



ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2014
Volume 17
Issue 2

Topic:
Agronomy

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Prusiński J. 2014. DYNAMICS AND DISTRIBUTION OF DRY MATTER AND TOTAL NITROGEN IN YELLOW LUPINE (*LUPINUS LUTEUS* L.) PLANTS, EJPAU 17(2), #04.
Available Online: <http://www.ejpau.media.pl/volume17/issue2/art-04.html>

DYNAMICS AND DISTRIBUTION OF DRY MATTER AND TOTAL NITROGEN IN YELLOW LUPINE (*LUPINUS LUTEUS* L.) PLANTS

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ABSTRACT

Pot experiment on yellow lupine cv. Mister covered the differentiated N_{min} content in plant growth medium and methods of seed preparation for sowing, including seed inoculation with *Bradyrhizobium lupini* and/or genistein. Abundance of N_{min} in the growth substrate as well as method of preparing seeds for sowing did not affect the formation of the mean dry matter of yellow lupine plants and their total N content. The highest dry matter of seeds per one lupine plant was obtained with a high (calculated for field conditions $80 \text{ kg N} \cdot \text{ha}^{-1}$) N_{min} content in the growth substrate. The total N content in the aboveground parts of plants significantly decreased since 35 days after emergence, and was significantly the lowest at full seed maturity, while in the underground parts the total N underwent significantly smaller changes during vegetation. The mean N content in yellow lupine seeds did not significantly depend on the abundance of N_{min} in the growth substrate, nor on the use of seed inoculation with rhizobia and genistein.

Key words: lupine, N_2 , N_{min} , *Bradyrhizobium lupini*, genistein.

INTRODUCTION

Nitrogen accumulation in plants remains under ontogenetic control, and its total content increases until all the leaves develop and just before flowering starts to decrease [12]. With the sufficient N content in the soil, its uptake is determined by the rate of plant growth, and decreases along with an increase in the plant weight [11,12,15]. The course of N uptake and fixing N_2 in legumes may be an important determinant of the yield and N content in a plant. The combined effect of the mineral and symbiotically fixed N on nodulation and legumes yield is generally well-known [16]. However, the dynamics and accumulation of N, and dry matter, in vegetative and generative parts of legume plants, depending on its availability and origin (mineral vs symbiotic), are interpreted in a very different way.

Seed inoculation with rhizobia does not vary significantly accumulation of the dry matter in white lupine organs [2], but it favorably affects the N content in seeds of this species [13]. On the other hand, the pre-sowing diversification of mineral N doses has no effect or slightly changes the content of the dry matter of white lupine [2] and nitrogen content in blue lupine plants [9] and field pea [26], which according to Vessey et al. [25], means that root nodules may successfully compete with pods for assimilates at the time of their filling. On the other hand, according to Merbach et al. [17], increase in the N doses for blue lupine causes increase in the dry matter of plants and in the total N content. Tang et al. [24] found that N concentration in legumes decreases along with an increase in the amount of N_{min} available in the growth substrate, which decreases the rate of N_2 assimilation most frequently observed in pea, and the least in white lupine. Barłóg [3] found a significant effect of nitrogen fertilization on the N content in seeds and leaves of blue lupine, however only until the stage of 6–7 leaves. On the other hand, Ciesiolka et al. [6], found that mineral N affects the vegetative growth in lupine plants much more than N_2 . Also isoflavonoids, eg. genistein, secreted by lupine roots [7], through a significant reduction of time until the formation of first nodules and stimulation of active assimilation of N_2 , increase the total N content in a plant [4], especially in the soil with a low content of N_{min} [18].

The author's research hypothesis assumes that diversification in the content of nitrogen available for yellow lupine will affect the rate of growth and accumulation of the dry matter of plants, including the total N content. The application of rhizobia and/or genistein should facilitate explanation of correlations between the effect of N_{\min} and N symbiotically fixed on the dynamics of plant growth and state of their nutrition with nitrogen.

MATERIALS AND METHODS

The two-factorial pot experiment was carried out in the years 2009–2011 under a fixed plastic tunnel in Mochelek, at the Experimental Station of the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences. The subject of research was conventional yellow lupine cultivar, Mister. The experimental design included the following factors:

A – N_{\min} content in $\text{mg} \cdot \text{kg}^{-1}$ of dry matter of a mixture of lessive soil (37.5%) and river sand (62.5%) determined according to the Polish Standard [20]:

- a1 – $4.45 \text{ mg } N_{\min} \cdot \text{kg}^{-1} \text{DM}$ – very low,
- a2 – $8.90 \text{ mg } N_{\min} \cdot \text{kg}^{-1} \text{DM}$ – low,
- a3 – $13.3 \text{ mg } N_{\min} \cdot \text{kg}^{-1} \text{DM}$ – medium,
- a4 – $17.8 \text{ mg } N_{\min} \cdot \text{kg}^{-1} \text{DM}$ – high,
- a5 – $22.5 \text{ mg } N_{\min} \cdot \text{kg}^{-1} \text{DM}$ – very high.

B – method of seed preparation for sowing:

- b1 – control – dressed seeds, not inoculated,
- b2 – dressed seeds, inoculated with an agar inoculation containing *Bradyrhizobium lupini*,
- b3 – dressed seeds, inoculated with an agar inoculation containing *Bradyrhizobium lupini* with an addition of genistein (4',5,7-trihydroxyisoflavone).

Carbendazim (Sarfun 50 WP at a dose of 200 g/100 kg seeds⁻¹) was used for dressing the seeds. In combination b2, the agar suspension of *Bradyrhizobium lupini* bacteria (10^8 – 10^{10} bacterial cells in 1 ml) prepared by the Department of Agricultural Microbiology of the Institute of Soil Science and Plant Cultivation (IUNG) in Pulawy (POLAND) was dissolved in distilled water, and next it was stirred for 24 hours in a laboratory shaker at a room temperature. In combination b3 – 60 μM genistein (SIGMA Aldrich) was added to the inoculation prepared in this way, previously dissolved in 1 ml of methanol, and again it was stirred for 24 hours. The seeds were sprayed with a suspension of *Bradyrhizobium lupini* (b2) or *Bradyrhizobium lupini* with genistein (b3), and after their drying up they were sown on the same day.

Each combination was set up in 8 replications (120 Koch pots of a diameter of 22.5 cm and height 25 cm with a full bottom), which were dug into the soil in order to protect them against excessive drying, and then placed in a plastic tunnel. The pots were filled with 9 kg of lessive soil mixture (37.5%) of a granulometric composition of light loamy sand and pH 6.1 – 6.4, taken from the arable land at the Experimental Station in Mochelek, of an average content of P, K and Mg and river sand (62.5%). After screening the lessive soil on sieves of 1 cm mesh diameter, it was first subjected to rinsing in running water, and next to thermal disinfection at a temperature of 70°C for 24 hours. With such a proportion of lessive soil and river sand, the N_{\min} content amounted to $4.45 \text{ mg} \cdot \text{kg}^{-1} \text{DM}$; the N_{\min} content in combinations a2 – a5 was obtained through addition of a proper amount of 34% ammonium saltpeter containing 50% N-NO₃ and N-NH₄. The growth substrate was also enriched with P and K, leading to their high content. Stable moisture of the growth substrate in pots was maintained on the level of 60% field water capacity.

Nine yellow lupine seeds, cv. Mister, were sown into each pot and after emergence 5 plants were left in each pot for further analyses. After 7 (c1), 14 (c2), 21 (c3), 28 (c4) and 35 (c5) days since the beginning of emergence, and next at the stage of full flowering (c6) and physiological maturity (c7) and full maturity (c8) of seeds, 15 pots were eliminated by turns. On all days, except c1 (an insufficient amount of material to determine N), the dry matter was determined as well as the total N content, with Kjeldahl method in the under- and aboveground parts (stems and leaves, with pod shells and without seeds) in each plant per pot separately (after drying at a temperature of 60°C for 24 hours). At full maturity, from each pot, the weight of seeds and vegetative parts was determined for each plant separately.

Dynamics of the dry matter and total N was determined based on the absolute rate of plant growth in yellow lupine (GR) and accumulation of total nitrogen (AR) on the basis of Evans' equations [8]:

$$\text{GR} = \frac{W_2 - W_1}{t_2 - t_1} \quad [\text{g} \cdot \text{day}^{-1}]$$

where:

GR – plant growth rate

W1 – plant dry matter [g] at the beginning of the measurement period

W2 – plant dry matter [g] at the end of the measurement period
t1 – beginning of the measurement period
t2 – end of the measurement period

$$AR = \frac{M2 - M1}{t2 - t1} \text{ [g}\cdot\text{day}^{-1}\text{]}$$

where:
AR – N accumulation
M1 – N weight [mg] in the plant dry matter [g] at the beginning of the measurement period
M2 – N weight [mg] in the plant dry matter [g] at the end of the measurement period
t1 – beginning of the measurement period
t2 – end of the measurement period

Also, the *Harvest Index* (HI) was calculated (quotient of the dry matter of seeds per plant divided by the dry matter of the whole plant, i.e. vegetative parts (above- and underground) and generative ones as well as *Nitrogen Harvest Index* (NHI) (quotient of the amount of N contained in the dry matter of collected lupine seeds per plant divided by its total N content at the stage of full maturity, both in the seeds and vegetative above- and underground parts) [10].

The obtained results were subjected to analysis of variance for the complete randomized block design with the use of the combined error model, while significance of differences was verified with Tukey test on the level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

In the author’s own research it was assumed that diversified content of N_{min} in pots, and seed inoculation with *Bradyrhizobium lupini* with an addition of genistein, would affect the growth rate in yellow lupine plants, including the increase in their dry matter and total N content in the above- and underground parts. The basic method of evaluating the degree to which a plant is nourished with nitrogen is chemical analysis of its parts, above all leaves [10]. The critical nitrogen content is defined as its minimum content, which allows for maximum growth rate of plants. Under conditions of suboptimal supplementation of plants with N in the growth substrate before sowing, N uptake depends on its availability and arrangement of the root system in plants [11], however a severe N deficiency leads to impairment of many growth processes and plant metabolism, and consequently to a decrease in the biomass of plants and their organs [28].

Plants accumulate in their tissues both nitrogen taken from the soil in the form of various compounds and ions [23] and, as in the case of legumes, N which is fixed symbiotically. In the author’s own research, N_{min} availability in the growth substrate and method of preparing seeds for sowing, despite significant diversification, had no significant effect on the formation of a mean dry matter of yellow lupine plants and total N content (Tab. 1), which confirms research results on different species of legume plants, from the studies of, among others, Ayisi et al. [2], Fan et al. [9], Vessey et al. [25], and Voisin et al. [26] but is inconsistent with observations made by e.g. Merbach et al. [17]. The mean dry matter of one plant (without seeds) amounted to 3.72 g, including its underground part, 0.80 g, while the aboveground part was 2.92 g. The N content in the aboveground parts (without seeds) was almost twice higher than in the underground parts. Only the dry matter of seeds per plant depended significantly on the N content, and was the highest (3.25 g) with the high N_{min} content in the growth substrate (a4 – calculated for field conditions 80 kg N·ha⁻¹), and significantly higher with the highest N_{min} content (a5 – calculated for field conditions 100 kg N·ha⁻¹) (Tab. 2). No interaction of both these factors was found in the formation of productivity of one lupine plant.

Table 1. The mean dry matter and the total N content in yellow lupine plants depending on the N_{min} content in the growth substrate and method of preparing seeds for sowing

Trait	Experimental factors		Mean
	N_{min} content in the growth substrate	Method of preparing seeds for sowing	
Dry matter of 1 plant [g]	ns	ns	3.72
Dry matter of under-ground part of 1 plant [g]	ns	ns	0.80
Dry matter of above-ground part of 1 plant [g]	ns	ns	2.92
N content in under-ground parts in DM [g·kg ⁻¹]	ns	ns	16.5
N content in above-ground parts in DM [g·kg ⁻¹]	ns	ns	31.2
N content in seeds [g·kg ⁻¹] in DM	ns	ns	62.3
Dry matter of seeds per plant [g]	s	ns	2.76
ns – the impact of factor not significant			
s – the impact of factor significant			

Table 2. The mean dry matter of seeds per yellow lupine plant depending on the used experimental factors

	Method of preparing seeds for sowing	

N _{min} content in the growth substrate mg·kg ⁻¹ in DM	Control Sarfun 50WP	Sarfun 50WP <i>Bradyrhizobium lupini</i>	Sarfun 50WP <i>Bradyrhizobium lupini</i> + genisteina(genistein)	Mean
Very low	2.60	2.79	2.52	2.64 ab
Low	2.70	2.85	2.58	2.71 ab
Medium	2.60	2.52	2.63	2.58 ab
High	3.10	3.35	3.29	3.25 a
Very high	2.42	2.61	2.77	2.60 b
Mean	2.68 A	2.82 A	2.76 A	2.76
Mean values followed by the same capital letters in rows and lower-case letters in columns did not differ significantly for $\alpha = 0.05$				

During the whole vegetation period, no significant differences were also observed in the formation of the mean dry matter of yellow lupine plants under the effect of diversified N_{min} content (Fig. 1a) and method of preparing seeds for sowing (Fig. 1b). According to Tang et al. [1999], concentration of N in pea plants decreases along with an increase of N in the growth substrate, which was not observed in the author's own research on yellow lupine. Up to 35 days after emergence (c5), statistically similar growth rate was observed in the dry matter of the above- and underground parts of lupine, after which the dry matter of the aboveground parts quickly and significantly increased until the full maturity (c8), while the dry matter of the underground parts remained without any significant changes from the full flowering (c6) until the end of the vegetation period (c8) (Fig. 2a). The highest increase in the dry matter of the aboveground parts (GR) was observed between 35 days since emergence (c5) until physiological maturity of seeds (c7), while the underground parts between 35 days (c5) since emergence and full flowering (c6) (Fig. 2b). In the field study, the maximum dry matter of yellow lupine plants of conventional cultivar Polo, was obtained a bit later, from the stage of flat pod to physiological maturity of seeds [21].

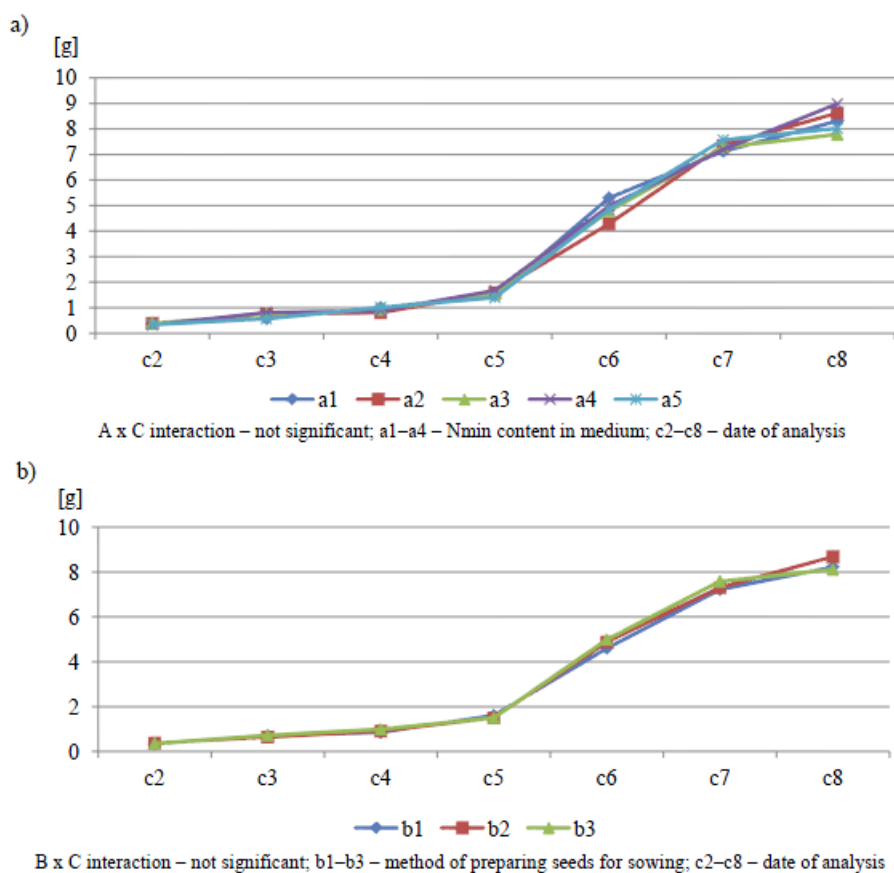


Fig. 1. Dynamics of the dry matter of yellow lupine plant depending on the N_{min} content in the growth substrate (a) and method of seed preparation for sowing (b)

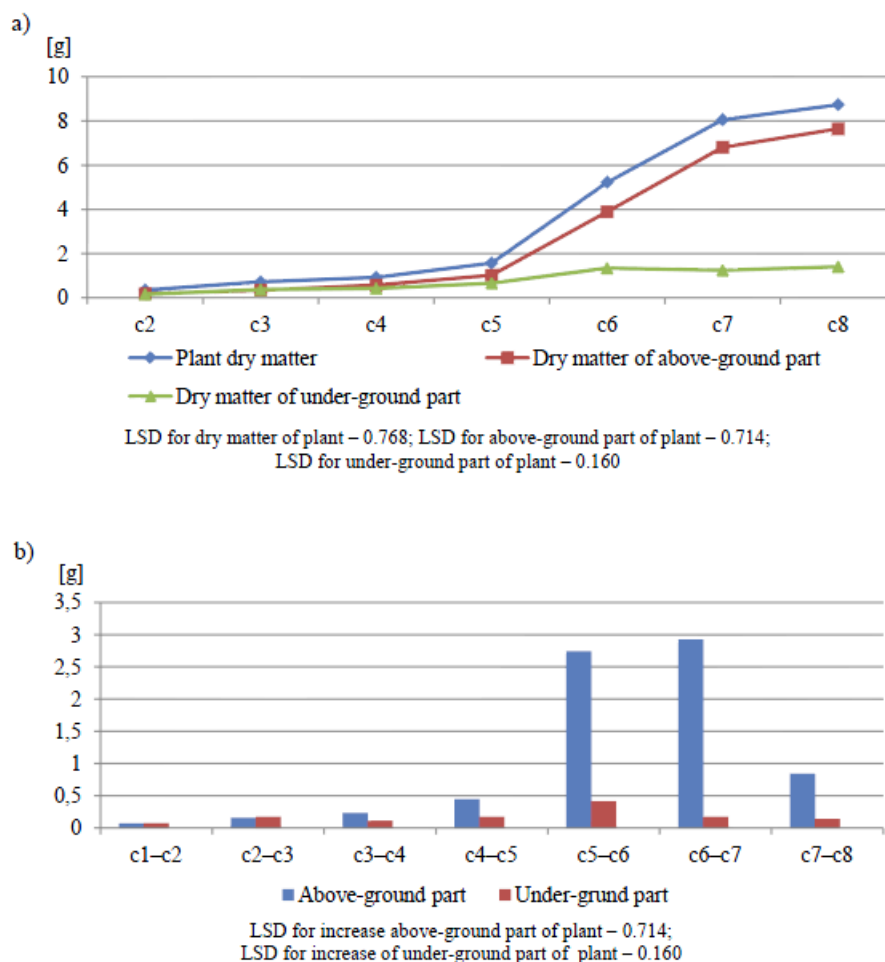


Fig. 2. Dynamics of dry mater of yellow lupine plant (a) and 24-hour increase (GR) of above- and under-ground part of plant (b)

The mean total N content in the dry matter of the underground parts of lupine was $16.5 \text{ g N} \cdot \text{kg}^{-1}$ (Tab. 1) and was significantly the highest between 28 (c4) and 35 (c5) days after emergence, after which it slightly decreased until the end of growing, reaching a condition as at the beginning of growing (between 14 and 21 days after emergence) (Fig. 3a). On the other hand, in the aboveground parts on average $31.2 \text{ g N} \cdot \text{kg}^{-1}$ were found in the dry matter, and since the beginning of vegetation, its gradual and significant decrease was observed until the end of the vegetation period. The N content, along with a plant growth decreased as a result of its slower accumulation as compared to the rate of the dry matter increase [15]. According to Greenwood et al. [12], with a plant development and accumulation of higher dry matter yields, there also occurs a specific dilution of the N content in a plant. At the end of the vegetation period, it was probably connected with aging of the nodules [27], a decrease in the physiological activity of the roots and redistribution of N from vegetative parts to pods and seeds [14] as well as reducing photosynthesis [28], which is represented in the author's own research by a rapid daily decrease in the N content in the aboveground parts in the period from physiological to full maturity (Fig. 3b). In the field study, the highest nitrogen content, over 4% in the total dry matter, was observed in the leaves of yellow lupine at the stage from budding to the beginning of flowering [21]. According to Flynn et al. [10], the optimum N content in soybean leaves in the period just before flowering, is 4.2–5.5%.

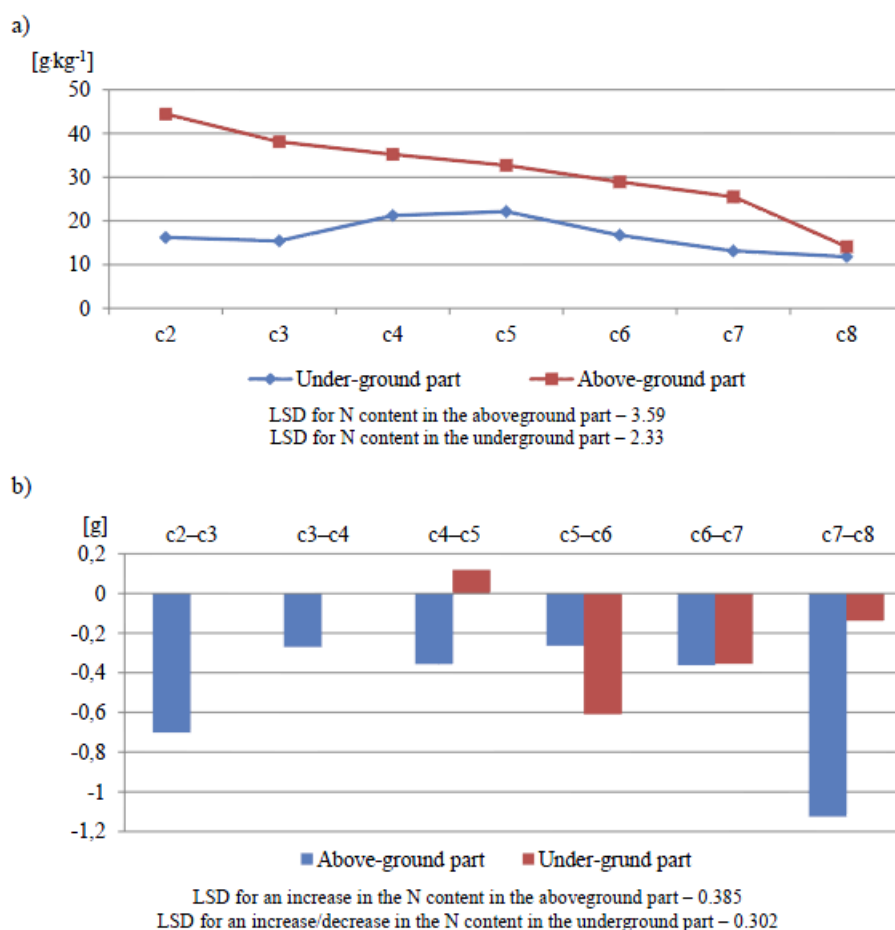


Fig. 3. Dynamics of the total N content (a) and its increase/decrease (AR) in the under- and above-ground parts of yellow lupine (c1–c8 – date of analysis) (b)

The mean N content in yellow lupine seeds was 62.3 g kg^{-1} (Tab. 1), and as in the research of Voisin et al. [26], did not significantly depend on the abundance of N_{\min} in the growth substrate. According to Ciesiolka et al. [6], N_2 as well as $N\text{-NO}_3$, as distinct from the ammonium form, favorably affect the N content in white lupine seeds and the vegetative matter of plants. However, in the author's own research proportion of $N\text{-NH}_4$ in the growth substrate of plants in their stage of seed filling constituted less than 20% [22]. Thus, no beneficial effect of an increasing N_{\min} content in the growth substrate was confirmed [3], nor of inoculating seeds with rhizobia [13], on the total nitrogen content in lupine seeds.

From the literature data it follows that isoflavonoids, eg. genistein, secreted by lupine roots [7] may affect an increase in the total N content in field pea plants [4] and soybean seeds [19], especially in the soil with a low N_{\min} content [18], which was not confirmed in the author's own research. Moreover, no significant interaction was observed of the availability of N_{\min} and genistein in the growth substrate, in the formation of the total N content at any stage of chemical analysis of plants, nor in the seeds.

The mean harvest index (HI) and nitrogen harvest index (NHI) were relatively high: 42.6% and 56.6%, respectively (Tab. 3). Values of both indices did not significantly depend on the studied factors nor their interaction, which confirms previous results concerning lack of the effect of both studied factors on the dry matter of plants and their N content. Under field conditions, HI for lupine is generally low, approximately 40% [21], with respect to high straw yields, mainly stems, and it depends more on the plant density. On the other hand, NHI mainly depends on the plant density, and even on the depth at which legume plants are sown [1].

Table 3. Seed yield and N indices and distribution of the dry matter and total N in a yellow lupine plant

Trait	Experimental factors		Mean
	N_{\min} content in the growth substrate	Method of preparing seeds for sowing	
Harvest Index [%]	ns	ns	42.4
N Harvest Index (NHI) [%]	ns	ns	65.4

ns – the impact of factor not significant

Distribution of the dry matter and the total N in a lupine plant on the harvest day is presented in Table 4. A little over 70% of N accumulated in a plant was found in seeds, which constituted only 23.5% of the dry matter of the whole plant. In the underground parts, the proportion of the dry matter and N content was similar, 11.8 and 13.3%, respectively, which confirms

previous results of the author's own field studies [22] on yellow lupine, as well as Książak's research [14] on faba bean.

Table. 4. Distribution of the dry matter and total N content in a yellow lupine plant on the day of harvest

Structure of a yellow lupine plant	Share – % by weight	
	Dry matter	N total
Seeds	23.5	70.7
Above-ground part	64.7	16.0
Under-ground part	11.8	13.3

According to Ciesiolka et al. [6], the ammonium form of mineral N has a more unfavorable effect than other N forms in various mineral fertilizers on the assimilation of N_2 and accumulation of nitrogen in white lupine plants, as well as on their vegetative matter, mainly because of a decreased intensity of photosynthesis [23]. After 45 days since sowing, the dry matter of vegetative parts in faba bean fertilized with NO_3 was twice higher than in the one fertilized with NH_4 [5]. In the author's own research, a dynamic change in the content of both N forms in the growth substrate of yellow lupine was observed, until 35 days after emergence, N- NO_3 form prevailed, and next N- NH_4 , while its proportion decreased along with a progressing vegetation [22]. Perhaps, the inhibiting effect of high N_{min} doses, including mainly NH_4^+ , and its fluctuation during vegetation, on N_2 assimilation was compensated through an increased uptake of mineral N, therefore probably no significant differences in the dry matter of plants and their total N content were found, despite the use of a highly diversified N_{min} content in the growth substrate (from 20 to 100 kg N·ha⁻¹).

CONCLUSION

Abundance of N_{min} in the growth substrate as well as method of preparing seeds for sowing, despite a significant diversification, remained without a significant effect on the formation of the mean dry matter of yellow lupine plants and their total N content. The highest dry matter of seeds per one lupine plant was obtained with a high (calculated for field conditions 80 kg N·ha⁻¹) N_{min} content in the growth substrate. Starting with the day 35, since the beginning of emergence, a significant increase in the dry matter of the aboveground parts was observed, while the dry matter of the underground parts did not undergo any significant changes until the end of vegetation. The total N content in the aboveground parts of plants significantly decreased since 35 days after emergence, and was significantly the lowest at full seed maturity, while in the underground parts the total N underwent significantly smaller changes during vegetation. The mean N content in yellow lupine seeds did not significantly depend on the abundance of N_{min} in the growth substrate, nor on the use of seed inoculation with rhizobia and genistein. No significant interaction between availability of N_{min} , seed inoculation with *Bradyrhizobium lupini* and genistein was confirmed in the formation of the total N content in plants at any stage of plants' chemical analysis

Acknowledgement

This work was carried out and financed by a research project of Scientific Research Committee N N310 148135 entitled *Mineral nitrogen vs. molecular nitrogen in the cultivation of legume plants*.

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Accepted for print: 14.04.2014

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