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DYNAMICS OF NITROGEN ACCUMULATION FROM VARIOUS SOURCES BY LUCERNE (*Medicago sativa* L.)

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Abstract. Biomass of lucerne harvested at the budding stage or at the beginning of flowering is a bulky feed with a high content of protein. This plant is less often harvested at the full maturity for seeds. Nitrogen fertilization is not frequently applied in lucerne cultivation, since it lives in symbiosis with nodule bacteria that reduce atmospheric nitrogen into ammonium forms available for plants. This work presents the result of the pot experiment that aimed to determine the effect of the development stage of lucerne on the dynamics of nitrogen uptake from the atmosphere, from the mineral fertilizer and from soil. The studied plant was harvested at the beginning of flowering and at full flowering, and after reaching full maturity. In fertilization of the test plant, ammonium sulphate with ^{15}N excess was applied and the method of isotopic dilution was used to assess the dynamics of nitrogen uptake. The control plant was spring barley. Systematic increase in the dry matter of lucerne was recorded at successive development stages. At the beginning of flowering and at full flowering of lucerne, the percentage of roots, stems and leaves in the whole plant mass was similar, whereas at the full maturity, the roots had the highest percentage in the dry matter yield (44%), and seeds (4%) and stripped pods (5%) the lowest. The average amount of nitrogen in the whole dry matter of the studied species was similar at successive harvest times. At the beginning of flowering and in full flowering, lucerne accumulated the highest amount of nitrogen in leaves, whereas after reaching the full maturity, in leaves and roots. The main source of nitrogen for the studied plant at successive developmental stages was the atmosphere. Nitrogen biologically reduced by lucerne harvested at the beginning of flowering and at full flowering accounted for 83.8 and 86.1%, respectively, and at the full maturity stage, 94.9% of the total uptake of this macroelement. The other small amount of nitrogen was taken up from the mineral fertilizer and from soil. At the beginning of flowering and at full flowering of lucerne the percentage of nitrogen taken up from the fertilizer did not exceed 10%, and from the soil was maximally 7%. At full maturity to harvest for seeds, the percentage of nitrogen taken up from the fertilizer and soil amounted to 2.4 and 2.7%, respectively.

Key words: biologically reduced N_2, lucerne, nitrogen, ^{15}N isotope

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INTRODUCTION

Nitrogen in the form of a two-atom particle N_2 is characterized with a high stability and it cannot be directly taken up either by plants or by the majority of microorganisms [Król 1999]. Deficiencies of available forms of this element is an essential factor limiting plant production [Kobus 1996]. Transformation of atmospheric nitrogen into forms available for plants occurs first of all in the process of chemical reduction during production of nitrogen fertilizers and biological reduction by microorganisms. Procaryotic organisms – bacteria and blue-green algae – have the ability to reduce N_2 to ammonium forms. This group of microorganisms includes bacteria of the genus *Rhizobium*, living in symbiosis with the Fabaceae. They reduce atmospheric nitrogen (N_2) to ammonia (NH_3) and may also include it in the amino acid glutamine and in this form pass to plant cells. Reduced nitrogen is accumulated in live tissues of plants, whereas it gets to soil with their fallen parts as a result of rhizodeposition [Pietrzak 2011]. The amount of reduced atmospheric nitrogen depends on many biotic and abiotic factors, for instance on the species, and even the variety of the Fabaceae plant, soil and climatic conditions, intensity of diseases and occurrence of pests, e.g. Sitona beetle destroying root nodules [Księżak 2000]. Nitrogen reduced by symbiotic microorganisms is of utmost importance for agriculture, since it is utilized by plants in 100%, and the utilization of nitrogen from mineral fertilizers the most often does not exceed 60% [Księżak 2000].

The Fabaceae plants are a good previous crop for cereal, industrial and root crops [Księżak 2000], since they provide the soil with considerable amounts of nitrogen [Wielgosz *et al.* 2004]. Introduction of the Fabaceae plants to the crop rotation allows reduction in the use of nitrogen fertilizers by 20-25% [Prusiński *et al.* 2008]. Additionally, post-harvest residues of those plants enrich soil in organic compounds [Jasińska and Kotecki 1997]. Their root system reduces soil degradation, through structure-forming and improving effect. The above traits result in no-outlay increase in yield of successive crops by 5-15% [Dubis and Budzyński 1998, Dzienia *et al.* 1989].

Lucerne is characterized by a high nutritive value of both fresh and preserved mass and high yield. This species, living in symbiosis with nodule microorganisms, use biologically reduced nitrogen [Radkowski and Grygierzec 2006, Zając *et al.* 2007]. Additional value of lucerne is its high adaptation ability, which allows this species to be grown under various soil and climatic conditions [Zając *et al.* 2007]. Favourable solution in plant biomass production is growing mixtures of lucerne with grasses, since nodule bacteria after satisfying their needs and the needs of the host plant, transfer a part of nitrogen to the soil, which is in favour for the grass component of the mixture. Moreover, decomposing roots and nodules of lucerne constitute an additional source of nitrogen for grasses collected in later cuts [Zając *et al.* 2008].

The aim of this study was to estimate the amount of nitrogen taken up by lucerne from different sources and accumulated in the roots, stems, leaves, stripped pods and seeds at different developmental stages. The research hypothesis assumed that the process of biological reduction of atmospheric N_2 carried out by the bacteria *Rhizobium* will be the main source of nitrogen for lucerne, and the amount of nitrogen taken up from the air will be dependent on the development stage during harvest.

MATERIAL AND METHODS

The vegetation experiment was conducted in 2006 at a glasshouse of the University of Siedlce University of Natural Sciences and Humanities. The experiment was aimed to determine the effect of the development stage of lucerne on the dynamics of taking up nitrogen from the atmosphere, from the mineral fertilizer and from the soil. In pots with a volume of 12 dm^3, filled with 13 kg of soil, lucerne cultivar Verko was grown in an amount of 10 plants per pot, in 3 replications. The method of isotopic dilution was used in order to determine the amount of nitrogen fixed in the process of biological reduction. It requires the use of a mineral fertilizer enriched in ^{15}N isotope and parallel cultivation of the control plant, not showing the ability to live in symbiosis with bacteria *Rhizobium* and not taking up nitrogen from the atmosphere. The control plant was the spring barley cultivar Bryl, grown in an amount of 10 plants per pot. Presowing fertilization with nitrogen in an amount of 0.642 $g \cdot pot^{-1}$ was used in cultivation of both plants in the form of ammonium sulphate with 10.2 atom % ^{15}N excess. Fertilization with phosphorus in the form of triple superphosphate and potassium in the form of potash salt was applied in an amount of 0.385 g P and 0.770 g $K \cdot pot^{-1}$, respectively, so as the N:P:K ratio amounted to 1:0.6:1.2. Lucerne seeds before sowing were dressed with nitragin containing symbiotic bacteria *Rhizobium melilotii*. Soil moisture during plant growth was kept on the level 50-60% of field water capacity.

The soil used in the experiment had the granulometric composition of heavy loamy sand (pgm), with a pH value measured in KCl with a concentration of 1 $mol \cdot dm^{-3}$ equal to 6.6. The total content of nitrogen and carbon in that soil, determined on the Perkin Elmer CHN autoanalyzer, amounted to 1.85 and 28.7 $g \cdot kg^{-1}$, respectively.

Harvest of lucerne and spring barley was performed at stages of the beginning and full flowering of lucerne (time I and II) and full maturity of both crops (time III). Collected plant material was divided into roots, stems and leaves (flowers were joined to the leaves) and at the full maturity stage additionally, seeds and stripped pods and grain and chaff, respectively, were distinguished. The amount of dry matter of individual organs of lucerne was found and the total nitrogen content and enrichment in isotope ^{15}N were determined. Additionally, the amount of nitrogen accumulated from different sources was calculated according to the formulas given by Kalembasa *et al.* [2014].

The obtained results of the study were subjected to the analysis of variance (Fischer Snedecor Ftest), and values of $NIR_{0,05}$ to compare averages were calculated using Tukey's test. Calculations were made using the software Statistica 10 Pl (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

The mass of individual organs of lucerne significantly depended on the development stage during the harvest (Table 1). At successive development stages, a significant increase in the amount of dry matter of lucerne roots, stems and leaves was observed, and consequently, of the mass of the whole plant. The percentage of stems in the dry matter of lucerne harvested at the beginning of flowering was the largest (36.3%), the percentage of leaves was slightly smaller (34.3%), and that of roots the smallest (29.5%). At the phase of full flowering, the structure of harvested mass of lucerne was at follows: roots – 34.9%, leaves – 33.6%, stems – 31.5%. After reaching full maturity, the percentage of roots, stems, leaves, seeds and stripped pods in the total yield accounted for, respectively: 44.1%, 23.4%, 23.7%, 4.0% and 4.8%. The total mass of individual organs of lucerne plants harvested at the beginning of flowering and at full flowering accounted for 25.0% and 38.1%, respectively, of the mass obtained at the full maturity stage.

Table 1. Dry matter of lucerne, g DM·pot^{-1}
Tabela 1. Suchej masa lucerny siewnej, g·wazon^{-1}

Harvested stage Faza rozwojowa podczas zbioru	Part of plant – Organy rośliny					Total mass Masa całkowita
	roots korzenie	stems łodygi	leaves liście	seeds nasiona	stripped pods strączyny	
Beginning of flowering Początek kwitnienia	6.62	8.16	7.71	–	–	22.49
Full flowering Pełnia kwitnienia	11.97	10.80	11.53	–	–	34.30
Full maturity Pełna dojrzałość	39.74	21.07	21.36	3.56	4.37	90.10
Mean – Średnia	19.44	13.34	13.53	–	–	48.96
$LSD_{0.05}$ – $NIR_{0,05}$	3.27	2.31	2.53	–	–	15.54

The lucerne yield under the soil and climatic conditions of Poland amounts on average to about 50 Mg·ha^{-1}, at the dry matter content reaching 20% [Maj *et al.* 2010]. Using the biomass of this plant to produce high protein bulky feed, one can to a large degree decrease protein deficit in feeding ruminants. Lucerne provides about 2300 kg of protein from one hectare, whereas 1400 kg are obtained from oilseed rape, and about 1000 kg from wheat. Only 800 kg of protein is obtained from cultivation of soya, which is the main plant providing protein feed used in nutrition of monogastric animals [Caillot 2008]. According to Andrzejewska *et al.* [2013], lucerne with the desired protein content and energy value can be obtained both in the budding and flowering stages.

Of the vegetative organs of lucerne, at all the development stages, the higher nitrogen content was recorded in the leaves (Table 2). It was more than two times higher than in the roots and stems. At the full maturity of lucerne plants the highest amount of nitrogen was determined in seeds. At this development stage the nitrogen content in lucerne stripped pods, stems and roots was similar. In the roots and stems of lucerne a higher nitrogen content was found at the full maturity stage than at the beginning of flowering and at full flowering (Table 2). The nitrogen content in lucerne leaves was similar at successive harvest times.

The content of ^{15}N isotope in individual organs of lucerne decreased along with the plant growth and was the lowest after reaching full maturity (Table 3). Considerable decrease in the content of ^{15}N isotope in the dry matter at the full maturity stage indicates a decreasing role of the applied fertilizer as a source of nitrogen for lucerne. A decrease in concentration of nitrogen ^{15}N in the dry matter of lucerne is the effect of higher uptake of nitrogen from other sources from the atmosphere or from the soil. Several times less content of nitrogen ^{15}N isotope was observed in individual organs of lucerne than spring barley (Table 3). This shows a smaller uptake of nitrogen from the applied mineral fertilizer enriched with ^{15}N isotope by lucerne than by barley, and consequently, it indicates uptake large amounts of nitrogen by lucerne from the atmosphere.

Table 2. The content of nitrogen in lucerne dry matter, g $N \cdot kg^{-1}$ DM
Tabela 2. Zawartość azotu w suchej masie lucerny siewnej, g $N \cdot kg^{-1}$ s.m.

Harvested stage Faza rozwojowa podczas zbioru	Part of plant – Organy rośliny					In total mass W całej masie
	roots korzenie	stems łodygi	leafs liście	seeds nasiona	stripped pods strączyny	
Beginning of flowering Początek kwitnienia	16.05	12.41	37.52	–	–	22.09
Full flowering Pełnia kwitnienia	13.35	12.51	35.19	–	–	20.43
Full maturity Pełna dojrzałość	18.85	15.12	36.12	56.19	16.99	23.46
Mean – Średnia	16.08	13.35	36.28	–	–	
$LSD_{0.05}$ – $NIR_{0,05}$	2.33	2.01	ns – ni	–	–	ns – ni

ns – ni – non-significant differences – różnice nieistotne

Table 3. Excess ^{15}N isotope of nitrogen in dry matter of lucerne and spring barley (% ^{15}N)
Tabela 3. Wzbogacenie biomasy lucerny siewnej i jęczmienia jarego w izotop ^{15}N, %

Cultivated plant Uprawiana roślina	Harvested stage Faza rozwojowa podczas zbioru	Part of plants – Organy roślin				
		roots korzenie	stems łodygi	leafs liście	seeds /grain* nasiona/ ziarno*	stripped pods/chaff* strączyny/ plewy*
Lucerne Lucerna siewna	beginning of flowering początek kwitnienia	0.898	0.964	0.984	–	–
	full flowering pełnia kwitnienia	0.611	0.721	0.739	–	–
	full maturity pełna dojrzałość	0.166	0.275	0.286	0.266	0.333
	Mean – Średnia	0.588	0.653	0.670	–	–
Spring barley Jęczmień jary	beginning of lucerne flowering początek kwitnienia lucerny	5.172	5.577	6.396	–	–
	full flowering of lucerne pełnia kwitnienia lucerny	4.727	5.291	5.126	–	–
	full maturity of barley pełna dojrzałość	4.294	4.934	4.934	4.965	5.219
	Mean – Średnia	4.731	5.267	5.485	–	–

* depending on cultivation plants: for lucerne, the columns concern the stripped pods and seeds, respectively; whereas for barley, they concern the chaff and grain, respectively – w zależności od uprawianych roślin: dla lucerny opisywane kolumny dotyczą odpowiednio strączyn i nasion, a dla jęczmienia – plew i ziarna

Irrespective of the development stage during harvest, lucerne accumulated the highest amount of nitrogen in leaves (Table 4). At the full maturity stage lucerne accumulated a similar amount of nitrogen in leaves and roots. The total amount of nitrogen taken up by the roots, stems and leaves of lucerne increased in successive lucerne development stages. At the full maturity stage lucerne accumulated more than four times more nitrogen than at the beginning of flowering and about three times more than at full flowering. At all plant development stages, as well as in all lucerne organs and totally in the whole dry matter, most nitrogen was taken up from the atmosphere.

Table 4. The amount and percentage of nitrogen taken up by lucerne from different sources, $mg N \cdot pot^{-1}$
Tabela 4. Ilość i procentowy udział azotu pobranego przez lucernę siewną z różnych źródeł, $mg N \cdot wazon^{-1}$

Harvested stage Faza rozwojowa roślin podczas zbioru	Sources of nitrogen Źródła azotu	Parts of plant – Organy rośliny										Sum – Suma	
		korzenie roots		łodygi stems		liście leaves		nasiona seeds		strączyny stripped pods			
		mg	%	mg	%	mg	%	mg	%	mg	%	mg	%
Beginning of flowering Początek kwitnienia	atmosphere atmosfera	87.9	82.7	83.7	82.7	244.7	84.6	–		–		416.3	83.8
	fertilizer nawóz	9.3	8.7	9.5	9.4	27.8	9.6					46.6	9.4
	soil – gleba	9.1	8.6	8.0	7.9	16.8	5.8					33.9	6.8
	sum – suma	106.3	100.0	101.2	100.0	289.3	100.0					496.8	100.0
Full flowering Pełnia kwitnienia	atmosphere atmosfera	139.2	87.1	116.7	86.4	347.3	85.6	–		–		603.2	86.1
	fertilizer nawóz	9.4	5.9	9.5	7.0	29.2	7.2					48.1	6.9
	soil – gleba	11.2	7.0	8.9	6.6	29.2	7.2					49.3	7.0
	sum – suma	159.8	100.0	135.1	100.0	405.7	100.0					700.6	100.0
Full maturity Pełna dojrzałość	atmosphere atmosfera	719.9	96.1	300.8	94.4	726.8	94.2	189.8	94.9	69.2	93.3	2006.5	94.9
	fertilizer nawóz	12.0	1.6	8.6	2.7	21.6	2.8	5.2	2.6	2.4	3.2	49.8	2.4
	soil – gleba	17.2	2.3	9.2	2.9	23.1	3.0	5.0	2.5	2.6	3.5	57.1	2.7
	sum – suma	749.1	100.0	318.6	100.0	771.5	100.0	200.0	100.0	74.2	100.0	2113.4	100.0
$LSD_{0.05}$ for total nitrogen uptaken by lucerne at different growth stages $NIR_{0,05}$ dla całkowitej ilości N pobranej przez lucernę w różnych fazach rozwojowych		87.8		47.7		96.0		–		–		329.2	

The proportion of biologically reduced nitrogen in the dry matter of this species at the beginning of flowering, at full flowering and at maturity was, respectively: 83.8; 86.1 and 94.9% of the total nitrogen taken up from different sources (atmosphere, fertilizer, soil). The similar percentage of nitrogen taken up from the atmosphere in individual organs of lucerne indicates its proportional distribution in the studied plant (Table 4).

The amount of nitrogen taken up from the mineral fertilizer and from the soil was small in comparison with that taken up from the atmosphere (Table 4). At the beginning of flowering lucerne took up slightly more nitrogen from the fertilizer (9.4%) than from the soil (6.6%). At the other development stages nitrogen uptake from the fertilizer and soil was similar and amounted to about 7.0% at full flowering and 2.4-2.7% at the full maturity of lucerne.

Walley [2013] claimed that it is not necessary to apply nitrogen fertilization in cultivation of lucerne, since this species takes up about 90% of this macroelement from the air, as a result of symbiosis with bacteria *Rhizobium*. However, other authors think that at the initial stage of lucerne growth, before the beginning of the effective fixing atmospheric nitrogen, a small amount of nitrogen is necessary for proper development of root nodules [Oliveira *et al.* 2004]. High level of nitrogen compounds in the soil has a negative effect on symbiosis. It leads to a decrease in the amount of nodules on the plant roots and inhibits the activity of bacterial nitrogenase, and consequently, reduces the amount of nitrogen fixed by the plant [Marek-Kozaczuk *et al.* 2006]. More than ninety per cent of nitrogen taken up from the atmosphere in the biomass of lucerne harvested at the full maturity stage obtained in the present study indicates the high effectiveness of reduction of atmospheric nitrogen by symbiotic bacteria. Similar conclusions were obtained by Mayer *et al.* [2003], who found that the amount of nitrogen derived from the atmosphere in plants at the full maturity stage may range from 80 to 93%.

The obtained data indicate that the largest amounts of nitrogen were accumulated in the leaves of lucerne, which confirms the thesis about accumulation of macroelements in the aboveground mass [Wilczewski 2007]. A part of nitrogen taken up by lucerne from the atmosphere is accumulated in the roots. In the present study at the full maturity stage of lucerne, in the roots there was 35.4% of the total amount of accumulated nitrogen (Table 4). By introducing this plant into crop rotation with annual plants, an increase in yield may be obtained caused by releasing nitrogen into soil from decomposing roots, nodules and post-harvest residues [Russelle *et al.* 1994].

CONCLUSIONS

1. The average content of nitrogen in the whole mass of lucerne harvested at the beginning of flowering and at full flowering and after reaching full maturity was similar. The largest amounts of nitrogen were accumulated in leaves.
2. The basic source of nitrogen for lucerne was atmosphere. At the beginning of flowering and at full flowering as well as at full maturity the percentage of biologically reduced nitrogen in the dry matter of lucerne was, respectively, 83.8; 86.1 and 94.9%,.
3. The percentage of nitrogen taken up by lucerne from the mineral fertilizer and from the soil reserve was, respectively, 9.4% and 6.8% at the beginning of flowering, 6.9% and 7.0% at full flowering, as well as 2.4% and 2.7% at the full maturity stage.
4. Taking up nitrogen mostly from the atmosphere indicates the ability to grown lucerne without fertilization with this macroelement.

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DYNAMIKA GROMADZENIA AZOTU Z RÓŻNYCH ŹRÓDEŁ PRZEZ LUCERNĘ SIEWNĄ (*Medicago sativa* L.)

Streszczenie. Biomasa lucerny siewnej zbieranej w fazie pąkowania lub początku kwitnienia stanowi paszę objętościową o wysokiej zawartości białka. Rzadziej roślina ta zbierana jest w fazie pełnej dojrzałości na nasiona. W uprawie lucerny często nie stosuje się nawożenia azotem, ponieważ żyje ona w symbiozie z bakteriami brodawkowymi redukującymi azot atmosferyczny do form amonowych dostępnych dla roślin. W pracy przedstawiono wyniki badań z doświadczenia wazonowego, w którym określono wpływ fazy rozwojowej lucerny siewnej na dynamikę pobierania azotu z atmosfery, z nawozu mineralnego i z gleby. Badaną roślinę zbierano w fazie początku i pełni kwitnienia oraz po uzyskaniu pełnej dojrzałości. W nawożeniu rośliny testowej zastosowano siarczan amonu wzbogacony w izotop ^{15}N i wykorzystano metodę izotopowego rozcieńczenia do oceny dynamiki pobierania azotu. Rośliną kontrolną był jęczmień jary. W kolejnych fazach rozwojowych odnotowano systematyczny przyrost suchej masy lucerny siewnej. W fazie początku i pełni kwitnienia lucerny udział korzeni, łodyg i liści w masie całej rośliny był zbliżony, natomiast w fazie pełnej dojrzałości największy udział w plonie suchej masy miały korzenie (44%), a najmniejszy nasiona (4%) i strączyny (5%). Średnia zawartość azotu w całej suchej masie badanego gatunku była zbliżona w kolejnych terminach zbioru. W fazie początku i pełni kwitnienia największą ilość azotu lucerna zgromadziła w liściach, natomiast po uzyskaniu pełnej dojrzałości w liściach i korzeniach. Głównym źródłem azotu dla badanej rośliny w kolejnych fazach rozwojowych była atmosfera. Azot biologicznie zredukowany przez lucernę zbieraną w fazie początku i pełni kwitnienia stanowił odpowiednio 83,8 i 86,1%, a w fazie pełnej dojrzałości 94,9% całkowitego pobrania tego makroelementu. Pozostała, niewielka ilość azotu była pobrana z nawozu mineralnego i z gleby. W fazie początku i pełni kwitnienia lucerny udział azotu pobranego z nawozu nie przekraczał 10%, a z gleby maksymalnie wyniósł 7%. W fazie pełnej dojrzałości do zbioru na nasiona udział azotu pobranego z nawozu i gleby wynosił odpowiednio 2,4 oraz 2,7%.

Słowa kluczowe: azot, izotop ^{15}N, lucerna, N_2 biologicznie zredukowany

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