąc	Monthly rainfall – Opady miesięczne mm		Mean monthly temperatures Średnie temperatury miesięczne °C	
	study period okres badawczy	multiyears – wielolecie (1981-2008)	study period okres badawczy	multiyears – wielolecie (1981-2008)
March – Marzec	68.9	28.5	1.7	2.0
April – Kwiecień	5.4	32.9	10.0	7.9
May – Maj	59.8	54.2	12.9	13.7
June – Czerwiec	163.6	68.8	15.7	16.1
July – Lipiec	56.5	64.9	19.3	18.3
August – Sierpień	95.7	62.7	17.3	17.7

Based on the obtained results of the study, the percentage and amount of nitrogen which in yellow lupine derived from different sources were calculated using the formulas given by Kalembasa [1995] as well as Azam and Farroq [2003]:

a) the percentage of nitrogen derived from the atmosphere:

$$\%\text{Ndfa} = \left[1 - \frac{\text{at\% } ^{15}\text{N wzbog. fx}}{\text{at\% } ^{15}\text{N wzbog. nfx}}\right] \cdot 100$$

- %Ndfa (nitrogen derived from the atmosphere) – % of nitrogen derived from the air (from the process of biological reduction of N_2),
- at% ^{15}N wzbog. fx – ^{15}N isotope enrichment in yellow lupine,
- at% ^{15}N wzbog. nfx – ^{15}N isotope enrichment in the control plant – spring triticale;

b) the percentage of nitrogen derived from the fertilizer:

$$\%\text{Ndff} = \frac{\text{at\% } ^{15}\text{N wzbog. fx}}{\text{at\% } ^{15}\text{N wzbog. nawozu}} \cdot 100$$

- %Ndff (nitrogen derived from the fertilizer) – % of nitrogen derived from the fertilizer,
- at% ^{15}N wzbog. fx – enrichment in yellow lupine,
- at% ^{15}N wzbog. nawozu – enrichment of the applied fertilizer;

c) the percentage of nitrogen derived from soil (this value covers nitrogen derived from all the other sources except biological reduction and fertilizer, including nitrogen from soil reserves, precipitation, introduced seeds; to simplify a description, the term "nitrogen from soil" will be used further in this study):

$$\%\text{Ndfs} = 100 - (\%\text{Ndfa} + \%\text{Ndff})$$

- %Ndfs (nitrogen derived from the soil) – % of nitrogen derived from soil,
- %Ndfa – % of nitrogen derived from the process of biological reduction,
- %Ndff – % of nitrogen derived from the fertilizer;

d) the amount of biologically reduced nitrogen:

$$Ndfa = \frac{\%Ndfa \cdot Ip\ N}{100}$$

- Ndfa – the amount of nitrogen derived from the biological reduction of N_2,
- %Ndfa – % of nitrogen derived from the air,
- Ip N – the total amount of nitrogen taken up by yellow lupine;

e) the amount of nitrogen derived from fertilizers:

$$Ndff = \frac{\%Ndff \cdot Ip\ N}{100}$$

- Ndff – the amount of nitrogen taken up by the plant from the fertilizer
- %Ndff – % of nitrogen derived from the fertilizer,
- IpN – total amount of nitrogen taken up by yellow lupine;

f) the amount of nitrogen derived from soil:

$$Ndfs = \frac{\%Ndfs \cdot Ip\ N}{100}$$

- Ndfs – the amount of nitrogen taken up from soil by the plant,
- %Ndfs – % of nitrogen derived from soil,
- IpN – the total amount of nitrogen taken up by yellow lupine.

The results of the study were subjected to statistical analysis using the analysis of variance. The significance of the effect of studied factors on values of individual features was estimated based on the Fisher-Snedecor F test, and values of LSD 0.05 for detailed comparision of the means were calculated using Tukey's test. Calculations were made using the program Statistica 10 PL (StatSoft, Tulsa, USA). The means for the content, enrichment and percentage of nitrogen derived from different sources in the test plants were given as weighted means.

RESULTS AND DISCUSSION

The amount of harvested biomass of individual parts and the total yield of yellow lupine were not significantly dependent on the applied rate of nitrogen, but a distinct tendency to its increase was observed in the full maturity phase (Table 2). The literature data ambiguously describe the effect of nitrogen fertilization on the yield of this plant. Some authors obtained an increase in the aboveground biomass yield (including the seeds) after the use of small, starting doses of nitrogen in yellow lupine cultivation [Faligowska and Szukała 2010], while others did not find a significant effect of nitrogen fertilization on the yield of this plant [Wojnowska *et al*. 1995, Wilczek 1997]. In the flowering phase of yellow lupine a larger mass of roots and a smaller mass of stems and leaves was collected, compared with the full maturity phase. The total amount of biomass of this plant harvested in the full maturity phase was two Times higher than in the flowering phase. In the flowering phase, the largest part of lupine yield consisted of leaves – 49.5%, the proportions of stems (30.3%) and of roots (17.3%) were smaller,

whereas the smallest percentage was in flowers – 2.9% (on average for all the rates of nitrogen fertilization). In the full maturity phase, the yield structure of lupine was as follows: leaves 27.8%, stems 25.6%, seeds 21.4%, stripped pods 17.9%, roots 7.3%.

Table 2. The yield of yellow lupine biomass, g D.M.·m^{-2}
Tabela 2. Plon biomasy łubinu żółtego, g s.m.·m^{-2}

Nitrogen rate Dawka azotu kg·ha^{-1}	Part of plant – Część rośliny					Total biomass Biomasa ogółem
	roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia						
0	42.6	70.7	116.9	8.2	–	238.4
30	40.0	72.2	120.5	6.7	–	239.4
150	40.7	73.4	115.3	6.1	–	235.5
Mean – Średnia	41.1	72.1	117.6	7.0	–	237.8
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	–	ns – ni
Full maturity – Pełna dojrzałość						
0	32.7	110.4	131.0	84.7	97.0	455.8
30	36.8	125.7	132.6	72.0	91.8	458.9
150	36.5	135.1	140.1	103.4	121.2	536.3
Mean – Średnia	35.3	123.7	134.6	86.7	103.3	483.7
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni
$LSD_{0.05}$ for harvest phase $NIR_{0.05}$ dla fazy zbioru	4.3	20.4	8.9	–	–	73.2

* depending on yellow lupine growth phase: for flowering phase the column concerns the flower, but for full maturity phase it concerns the stripped pods – w zależności od fazy wzrostu łubinu żółtego: dla fazy kwitnienia kolumna dotyczy kwiatów, natomiast dla fazy dojrzałości pełnej – strączyn
ns – differences among averages non significant – ni – różnice pomiędzy średnimi nieistotne

Podleśny and Podleśna [2012] in the pot study obtained the largest proportion of stems, smaller of leaves, and the smallest of roots in the vegetative mass of yellow lupine. The authors used only a small starting rate of nitrogen in the initial period of lupine growth, whereas in the present study the plant was in one treatment fertilized with 150 kg N·ha^{-1} and grown in a soil abundant in nitrogen, which apart from varietal predispositions could affect its abundance of leaves. In yellow lupine cultivation for seeds, the basic element which is taken into consideration by the producer is their yield obtained from the defined area. The majority of results of the conducted studies indicate that the seed yields of this plant most often do not exceed 2.5 t·ha^{-1} [Florek *et al*. 2012, FAOSTAT 2013]. Seed yields of yellow lupine in the experiments by Kotecki [1990] ranged from 0.86 to 2.16 t·ha^{-1} (on average 1.38), and straw yields from 2.26 to 8.02 t·ha^{-1} (on average 4.43), and the values of coefficients of variation of those parameters during the eight years of the study for seeds and straw amounted to 32.2 and 42.0%, respectively. Krześlak and Sadowski [1997] report that in their study the seed yields of this plant also were highly varied and ranged from 0.69 to 2.49 t·ha^{-1}. In the present study, the seed yields ranged from 0.92 to 1.21 t·ha^{-1} (on average 1.03 t·ha^{-1}) and in comparison with the data by other authors, they should be regarded as small. The proportion of seeds in the total biomass yield of the test cultivar was on average 21.4%. Prusiński [2005] reports that the percentage of seeds in the total yield of yellow lupine of the cultivar Polo amounted to 39% and of the cultivar Legat 42%. Comparing the

seed yields of Fabaceae plants most frequently cultivated in Poland, it was found that they were the lowest for yellow lupine (1.1-1.7 t·ha^{-1}), higher for pea (1.9-2.6 t·ha^{-1}), and the highest in the case of faba bean (2.0-2.8 t·ha^{-1}) [Florek *et al*. 2012].

The content of nitrogen in all parts and whole plants of yellow lupine, irrespective of the harvest phase, was not significantly dependent on the nitrogen fertilization applied (Table 3). The amount of nitrogen in the roots and stems of lupine harvested in the flowering phase was larger than in the full maturity phase, whereas the amount of nitrogen determined in the leaves was not significantly dependent on the developmental phase of this plant. Also other studies indicate the fact of decreasing the content of nitrogen of vegetative organs during the growth of plants [Kocoń 1999, Kalembasa and Wysokiński 2010]. In the flowering phase of lupine, the highest content of nitrogen was found in flowers, slightly lower in roots, whereas the lowest in stems and leaves. In the full maturity of lupine, the highest nitrogen content was determined in seeds. The content of nitrogen in seeds was about three times higher than in the roots, stems and striped pods, and about two times higher than in leaves.

Table 3. Nitrogen content in yellow lupine biomass, g N·kg^{-1} D.M.
Tabela 3. Zawartość azotu w biomasie łubinu żółtego, g N·kg^{-1} s.m.

Nitrogen rate Dawka azotu kg·ha^{-1}	Part of plant – Część rośliny					Mean in biomass Średnio w biomasie
	roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia						
0	33.4	29.1	27.8	42.4	–	29.7
30	38.4	28.3	28.4	41.9	–	30.4
150	37.4	31.7	30.9	40.3	–	32.5
Mean – Średnia	36.4	29.7	29.0	41.5	–	30.9
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	–	ns – ni
Full maturity – Pełna dojrzałość						
0	17.8	16.5	28.1	16.7	52.8	27.7
30	20.5	16.2	28.3	18.3	53.8	27.9
150	17.6	16.7	28.0	16.8	53.1	28.0
Mean – Średnia	18.6	16.5	28.1	17.3	53.2	27.9
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni
$LSD_{0.05}$ for harvest phase $NIR_{0,05}$ dla fazy zbioru	5.0	2.9	ns – ni	–	–	ns – ni

* for explanations see Table 2 – objaśnienia w tabeli 2

Enriching in ^{15}N isotope of individual organs and on average of the whole yellow lupine fertilized with the higher nitrogen rate (150 kg·ha^{-1}) was smaller than after the application of a lower rate – 30 kg N·ha^{-1} (Table 4). On average ^{15}N isotope enrichment of the biomass of lupine harvested in the flowering phase was larger than in the full maturity phase. The given relationship indicates that after the flowering period the role of mineral fertilizer applied as a source of this element for yellow lupine decreased.

Comparing biomass enrichment of spring triticale and yellow lupine in ^{15}N nitrogen isotope it was found that in the same fertilizing treatments enrichment of individual parts of lupine was smaller than of triticale organs (Tables 4 and 5). This indicates the "dilution" of the amount of ^{15}N isotope contained in lupine and applied in fertilizer not

only with nitrogen derived from soil reserves but also with nitrogen fixed in the process of biological reduction (derived from air).

Table 4. ^{15}N isotope enrichment of nitrogen in biomass of yellow lupine, at. % ^{15}N at.
Tabela 4. Wzbogacenie biomasy łubinu żółtego w izotop azotu ^{15}N

Nitrogen rate Dawka azotu kg·ha^{-1}	Part of plant – Część rośliny					Mean in biomass Średnio w biomasie
	roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia						
30	0.552	0.725	0.750	0.687	–	0.699
150	2.045	2.719	2.612	2.583	–	2.531
Mean – Średnia	1.299	1.722	1.681	1.635	–	1.615
Full maturity – Pełna dojrzałość						
30	0.598	0.677	0.714	0.426	0.573	0.617
150	1.888	2.020	1.940	1.186	1.310	1.572
Mean – Średnia	1.243	1.349	1.327	0.806	0.941	1.095

* for explanations see Table 2 – objaśnienia w tabeli 2

Table 5. ^{15}N isotope enrichment of nitrogen in biomass of spring triticale, at. % ^{15}N
Tabela 5. Wzbogacenie biomasy pszenżyta jarego w izotop azotu ^{15}N

Nitrogen rate Dawka azotu kg·ha^{-1}	Part of plant – Część rośliny					Mean in biomass Średnio w biomasie
	roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia						
30	1.618	1.882	1.850	1.397	–	1.755
150	4.406	4.700	4.804	4.571	–	4.672
Mean – Średnia	3.012	3.291	3.327	2.984	–	3.214
Full maturity – Pełna dojrzałość						
30	0.976	1.377	1.463	0.957	1.113	1.167
150	3.299	3.321	3.920	3.187	3.109	3.254
Mean – Średnia	2.138	2.349	2.691	2.072	2.111	2.211

* depending on spring triticale harvest phase: for flowering stage of lupine the column concerns the ears, but for full maturity stage it concerns the chaff – w zależności od fazy zbioru pszenżyta jarego: dla stadium kwitnienia łubinu kolumna dotyczy kłosów, natomiast dla stadium dojrzałości pełnej – plew

Amounts of nitrogen taken up by individual parts and the total uptake of this element by yellow lupine harvested in the flowering and full maturity phases were not significantly dependent on the applied nitrogen fertilization (Table 6). The amount of nitrogen accumulated in roots was larger in flowering than in full maturity, whereas in leaves this relationship was reverse. The amount of nitrogen accumulated in stems was not significantly dependent on the harvest time of the test plant. In the flowering phase the percentage of nitrogen accumulated in leaves amounted to 46.5%, in stems 29.2%, in roots 20.3% and in flowers 4.0% of the total amount taken up by lupine. In the full maturity phase the percentage of nitrogen accumulated by lupine in seeds, leaves, stems, stripped pods and roots, respectively, amounted to: 40.8; 28.1; 15.1; 11.1 and 4.9% of the total amount. The total amount of nitrogen taken up by yellow lupine was about two times larger in the full maturity phase than in the flowering phase. The test lupine

cultivar accumulated 79.7% of nitrogen taken up in the flowering phase in the aboveground biomass, and after achieving full maturity in the aboveground parts of this plant was 95.1 % of taken up nitrogen, a considerable part of which was accumulated in seeds. In other studies it was found that nitrogen present in the roots amounted to 25% of the total element taken up by Fabaceae plants [Peoples and Craswell 1992, Peoples *et al*. 1995, Peoples 2001]. In the present study, the percentage of nitrogen accumulated in the roots of lupine harvested in the flowering and full maturity phases amounted to 20.3 and 4.9 %, respectively. Apart from a decrease in the percentage of nitrogen contained in roots after reaching full maturity of lupine, also a decrease in the amount of nitrogen in the roots of this test plant was observed, which indicates transferring of this macroelement from the roots to generative organs. Nitrogen allocation to generative parts occurred not only from the roots but also from the stems. A decrease in the amount and percentage of nitrogen in the roots of yellow lupine was also observed in other studies [Kalembasa and Wysokiński 2010].

Table 6. The uptake of nitrogen (total amount) by yellow lupine, g $N \cdot m^{-2}$
Tabela 6. Ilość azotu pobranego przez łubin żółty, g $N \cdot m^{-2}$

Nitrogen rate Dawka azotu $kg \cdot ha^{-1}$	Part of plant – Część rośliny					Total Ogółem
	roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia						
0	1.42	2.06	3.25	0.35	–	7.08
30	1.54	2.04	3.42	0.28	–	7.28
150	1.52	2.33	3.56	0.25	–	7.66
Mean – Średnia	1.49	2.14	3.41	0.29	–	7.34
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	–	ns – ni
Full maturity – Pełna dojrzałość						
0	0.58	1.82	3.68	1.41	5.12	12.62
30	0.75	2.04	3.75	1.32	4.94	12.80
150	0.64	2.26	3.92	1.74	6.44	14.99
Mean – Średnia	0.66	2.04	3.78	1.49	5.50	13.47
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni
$LSD_{0.05}$ for harvest phase $NIR_{0,05}$ dla fazy zbioru	0.17	ns – ni	0.32	–	–	2.17

* for explanations see Table 2 – objaśnienia w tabeli 2

Taking into consideration enriching the biomass of yellow lupine (Table 4), spring triticale (Table 5) and mineral fertilizer introduced into soil in ^{15}N nitrogen isotope, the percentage proportion of nitrogen in lupine derived from different sources was calculated, i.e. from the process of biological reduction of N_2, from the fertilizer and from soil (Table 7). In the total pool of nitrogen taken up by lupine, nitrogen derived from the atmosphere constituted the higher proportion. The percentage of nitrogen derived from biological reduction in the total amount of this element accumulated in the roots, stems, leaves and flowers of lupine harvested in the flowering phase amounted to: 59.7; 51.8; 52.5 and 47.2%, respectively (in total in the whole plant 53.6% – average values for both nitrogen rates), and in the full maturity phase in roots, stems, leaves, stripped pods and seeds, respectively, it amounted to: 40.8; 45.0; 50.9; 59.1 and 53.2%

(on average in the whole plant 51.6%). In the total amount of nitrogen derived from all the sources, the percentage of this element taken up by lupine from the fertilizer amounted to 16.1% in the flowering phase and 11.4% in the full maturity phase, and the percentage of nitrogen derived from soil amounted to 30.3% in the first harvest time and 37.0% in the second.

Table 7. Proportional part of nitrogen taken up by yellow lupine from different sources, %
Tabela 7. Udział azotu pobranego przez łubin żółty z różnych źródeł, %

Nitrogen rate Dawka azotu kg·ha^{-1}	Nitrogen sources Źródła azotu	Part of plant – Część rośliny					Mean by plant Średnio na roślinę
		roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia							
30	Ndfa	65.9	61.5	59.5	50.8	–	61.1
	Ndff	5.5	7.2	7.5	6.9	–	7.0
	Ndfs	28.6	31.3	33.0	42.3	–	31.9
150	Ndfa	53.6	42.1	45.6	43.5	–	46.1
	Ndff	20.4	27.2	26.1	25.8	–	25.3
	Ndfs	26.0	30.7	28.3	30.7	–	28.6
Mean Średnia	Ndfa	59.7	51.8	52.5	47.2	–	53.6
	Ndff	13.0	17.2	16.8	16.3	–	16.1
	Ndfs	27.3	31.0	30.6	36.5	–	30.3
Full maturity – Pełna dojrzałość							
30	Ndfa	38.7	50.8	51.2	55.5	48.5	49.8
	Ndff	6.0	6.8	7.1	4.3	5.7	6.2
	Ndfs	55.3	42.4	41.7	40.2	45.8	44.0
150	Ndfa	42.8	39.2	50.5	62.8	57.9	53.1
	Ndff	18.9	20.2	19.4	11.9	13.1	15.9
	Ndfs	38.3	40.6	30.1	25.3	29.0	31.0
Mean Średnia	Ndfa	40.8	45.0	50.9	59.1	53.2	51.6
	Ndff	12.4	13.5	13.3	8.1	9.4	11.4
	Ndfs	46.8	41.5	35.9	32.8	37.4	37.0

* for explanations see Table 2 – objaśnienia w tabeli 2
Ndfa – nitrogen derived from atmospheric air – azot pochodzący z powietrza atmosferycznego
Ndff – nitrogen derived from fertilizer – azot pochodzący z nawozu
Ndfs – nitrogen derived from soil – azot pochodzący z gleby

The amount of nitrogen derived from the atmosphere in individual parts and in total in the whole yellow lupine harvested in the flowering phase was smaller after the application of the rate of 150 kg N·ha^{-1} than after introducing 30 kg N·ha^{-1} (Table 8). In the full maturity phase of lupine the amount of nitrogen taken up from the atmosphere by roots, stems and leaves was not significantly dependent on the level of nitrogen fertilization. The amount of nitrogen derived from this source in stripped pods and seeds and in the whole lupine was higher after the application of 150 kg N·ha^{-1} than after introducing 30 kg N·ha^{-1}. Almost two times more biologically reduced nitrogen was found in lupine harvested in the full maturity phase, compared with the harvest which was performed in the flowering phase. In the full maturity phase of lupine the proportion of nitrogen derived from biological reduction in roots, stems, leaves, stripped pods and seeds, respectively, amounted to 4.0; 13.4; 27.2; 12.7 and 42.7% of the total amount of

this macroelement taken up from the source in question by the whole plant. In the flowering phase, a distribution of nitrogen derived from biological reduction in the roots, stems, leaves and flowers amounted to, 22,9; 28,1; 45,9, respectively, and 3,1% of the total amount of this macroelement taken up from the source in question by lupine.

Table 8. The uptake of nitrogen by yellow lupine from different sources, g $N \cdot m^{-2}$
Tabela 8. Ilość azotu pobranego przez łubin żółty z różnych źródeł, g $N \cdot m^{-2}$

Nitrogen rate Dawka azotu $kg \cdot ha^{-1}$	Nitrogen sources Źródła azotu	Part of plant – Część rośliny					Total Ogółem
		roots korzenie	stems łodygi	leaves liście	flowers/ stripped pods* kwiaty/strączyny	seeds nasiona	
Full flowering – Pełnia kwitnienia							
30	Ndfa	1.012	1.256	2.036	0.143	–	4.447
	Ndff	0.085	0.147	0.257	0.019	–	0.508
	Ndfs	0.439	0.640	1.129	0.119	–	2.327
150	Ndfa	0.816	0.980	1.625	0.107	–	3.528
	Ndff	0.310	0.633	0.930	0.063	–	1.936
	Ndfs	0.396	0.714	1.008	0.076	–	2.194
Mean Średnia	Ndfa	0.914	1.118	1.830	0.125	–	3.987
	Ndff	0.198	0.390	0.593	0.041	–	1.222
	Ndfs	0.417	0.677	1.069	0.097	–	2.261
$LSD_{0.05}$ $NIR_{0,05}$	Ndfa	0.173	0.236	0.347	0.023	–	0.614
	Ndff	0.058	0.103	0.142	0.010	–	0.316
	Ndfs	ns – ni	ns – ni	ns – ni	ns – ni	–	ns – ni
Full maturity – Pełna dojrzałość							
30	Ndfa	0.292	1.034	1.922	0.731	2.395	6.374
	Ndff	0.045	0.139	0.266	0.057	0.282	0.789
	Ndfs	0.417	0.863	1.565	0.530	2.262	5.637
150	Ndfa	0.275	0.884	1.981	1.091	3.726	7.957
	Ndff	0.121	0.456	0.761	0.207	0.843	2.388
	Ndfs	0.246	0.916	1.181	0.439	1.867	4.649
Mean Średnia	Ndfa	0.284	0.959	1.951	0.911	3.061	7.166
	Ndff	0.083	0.297	0.513	0.132	0.563	1.588
	Ndfs	0.331	0.890	1.373	0.484	2.065	5.143
$LSD_{0.05}$ $NIR_{0,05}$	Ndfa	ns	ns	ns	0.191	0.655	0.964
	Ndff	0.014	0.070	0.119	0.034	0.131	0.393
	Ndfs	0.061	ns	0.258	0.074	0.314	0.519
$LSD_{0.05}$ for harvest phase $NIR_{0,05}$ dla fazy zbioru	Ndfa	0.166	0.156	ns – ni	–	–	1.250
	Ndff	0.026	0.065	ns – ni	–	–	0.307
	Ndfs	0.071	0.162	0.163	–	–	0.863

* for explanations see Table 2 – objaśnienia w tabeli 2

The amount of nitrogen taken up by yellow lupine from the mineral fertilizer was, on average for all treatments, four times smaller than from the atmosphere (Table 8). All parts of yellow lupine harvested in both developmental phases from the treatments fertilized with the rate of 150 kg $N \cdot ha^{-1}$ took up more nitrogen from the fertilizer than after the application of 30 kg $N \cdot ha^{-1}$. In total, the whole test plant fertilized with a higher rate of nitrogen took up more than three times more of this element from the fertilizer

than after the application of the lower rate. The harvest time of yellow lupine did not significantly differentiate the amount of nitrogen taken up from the fertilizer only in leaves. Amounts of nitrogen derived from the fertilizer accumulated in the roots and stems of this test plant were higher in the 1st harvest time than in the 2nd. The amount of nitrogen derived from the fertilizer in total in the whole biomass of lupine was larger in the full maturity phase than in flowering. This state was affected by a relatively large amount of this macroelement derived from the fertilizer accumulated in the lupine seeds. Lupine accumulated in seeds about 1/3 of nitrogen taken up from the fertilizer by the whole plant harvested in the full maturity phase. In the flowering phase, lupine accumulated the most of nitrogen derived from the fertilizer in leaves (almost the half of the total amount taken up from this source).

The amount of nitrogen derived from soil in all the parts and in total, in the whole biomass of yellow lupine harvested in the flowering phase, was not dependent on different fertilization with nitrogen, whereas in the phase of full maturity it was higher in the treatments fertilized with a smaller nitrogen rate (30 kg $N \cdot ha^{-1}$) than after the application of the larger rate (150 kg $N \cdot ha^{-1}$, table 8). The roots of lupine harvested in the flowering phase contained more nitrogen taken up from soil, compared with the harvest performed in the full maturity phase. The amount of nitrogen derived from this source in stems and leaves and totally in whole lupine was higher in the 2nd time of harvest than in the 1st. The most nitrogen taken up from soil in the flowering phase was accumulated by lupine in leaves, whereas in the phase of full maturity in seeds.

Economic analyses showed that cultivation of yellow lupine should not be considered only in the aspect of the seed yields obtained [Majchrzak *et al*. 2010, Czerwińska-Kayzer and Florek 2012]. Taking up of biologically reduced nitrogen from the air should be also taken into consideration. In the present experiment, the percentage of nitrogen derived from the air in yellow lupine harvested in the flowering phase was on average in the whole plant 53.4%, and in full maturity 51.6%. In the quantitative aspect, yellow lupine harvested in the 1st and 2nd time took up 39.9 and 71.7 kg of biologically reduced nitrogen per 1 hectare, respectively. In the study conducted by Kalembasa and Wysokiński [2010] in a greenhouse (a pot experiment) a considerably smaller proportion of nitrogen derived from the atmosphere in the individual organs of yellow lupine was obtained (on average from the whole plant 43.2 % in the flowering phase and 29.2 % after reaching full maturity). It may be supposed that the increased temperature prevailing in the glasshouse may have been the factor limiting the activity of symbiotic bacteria. Both data given by those authors and the present study indicate decreasing percentage share of biologically reduced nitrogen along with the growth and development of yellow lupine. Both Andrzejewska and Ignaczak [1997] and Kocoń [1999] informed about the phenomenon of slowing the rate of fixing nitrogen from the air by Fabaceae plants after the period of their flowering.

Nitrogen fertilization is a factor affecting the intensity of atmospheric nitrogen fixation, and consequently, the amount and quality of yield of Fabaceae plants. Mineral forms of nitrogen may have an unfavorable effect on the development and stability of nodules, the activity of nitrogenase, and as a result, the reduction of N_2 nitrogen – the effect of symbiosis [Mengel 1994, Filek *et al*. 1997, Borowiecki 2004]. Nitrogen present in soil in the forms assimilable for plants may also inhibit the process of nitrogen biological reduction, but at the same time, it does not always reduce microorganism multiplication and plant growth or decrease the amount of nitrogen fixed in the process of biological reduction [Voisin *et al*. 2002]. In the present study the

amount of nitrogen deriving from reduction of N_2 in the flowering phase of lupine decreased after increasing the nitrogen rate from 30 to 150 kg·ha^{-1}, whereas in the full maturity phase this relationship was reverse. Increasing nitrogen fertilization from 30 to 150 kg·ha^{-1} irrespective of the developmental phase of lupine increased the amount of nitrogen taken up from the applied fertilizer.

CONCLUSIONS

1. Fertilization with nitrogen in a rate of 30 and 150 kg N·ha^{-1} did not have a significant effect on the yield of seeds and whole biomass of yellow lupine and the content and total amount of nitrogen accumulated in seeds and whole plants.

2. Percentage of nitrogen deriving from the process of biological reduction in the yellow lupine biomass was similar in the flowering and full maturity phases and amounted to 53.4 and 51.6% respectively. The proportion of nitrogen from the fertilizer was larger in the flowering phase than in the full maturity phase of lupine, whereas for nitrogen taken from soil this relationship was reverse. No significant effect of different nitrogen fertilization on the percentage of this element deriving from biological reduction in lupine was observed. Lupine fertilized with a higher nitrogen rate had a larger proportion of this element deriving from the fertilizer and smaller from soil reserves, in comparison with fertilization with the lower rate.

3. In the flowering phase of yellow lupine the amount of nitrogen deriving from the process of biological reduction decreased after increasing the rate from 30 to 150 kg N·ha^{-1}, whereas in the phase of full maturity this relationship was reverse. The amount of nitrogen in lupine deriving from fertilizers increased after increasing a rate of this component in fertilization. Amounts of nitrogen deriving from all sources (biological reduction, fertilizer and soil) were larger in the full maturity phase than in the flowering phase of lupine.

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ILOŚCIOWA OCENA PROCESU BIOLOGICZNEJ REDUKCJI AZOTU CZĄSTECZKOWEGO PRZEZ ŁUBIN ŻÓŁTY (*Lupinus luteus* L.)

Streszczenie. W badaniach określono ilość azotu pobranego przez łubin żółty z atmosfery, nawozu mineralnego i gleby przy zróżnicowanym nawożeniu azotem i fazie rozwojowej tej rośliny testowej. Łubin żółty uprawiano w trzech wariantach nawozowych: bez nawożenia azotem oraz po zastosowaniu dawki 30 i 150 kg $N \cdot ha^{-1}$. Zbiór przeprowadzono w fazie kwitnienia i pełnej dojrzałości. W prowadzonych badaniach wykorzystano izotop azotu ^{15}N – wprowadzony do gleby w postaci siarczanu amonu – i metodę izotopowego rozcieńczenia. Plon biomasy łubinu żółtego i zawartość azotu w poszczególnych jego częściach nie były istotnie uzależnione od dawki azotu. Ilość zebranej biomasy łubinu żółtego w fazie pełnej dojrzałości była ponad dwukrotnie większa niż w fazie kwitnienia. Zawartość azotu w łubinie zbieranym w fazie kwitnienia była większa niż w fazie pełnej dojrzałości. Zróżnicowane nawożenie azotem, niezależnie od terminu zbioru łubinu, nie wpłynęło istotnie na całkowitą ilość pobranego azotu, która była około dwukrotnie większa w fazie pełnej dojrzałości niż w fazie kwitnienia. Ilość azotu pochodzącego z procesu biologicznej redukcji w biomasie łubinu żółtego zbieranego w fazie kwitnienia była mniejsza po zastosowaniu większej dawki tego składnika, natomiast w fazie pełnej dojrzałości zależność ta była odwrotna. Ilość azotu pochodzącego z nawozu zwiększała się wraz ze wzrostem dawki. Procentowy udział azotu pochodzącego z procesu biologicznej redukcji w biomasie łubinu żółtego był zbliżony w fazie kwitnienia (53,4 %) i pełnej dojrzałości (51,6 %). Udział azotu pochodzącego z nawozu był większy w I terminie zbioru łubinu niż w terminie II, natomiast w przypadku azotu pobranego z gleby zależność ta była odwrotna. Zróżnicowane nawożenie azotem nie wpłynęło istotnie na procentowy udział azotu pochodzącego z biologicznej redukcji w łubinie żółtym. Łubin żółty nawożony większą dawką azotu zawierał większy procentowy udział tego pierwiastka pochodzącego z nawozu, a mniejszy z zapasów glebowych, niż po zastosowaniu mniejszej dawki.

Słowa kluczowe: azot, biologiczna redukcja N_2, łubin żółty

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