ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 7 2010

Stanisław KALEMBASA[1] and Andrzej WYSOKIŃSKI[1]

CUMULATION OF BIOLOGICALLY REDUCED NITROGEN IN THE BIOMASS OF YELLOW LUPINE (*Lupinus luteus*) AT ITS DIFFERENT GROWING STAGES

KUMULACJA BIOLOGICZNIE ZREDUKOWANEGO AZOTU PRZEZ ŁUBIN ŻÓŁTY (*Lupinus luteus*) W RÓŻNYCH JEGO FAZACH ROZWOJOWYCH

Abstract: The content of biological reduced nitrogen from air by yellow lupine (*Lupinus luteus*) living in the symbiosis with *Rhizobium lupine* which biomass was harvested in differentiated developing stages was determinated in pot experiment. The highest share in total yield of yellow lupine harvested in the blooming stage were leaves (51.4 %) but at full maturity steams (45.7 %). The roots of tested plant were in relation to total yield 14.7 % harvested at first term and 6.7 % at second term. The total yield of yellow lupine harvested at the stage of full maturity was almost twofold higher that at blooming stage. The content of total nitrogen in vegetative parts of yellow lupine was, the highest in the leaves and in roots and steams was about 1/3 lower than in leaves but in and seeds nearly two and three times higher that in leaves, relatively. The enrichment in ^{15}N in particularly parts of mustard (plant which takes up nitrogen only from two sources, namely from fertilizer and soil) was higher in comparison with relatively parts of yellow lupine (which is able to take up the nitrogen from three sources: fertilizer, soil and atmospheric air). The share of nitrogen which was biologically reduced in the total content of those element got lured in biomass of roots, steams and leaves harvested at blooming stages reached respectively 9.8; 16.9 and 59.8 % (total in the whole plant 43.2 %). In the biomass of yellow lupine harvested at maturity stage the share of nitrogen coming form the biological reduction process in the total amount of those element cumulated in roots, steams, leaves, stripped pods and seeds reached 6.7; 22.8; 26.0; 26.6 and 35.6 % respectively (total the in whole plant 29.2 %). In total amount of nitrogen coming from different sources the amount of this element taken up by yellow lupine reached from: fertilizer 23.1 % in blooming stage and 31.3 % in full maturity stage, but the share of nitrogen taken up from soil reached 83 % at the first term and 38.9 % in the second term of harvesting.

Keywords: nitrogen, the biological reduction of nitrogen by plant, yellow lupine, white mustard, isotope ^{15}N

Introducing the legume family crops living in a symbiosis with *Rhizobium* genus bacteria into the crop rotation system, is the most energy-saving and efficient form (besides natural and organic fertilizers) of nitrogen nutrition that allows for considerable

[1] Department of Soil Science and Plant Nutrition, Siedlce University of Natural Sciences and Humanities, ul. B. Prusa 14, 08–110 Siedlce, Poland, phone/fax: 25 643 12 87, email: kalembasa@ap.siedlce.pl

reductions in the element amounts introduced into the soil environment in a form of mineral fertilizers [1].

During the last twenty years, the area of legume family crops cultivation has been gradually decreasing to the advantage of cereals. Current percentage of these crops in sown structure in Poland is less than 1 %. Import of the crushed soybean seed as a cheaper protein source along with instability of legume family crops yielding due to the surplus of vegetative over generative organs greatly affect the poor popularity of these plants. However, domestic legume family plant species should play a major role when popularizing the high-quality, natural, and genetically non-modified food for people and fodder for animals. Moreover, that group of crops has out-of-production virtues manifesting as the improvements in soil fertility and its abundance in nutrients, mainly nitrogen. Ability to bind atmospheric nitrogen reduces inputs associated with nitrogen nutrition, both papilionaceous and consequent crops [2–4]. At promoting the balanced agriculture development and caring the natural environment, problems of elevated nitrogen quantities, namely its mineral or readily mineralizable forms in soils, attracts more and more attention. Amount of nitrogen bound within biological reduction processes depends on many natural and anthropogenic factors, including: crop species (even the variety), *Rhizobium* genus bacteria presence, nitrogen nutrition, temperature, rainfalls, and their distribution during vegetation season. Elevating the nitrogen content in the soil where plants binding atmospheric nitrogen grow, depends on their purpose. Considerably large amounts of the element may be introduced into the soil in the case of plants grown for green forage. Then, nitrogen from biological reduction should be taken into account at balancing the nitrogen fertilization not to let its excessive accumulation in soil and cultivated consequent crops.

Yellow lupine may be a plant utilized for many ways. Its green matter harvested at flowering stage has significant nutritional value, because lignifications processes begins when pods are formed [5]. Moreover, the species can be considered as a green fertilizer, which can be completely ploughed or seeds can be harvested at full maturity stage, while post-crop remains introduced into the soil.

The study aimed at evaluating the biologically reduced gaseous nitrogen amounts by yellow lupine (*Lupinus luteus)* growing in a symbiosis with *Rhizobium lupini* bacteria, harvested at various growth and development stages.

Material and methods

The yellow lupine (cv. Parys) was grown in pots of 10 dm^3 capacity and filled with 10 kg of soil (5 plants $\cdot$ pot^{-1}); pots were placed in a greenhouse. In order to determine the amount of nitrogen bound during biological reduction, the isotope dilution method was applied, which required to use mineral fertilizers amended with ^{15}N isotope, along with parallel cultivation of the control plants that have not the ability of symbiosis with nodule bacteria. White mustard (cv. Rota) was the control. Nitrogen fertilization was applied before seed sowing at the amount of 1 g N $\cdot$ pot^{-1}, while phosphorus and potassium at such quantities to achieve N:P:K ratio 1:0.3:1. Phosphorus and potassium were applied in forms of triple superphosphate and potassium salt, whereas nitrogen was introduced as ammonia sulfate enriched with ^{15}N 10.0 at. % isotope. Applying the fertilizer with elevated ^{15}N isotope content allowed for determining the quantity of nitrogen uptaken by lupine from different sources (air, fertilizer, soil resources). Before sowing, lupine seeds were dressed using anti-fungal preparation Dithane M-45 80 WP as well as nitragine containing symbiotic bacteria *Rhizobium lupini*. The soil used in the experiment had granulometric composition of light loamy sand; pH in KCl was 5.2; total nitrogen content 0.81 $g \cdot kg^{-1}$, and organic carbon content 9.0 $g \cdot kg^{-1}$.

Lupine and mustard were harvested at lupine's blooming stage (date I) as well as full maturity of both crops (date II). Collected plant material was divided into roots, stems, and leaves, whereas additionally at full maturity stage, into seeds and pods or hulls. Water content was determined in collected material. Achieved yields were recalculated onto dry matter content. Total amounts of nitrogen, enrichment in ^{15}N isotope, and quantity of nitrogen uptaken by particular parts of yellow lupine from various sources, were calculated according to method given by Kalembasa [6].

Achieved study results were subjected to variance analysis in completely randomized design (F-Fischer–Snedecor test), while $LSD_{0.05}$ to compare mean values were calculated using Tukey test.

Results and discussion

Leaves made up the largest percentage in yellow lupine biomass (Table 1) harvested at flowering stage (51.4%), while stems – at full maturity stage (45.7 %).

Table 1

The yield of yellow lupine [g dm $\cdot$ pot^{-1}]

Harvesting stage	Part of plant					Total yield
	roots	stems	leaves	stripped pods	seeds	
Flowering stage	2.11 *14.7**	4.86 *33.9**	7.36 *51.4**	— —	— —	14.33
Full maturity stage	1.79 *6.7**	12.27 *45.7**	10.56 *39.3**	1.65 *6.1**	0.59 *2.2**	26.86
Average	1.95	8.57	8.96	—	—	—
$LSD_{0.05}$	ns	3.51	ns	—	—	9.63

* Proportional (%) participation in total yield.

Roots of the plant made up 14.7 % of the total yield on date I, whereas 6.7 % at date II. Weight of yellow lupine roots collected at full maturity was slightly lower than at blooming stage, while for leaves the dependencies were opposite, and in both cases the differences between mean values were not statistically proven. Only the stem weight harvested at date II was significantly higher than at date I. Total yield of lupine harvested at full maturity stage was almost twice as high as at flowering stage. Literature data also underline considerable share of stems and leaves at the initial growth period, while at full maturity stage, seeds play significant role in accumulating dry matter by yellow lupine [7, 8]. In present study, lupine's generative parts (seeds and pods) made up 8.3 % of the total yield. Low seed percentage in the yield was reflected in great failures of the plant cultivation for seeds [9]. A necessary condition leading to the increase of lupine cultivated area should consist in intensive breeding works tending improve the fertility and stability of the plant yielding in years with varied agricultural and climatic conditions [10].

Among vegetative parts of lupine plants, leaves contained the largest amounts of total nitrogen, regardless of the harvest date (Table 2). Nitrogen content in roots and stems was about 1/3 lower than in leaves, while in pods and seeds, it was about two and three times higher than in leaves. Nitrogen content in leaves increases till all leaves are developed and it begins to gradually decrease just before plant blooming [11]. Own study confirmed higher nitrogen contents in leaves, stems, and roots of lupine plants harvested at flowering rather than full maturity stage. The phenomenon should be attributed to the damage of root nodules, which progresses after flowering, as well as transport of nitrogen from vegetative to generative parts of plants – pods and seeds [12].

Table 2

The content of nitrogen in yellow lupine [$g \cdot kg^{-1}$ dm]

Harvesting stage	Part of plant					Average arithmetical
	roots	stems	leaves	stripped pods	seeds	
Flowering stage	20.9	20.7	33.4	—	—	25.0
Full maturity stage	17.0	16.7	27.1	49.0	70.0	36.0
Average	19.0	18.7	30.3	—	—	—
$LSD_{0.05}$	2.6	3.0	2.8	—	—	1.7

Enriching particular parts of white mustard (plant that uptakes nitrogen only from two sources: fertilizer and soil) in ^{15}N was higher as compared with corresponded parts of lupine plants (that additionally uptakes nitrogen from the air) (Table 3). The quantity of ^{15}N isotope determined in lupine biomass harvested at full maturity stage was higher than at flowering stage, which indicates the elevated amounts of uptaken nitrogen from a fertilizer, as well as slower rate of biological reduction process.

Table 3

Enrichment ^{15}N isotope of nitrogen in yellow lupine and white mustard [% ^{15}N]

Cultivation plant	Harvesting stage	Part of plant					Average arithmetical
		roots	stems	leaves	stripped pods, siliques	seeds	
Yellow lupine	flowering stage	2.779	3.207	1.939	—	—	2.642
	full maturity stage	3.136	3.441	3.433	3.414	3.142	3.313
Average		2.698	3.040	2.686	—	—	—
White mustard	flowering stage	3.079	3.861	4.825	—	—	3.922
	full maturity stage	3.362	4.456	4.641	4.651	4.876	4.397
Average		3.221	4.158	4.733	—	—	—

Regardless the harvest date, the largest amounts of total nitrogen were accumulated by lupine in leaves, which confirms the assumption on accumulating majority of macronutrients in aboveground biomass by papilionaceous plant species [13]. Percentage of nitrogen originating from biological reduction of gaseous N_2 in its total amount uptaken by roots, stems, and leaves collected at flowering stage was: 9.8, 16.9, and 59.8 %, respectively (or 43.2 % for the whole plant). At full maturity stage, the share of nitrogen from biological reduction in its total quantity accumulated in roots, stems, leaves, and seeds amounted to: 6.7, 22.8, 26.0, 26.6, and 35.6 % (or 29.2 % for the whole plant). In the total amount of nitrogen originating from all sources, its quantities uptaken by lupine plants from fertilizers made up 23.1 % at blooming and 31.9 % at full maturity stage, whereas the share of nitrogen originating from the soil resources made up 33.7 % at date I and 38.9 % at date II of the harvest (Table 4).

Table 4

The quantity of nitrogen taken up through yellow lupine [$mg \cdot pot^{-1}$]

Harvesting stage	Source of nitrogen	Part of plant					Sum
		roots	stems	leaves	stripped pods	seeds	
Flowering stage	biologically reduction N_2	4.2 *9.8**	17.0 *16.9**	146.4 59.8*	—	—	167.7 *43.2**
	fertilizer	11.8 *27.2**	31.6 *31.4**	46.5 *19.0**	—	—	89.9 *23.1**
	soil	27.2 *63.0**	51.9 *51.6**	51.8 *21.2**	—	—	130.9 *33.7**
	sum	43.2	100.5	244.7	—	—	388.4
Full maturity stage	biologically reduction N_2	2.1 *6.7**	47.6 *22.8**	75.1 *26.0**	21.5 *26.6**	14.7 *35.6**	161.0 *29.2**
	fertilizer	9.6 *30.7**	70.5 *33.7*	97.1 *33.7**	27.1 *33.5**	12.7 *30.8**	217.0 *31.9**
	soil	19.4 *52.5**	90.9 *43.5**	116.4 *40.3**	32.3 *39.9**	13.9 *33.6**	272.9 *38.9**
	sum	31.1	209.0	288.6	80.9	41.3	650.9
Average total quantity of nitrogen taken up by lupine's individual parts		37.2	154.8	266.7	—	—	—
$LSD_{0.05}$ for total quantity of nitrogen taken up through yellow lupine harvested in flowering and full maturity stage		ns	82.7	ns	—	—	221.0

* Proportional (%) participation in total quantity of nitrogen taken up from all source.

Conclusions

1. The dry matter yield of yellow lupine harvested at full maturity stage was almost twice as high as that at flowering stage. Regardless of the harvest date, leaves and stems made up the largest share in the total yield (about 85 %).

2. Nitrogen content in vegetative parts of lupine was lower at full maturity rather than blooming stage.

3. Share of nitrogen originating from a biological reduction in its total amount uptaken by yellow lupine from different sources was 43.2 % at flowering and 29.2 % at full maturity stage. Quantity of nitrogen from fertilizers and soil made up 23.1 % and 33.7 % of its total amount uptaken by yellow lupine harvested at date I, as well as 31.9 % and 38.9 % collected at date II.

References

[1] Jasińska Z. and Kotecki A.: Zesz. Nauk. AR Kraków 2001, **373**(76), 47–54.
[2] Wojnowska T., Sienkiewicz S. and Wojtas A.: Acta. Acad. Agric. Techn. Olst., Agric. 1995, **61**, 157–166.
[3] Podleśny J.: Zesz. Probl. Post. Nauk. Roln. 1997, **446**, 287–290.
[4] Kocoń A.: Fragm. Agron. 1999, **2**(62), 50–61.
[5] Jasińska Z. and Kotecki A.: Rośliny strączkowe. PWN Warszawa 1993, s. 205.
[6] Kalembasa S.: Zastosowanie izotopów ^{15}N i ^{13}N w badaniach gleboznawczych i chemiczno-rolniczych. WNT Warszawa 1995, s.252.
[7] Jasińska Z. and Dmowski Z.: Roczn. Nauk Roln. 1973, **70C**(1), 31–38.
[8] Prusiński J.: Acta Sci. Polon., Agricultura 2005, **4**(2), 57–72.
[9] Sadowski T., Krześlak S. and Zawiślak K.: Zesz. Nauk. AR Wrocław 2001, **426**, 135–142.
[10] Prusiński J.: Zesz. Probl. Post. Nauk. Roln. 2007, **522**, 23–37.
[11] Greenwood D., Lemaire G., Gosse G., Cruz P., Draycott A. and Neeteson J.J.: Ann. Bot. 1990, **66**, 425–436.
[12] Księżak J.: Dynamika gromadzenia składników pokarmowych w organach roślin tradycyjnych i samokończących odmian bobiku w okresie od kwietnia do dojrzałości pełnej, Wyd. IUNG Puławy, Monogr. i Rozpr. Nauk. 2002, **5**, 1–95.
[13] Wilczewski E.: Acta Sci. Polon., Agricultura 2007, **6**(1), 35–44.

KUMULACJA BIOLOGICZNIE ZREDUKOWANEGO AZOTU PRZEZ ŁUBIN ŻÓŁTY (*Lupinus luteus*) W RÓŻNYCH JEGO FAZACH ROZWOJOWYCH

Katedra Gleboznawstwa i Chemii Rolniczej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach

Abstrakt: W doświadczeniu wazonowym określono ilość azotu biologicznie zredukowanego z powietrza przez łubin żółty (*Lupinus luteus)* żyjący w symbiozie z bakteriami *Rhizobium lupini,* zbierany w różnych fazach wzrostu i rozwoju. Największy udział w plonie całkowitym łubinu żółtego zbieranego w fazie kwitnienia stanowiły liście (51,4%), a w fazie dojrzałości pełnej – łodygi (45,7 %). Korzenie tej badanej rośliny stanowiły 14,7 % plonu całkowitego w I terminie zbioru i 6,7 % w II terminie. Plon całkowity łubinu zbieranego w fazie pełnej dojrzałości był prawie dwukrotnie większy niż w fazie kwitnienia. Zawartość azotu ogółem w częściach wegetatywnych łubinu była największa w liściach. Zawartość azotu w korzeniach i łodygach była o około 1/3 mniejsza niż w liściach, natomiast w strączynach i nasionach odpowiednio około dwu- i trzykrotnie większa niż w liściach. Wzbogacenie w azot ^{15}N poszczególnych części gorczycy (rośliny pobierającej azot tylko z nawozu i zapasów glebowych) było większe, w porównaniu z odpowiednimi częściami łubinu (korzystającego dodatkowo z azotu atmosferycznego). Udział azotu pochodzącego z biologicznej redukcji N_2 w całkowitej ilości tego pierwiastka zgromadzonej w korzeniach, łodygach i liściach łubinu zbieranego w fazie kwitnienia stanowił kolejno 9,8, 16,9 i 59,8 % (ogółem w całej roślinie 43,2 %). W fazie dojrzałości pełnej łubinu udział azotu pochodzącego z biologicznej redukcji w całkowitej ilości tego pierwiastka zgromadzonej w korzeniach, łodygach, liściach, strączynach i nasionach stanowił odpowiednio: 6,7; 22,8; 26,0; 26,6 i 35,6 % (ogółem w całej roślinie 29,2 %). W całkowitej ilości azotu pochodzącego ze wszystkich źródeł ilość tego pierwiastka pobrana przez łubin z nawozu stanowiła 23,1 % w fazie kwitnienia i 31,9 % w fazie pełnej dojrzałości, natomiast udział azotu pochodzącego z zapasów glebowych wynosił 33,7 % w I terminie zbioru i 38,9 % w II terminie.

Słowa kluczowe: azot, biologiczna redukcja azotu, łubin żółty, gorczyca biała, izotop ^{15}N