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ORIGINAL PAPER

EFFECT OF VARIOUS NITROGEN DOSES ON THE ACCUMULATION OF MOLYBDENUM, BORON AND IRON IN YELLOW LUPINE BIOMASS

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Abstract

The availability of Mo, B and Fe is particularly important for legume plants since they live in symbiosis with bacteria in root nodules, which reduce atmospheric nitrogen to species available to plants. This field experiment was conducted to determine the content and accumulation of Mo, B and Fe by roots, stems, leaves, flowers, pods and seeds of yellow lupine. The growth phase (65 BBCH and 90 BBCH) in which the plants were harvested and the amount of nitrogen (0, 30 and 120 kg N ha^{-1}) introduced to the soil before sowing were the factors under study. The content of B and Fe was smaller in lupine harvested during the flowering phase (19.5 and 416.2 mg kg^{-1}, respectively) than after it had reached full ripeness (22.9 and 457.7 mg kg^{-1}, respectively). The growth phase in which lupine was harvested was found to have no effect on the content of Mo in the biomass obtained. Yellow lupine fertilised with 120 kg N ha^{-1} contained less Mo and Fe compared to its cultivation with no nitrogen fertilisation and to the application of nitrogen at 30 kg N ha^{-1}. Different nitrogen fertilisation did not have a significant effect on the content of B in the lupine biomass. The amount of boron and iron taken up by lupine did not depend on the nitrogen fertilisation. A larger amount of Mo was taken up by lupine grown with no nitrogen fertilisation than when doses of 30 and 120 kg N ha^{-1} were applied. The amount of Mo, B and Fe taken up by lupine harvested in the full ripeness phase was greater by 82%, 130% and 126%, respectively, than in the flowering phase. The greatest amount of Mo was accumulated in stems during the flowering phase and in seeds during the full ripeness phase. The greatest amounts of B and Fe were accumulated in leaves, regardless of the growth phase. The correlation coefficients calculated in the experiment did not reveal any significant relationships between the content of Mo, B and Fe in the biomass of yellow lupine and the amount of nitrogen taken up from the atmosphere.

Keywords: molybdenum, boron, iron, yellow lupine, nitrogen fertilization, growth stage.

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INTRODUCTION

Molybdenum, boron and iron are needed by legumes for the chemical reduction of atmospheric nitrogen (N_2) and nitrogen nutrition (Brown et al. 2002, Brear et al. 2013, Weisany at al. 2013, Gonzalez-Guerrero at al. 2016, Hemantaranjan at al. 2016). Their deficiency may be manifested as a deficiency of plant N (Pollock et al. 2002). Enzymes in nodule plants participate in nitrogen metabolism, including the nitrogen fixation process by legumes (Mendel, Haensch 2002, Bell et al. 2003, Alam et al. 2015). The symbiotic bacterial enzyme nitrogenase comprises two subunits, one of which is the MoFe protein directly involved in the reduction of N_2 to NH_3. The supply of molybdenum and iron to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes (Cao et al. 2005, Kaiser et al. 2005, Hu, Ribbe 2013, Weisany at al. 2013). Availability of molybdenum and boron is closely correlated with nodule development. Increased availability of these micronutrients can enhance nitrogen-fixing symbiosis through increased nitrogenase activity and larger nodules (Vieira et al. 1998*a*,*b*, Redondo-Nieto et al. 2003). Molybdenum deficiencies would also impact the ability of the plant to efficiently export reduced nitrogen from nodules (Pastori, Rio 1997, Hesberg et al. 2004). In boron-deficient plants, the number of Rhizobia infecting the host cells and the number of infection threads were reduced and the infection threads developed morphological aberrations. The cell walls of root nodules of boron-deficient plants showing structural aberrations were reported to lack the covalently-bound hydroxyproline/proline rich proteins, which contribute to an O_2 barrier, preventing inactivation of nitrogenase and an associated decrease in N fixation (Weisany at al. 2013).

The aim of this experiment was to determine the effect of different nitrogen fertilisation regimes and the growth phase on the content and amounts of molybdenum, boron and iron accumulated in different organs of yellow lupine (*Lupinus luteus* L.).

MATERIAL AND METHODS

A field experiment was conducted in Siedlce (N52°10′12.04″ E22°17′15.40″), in 2008 and 2011. 1 m^2 plots were delineated in a field of the yellow lupine cultivar Mister. A two-factorial experiment was set up in a randomised split--block design, with three replications. Nitrogen fertilisation was the first factor: a) control, with no nitrogen fertilisation; b) with nitrogen applied at a dose equivalent to 30 kg N ha^{-1}; c) with nitrogen applied at a dose equivalent to 120 kg N ha^{-1}. The time of harvest was the second factor (determined as per Bleinholder et al. 2001): a) full flowering phase, 65 BBCH (date I);

b) full ripeness phase, 90 BBCH (date II). Mineral nitrogen was introduced to the soil as ammonium sulphate $(NH_4)_2SO_4$ before yellow lupine was sown. The amounts of phosphorus and potassium were established according to the amounts of the available element species in soil. Potassium was introduced to the soil in all plots as potassium salt dosed at 100 kg K ha^{-1}. Because of a very high amount of available phosphorus (Table 1), no phosphorus fertilisation was applied. Before sowing, seeds of yellow lupine were inoculated with a vaccine containing *Rhizobium lupini*. Sowing was performed in early April

Table 1

Selected properties of soil in the humus layer prior to the field experiments conducted in 2008 and 2011

Soil properties	Unit	Years of the experiment	
		2008	2011
pH_{KCl}	–	5.9	5.8
C_{tot}	(g kg^{-1})	25.7	23.8
N_{tot}		2.04	1.92
P_{tot}		1.10	1.15
K_{tot}		0.845	0.810
Mg_{tot}		0.961	0.927
S_{tot}		0.448	0.561
$N\text{-}NH_4^+$	(mg kg^{-1})	4.091	2.557
$N\text{-}NO_3^-$		9.016	8.605
P_{av}		369.0	314.0
K_{av}		67.0	59.0
Mg_{av}		43.6	39.2
Mo_{tot}		0.120	0.086
B_{tot}		4.245	0.987
Mn_{tot}		155.2	157.9
Cu_{tot}		20.3	18.0
Fe_{tot}		5243.3	5135.0
Zn_{tot}		219.1	176.0
Mo_{1MHCl}		0.016	0.014
B_{1MHCl}		3.421	0.794
Mn_{1MHCl}		128.1	116.7
Cu_{1MHCl}		15.4	12.8
Fe_{1MHCl}		1497.8	1456.5
Zn_{1MHCl}		102.5	67.2

P_{av}, $K_{av,}$ Mg_{av} – forms available for plants, X_{tot} – total content, X_{1MHCl} – forms extracted with 1 mol dm^{-3} HCl

at a density of 100 germinating seeds per 1 m^2. Soil was sprayed with the herbicide Stomp 330 EC at a dose of 4 dm^3 ha^{-1} on the day following the sowing of lupine. Lupine plants were sprayed with Amistar 250 SC at 1.0 dm^3 ha^{-1} against anthracnose at the beginning of the budding phase; this procedure was repeated after 10 days. Plants harvested manually during the flowering phase were divided into roots, steams, leaves and flowers, whereas those harvested during the full ripeness phase were divided into roots, steams, leaves, pods and seeds.

The content of molybdenum, boron and iron in the plant material was determined by the ICP-AES method in bulk solution obtained by the mineralisation of samples at 450°C. The ash generated by mineralisation was dissolved in HCl 6 mol dm^{-3} in order to degrade carbonates, and then evaporated to dryness on a sand bath. A 10% solution of HCl was used to transfer chlorides to volumetric flasks. Data such as the amounts and percentage of nitrogen taken up by yellow lupine from the atmosphere were calculated by the isotopic dilution method using ^{15}N isotope in fertilization and published by Wysokiński (2013). The percentage of nitrogen which originated in yellow lupine from different sources was calculated using the formulas given by Kalembasa (1995) as well as Azam and Farroq (2003):

a) the percentage of nitrogen derived from the atmosphere:

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

- %Ndfa – % of nitrogen derived from the air,
- at% ^{15}N excess fx – ^{15}N isotope excess in yellow lupine,
- at% ^{15}N excess nfx – ^{15}N isotope excess in the control plant (spring triticale, a plant not fixing nitrogen);

b) the percentage of nitrogen derived from the fertiliser:

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

- %Ndff – % of nitrogen derived from the fertiliser,
- at% ^{15}N excess fx – ^{15}N isotope excess in yellow lupine,
- at% ^{15}N fert. excess – ^{15}N isotope excess of the applied fertiliser.

c) the percentage of nitrogen derived from soil:

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

- %Ndfs – % of nitrogen derived from soil,
- %Ndfa – % of nitrogen derived from the air,
- %Ndff – % of nitrogen derived from the fertiliser.

The results were worked out statistically with an analysis of variance. Conclusions regarding the significance of the impact of the factors under

study on individual features were based on the Fisher-Snedecor *F*-test, and the $LSD_{0.05}$ for comparison of the calculated means were calculated by the Tukey test. Moreover, linear correlation coefficients for the content, uptake of Mo, B, Fe as well as the amounts and percentage of nitrogen taken up by yellow lupine from the atmosphere were calculated. These calculations were supported by a Statistica 10 PL software package (StatSoft, Tulsa, USA) was used.

The experiment was conducted on soil with the textural composition of loamy sand, slightly acidic in reaction and assigned to the quality class IVa very good rye complex. The content of selected macro- and micronutrients in the soil before the experiment was set up is shown in Table 1.

The total rainfall in individual months and mean monthly air temperature during the growing season for yellow lupine are given in Table 2. Both

Table 2

Rainfall and air temperatures during the test crop cultivation (IMGW PIB Warsaw 2017)

Weather parameter	Month	Study period		Multiyear (1981-2007)
		2008	2011	
Monthly rainfall (mm)	April	43.5	38.1	32.9
	May	72.7	55.6	54.2
	June	56.7	44.3	68.8
	July	108.8	204.2	64.9
	August	85.1	55.4	61.8
Average monthly temperatures (°C)	April	8.7	9.8	7.9
	May	12.5	13.5	13.7
	June	17.0	18.1	16.1
	July	18.1	18.1	18.3
	August	18.3	18.1	17.6

growing seasons were quite favourable for the growth, development and yielding of yellow lupine. The total rainfall during the 2008 and 2011 growing seasons fully satisfied the plants' needs. However, the precipitation was not properly distributed over the months of plant growth. The amount of rainfall in June 2008 and in May and June 2011 was lower than required for yellow lupine, as reported by Dzieżyc et al. (1987). In addition to a higher water deficit during the intensive growth of lupine (May-June) in 2011, higher temperatures were recorded during that period than in 2008, which probably exacerbated the water deficit and decreased the yield (Wysokiński 2013).

RESULTS AND DISCUSSION

The mean content of molybdenum, boron and iron in the whole plants of lupine during the two years of the experiment was 5.117; 21.05 and 437.0 mg kg^{-1}, respectively (Tables 3-5). A lower content of boron and iron was determined in lupine harvested during the flowering phase (19.47 and 416.2 mg kg^{-1}, respectively) than during the full ripeness phase (22.63 and 457.7 mg kg^{-1}, respectively). The development phase in which lupine was harvested was found to have no effect on the content of molybdenum in the biomass obtained. The highest content of molybdenum and iron during the blooming phase was found in roots, and the highest content of boron was

Table 3

Molybdenum content in yellow lupine (mg kg^{-1} DM), the means for investigated factors

Investigated factor		Parts of plant						Mean in plant (weighted average)
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	7.278^{c}	6.688^{b}	4.760^{b}	5.160^{b}	4.450^{b}	9.625^{b}	6.365^{c}
	30	6.183^{b}	4.255^{a}	3.595^{a}	5.670^{b}	4.395^{b}	6.655^{a}	4.835^{b}
	120	4.643^{a}	3.618^{a}	3.178^{a}	4.385^{a}	3.065^{a}	6.895^{a}	4.150^{a}
Growth stage (BBCH)	65	9.702^{b}	6.267^{b}	2.818^{a}	5.072	-	-	5.323^{a}
	90	2.367^{a}	3.440^{a}	4.870^{b}	-	3.970	7.725	4.910^{a}
Years of study	1st	5.680^{a}	4.328^{a}	3.423^{a}	5.297^{a}	4.013^{a}	6.857^{a}	4.765^{a}
	2nd	6.388^{b}	5.378^{b}	4.265^{b}	4.847^{a}	3.927^{a}	8.593^{b}	5.468^{b}

a, b, c – means with different letters in the columns are significantly different

Table 4

Boron content in yellow lupine (mg kg^{-1} DM), the means for investigated factors

Investigated factor		Parts of plant						Meanly in plant (weighted average)
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	15.88^{a}	17.75^{a}	23.08^{a}	26.65^{a}	27.65^{a}	22.20^{a}	20.98^{a}
	30	15.98^{a}	17.39^{a}	23.73^{a}	27.90^{a}	30.72^{b}	22.05^{a}	21.53^{a}
	120	15.86^{a}	17.35^{a}	22.38^{a}	27.55^{a}	27.09^{a}	21.95^{a}	20.65^{a}
Growth stage (BBCH)	65	16.68^{a}	17.90^{a}	21.10^{a}	27.37	-	-	19.47^{a}
	90	15.13^{a}	17.07^{a}	25.02^{a}	-	28.47	22.07	22.63^{b}
Years of study	1st	17.82^{b}	19.15^{b}	25.97^{b}	32.03^{b}	31.13^{b}	22.84^{a}	23.50^{b}
	2nd	14.00^{a}	15.82^{a}	20.15^{a}	22.70^{a}	25.80^{a}	21.37^{a}	18.70^{a}

a, b – means with different letters in the columns are significantly different

Table 5

Iron content in yellow lupine (mg kg^{-1} DM), the means for investigated factors

Investigated factor		Parts of plant						Meanly in plant (weighted average)
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	1089.2^{b}	211.9^{b}	651.0^{a}	195.9^{a}	275.8^{b}	92.3^{a}	466.2^{b}
	30	895.4^{a}	142.2^{a}	739.3^{b}	172.9^{a}	301.4^{b}	105.7^{a}	442.5^{b}
	120	839.8^{a}	158.7^{a}	683.0^{ab}	324.2^{b}	241.1^{a}	150.2^{b}	402.2^{a}
Growth stage (BBCH)	65	826.8^{a}	148.6^{a}	465.2^{a}	231.0	-	-	416.2^{a}
	90	1056.2^{b}	193.3^{b}	917.0^{b}	-	272.8	116.0	457.7^{b}
Years of study	1st	974.4^{a}	163.1^{a}	757.7^{b}	161.7^{a}	233.1^{a}	93.1^{b}	431.3^{a}
	2nd	908.5^{a}	178.7^{a}	624.4^{a}	300.2^{b}	312.4^{b}	138.9^{a}	442.6^{a}

a, b – means with different letters in the columns are significantly different

determined in flowers. The highest content of iron was found in roots during the full ripeness phase, whereas the highest concentration of molybdenum during the same phase was detected in seeds and the highest concentration of boron was determined in pods.

Yellow lupine fertilised with 120 kg N ha^{-1} contained less molybdenum and iron in the whole biomass compared with that grown with no nitrogen fertilisation and with 30 kg N ha^{-1} (Table 3). Compared to the control, fertilisation with nitrogen at both doses reduced the content of molybdenum in roots, stems, leaves and seeds of lupine. The content of molybdenum in flowers and pods was reduced only after the higher dose of nitrogen was applied (120 kg ha^{-1}). The content of iron in lupine roots and stems with no nitrogen fertilisation was higher than after nitrogen had been applied at either of the doses. The highest concentration of this micronutrient in leaves was obtained when nitrogen was applied at 30 kg ha^{-}1 and in seeds and flowers when it was applied at 120 kg N ha^{-1}, whereas in pods in the control and in the plot where it was applied at 30 kg N ha^{-1}.

The different amounts of nitrogen applied did not affect the content of boron in any of the parts of the plants or on average in the whole lupine biomass (Table 4).

The content of molybdenum in different parts of lupine and on average in the whole plants was usually higher in the year when the thermal and water conditions were less favourable (2011) than in 2008, when the rainfall was distributed more favourably and when the temperatures during the growing season were slightly lower. A reverse relationship was observed for the level of boron. The average content of iron in the entire plant was not significantly differentiated in the different years of growing lupine.

The amounts of boron and iron accumulated in lupine biomass did not depend on the nitrogen fertilisation (Tables 6 and 7). A larger amount of

Table 6

Uptake of boron by yellow lupine (g ha^{-1}), the means for investigated factors

Investigated factor		Parts of plant						Total by plant
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	7.119^{a}	19.157^{a}	35.053^{a}	3.000^{a}	28.487^{a}	27.364^{a}	90.755^{a}
	30	7.626^{a}	20.324^{a}	37.835^{a}	3.615^{b}	38.447^{b}	31.116^{a}	101.374^{a}
	120	7.795^{a}	22.523^{a}	38.892^{a}	3.313^{ab}	34.509^{b}	38.984^{b}	107.614^{a}
Growth stage (BBCH)	65	9.389^{b}	18.646^{a}	29.145^{a}	3.309	-	-	60.489^{a}
	90	5.638^{a}	22.690^{b}	45.375^{b}	-	33.148	32.488	139.339^{b}
Years of study	1st	8.455^{b}	23.625^{b}	45.338	3.716^{b}	40.789^{b}	35.614^{b}	117.483^{b}
	2nd	6.572^{a}	17.711^{a}	29.182^{a}	2.903^{a}	25.497^{a}	29.363^{a}	82.346^{a}

a, b – means with different letters in the columns are significantly different

Table 7

Uptake of iron by yellow lupine (g ha^{-1}), the means for investigated factors

Investigated factor		Parts of plant						Total by plant
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	484.1^{b}	237.9^{c}	1053.4^{a}	22.7^{a}	278.7^{a}	115.1^{a}	1983.7^{a}
	30	411.9^{a}	166.1^{a}	1200.8^{b}	22.4^{a}	343.8^{b}	150.8^{b}	2037.3^{a}
	120	381.5^{a}	203.3^{b}	1253.2^{b}	40.9^{b}	298.1^{a}	241.3^{c}	2128.1^{a}
Growth stage (BBCH)	65	456.6^{b}	150.3^{a}	621.3^{a}	28.7	-	-	1256.8^{a}
	90	395.1^{a}	254.6^{b}	1717.0^{b}	-	306.9	169.0	2842.5^{b}
Years of study	1st	429.2^{a}	204.8^{a}	1447.1^{b}	18.8^{a}	305.0^{a}	142.8^{a}	2314.5^{b}
	2nd	422.5^{a}	200.0^{a}	891.2^{a}	38.5^{b}	308.8^{a}	195.2^{b}	1784.9^{a}

a, b, c – means with different letters in the columns are significantly different

molybdenum was taken up by lupine grown with no nitrogen fertilisation than when 30 and 120 kg N ha^{-1} were applied (Table 8). The amounts of molybdenum, boron and iron taken up by lupine harvested in the full ripeness phase were greater by 82%, 130% and 126%, respectively, than in the blooming phase. The greatest amount of molybdenum was accumulated in stems during the blooming phase and in seeds during the full ripeness phase. The greatest amounts of boron and iron were accumulated in leaves, regardless of the development phase.

The critical deficiency concentration of molybdenum in most crop plants is quite low, normally between 0.1 and 1.0 (3.0 in most forages) mg Mo in kg dry tissue (Gupta, Lipsett 1981). Because molybdenum is very mobile within the plant, its deficiency can be observed in the whole plant, most often in the

Table 8

Uptake of molybdenum by yellow lupine (g ha^{-1}), the means for investigated factors

Investigated factor		Parts of plant						Total by plant
		roots	stems	Leaves	flowers	stripped pods	seeds	
N dose (kg ha^{-1})	0	3.651^{c}	6.997^{b}	7.314^{b}	0.579^{a}	4.582^{b}	11.984^{b}	26.534^{b}
	30	3.243^{b}	4.744^{a}	5.651^{a}	0.734^{b}	5.022^{b}	9.343^{a}	21.187^{a}
	120	2.551^{a}	4.554^{a}	5.551^{a}	0.530^{a}	3.991^{a}	11.706^{b}	20.769^{a}
Growth stage (BBCH)	65	5.392^{b}	6.380^{b}	3.807^{a}	0.614	-	-	16.193^{a}
	90	0.905^{a}	4.482^{a}	8.537^{b}	-	4.532	11.011	29.467^{b}
Years of study	1st	3.108^{a}	4.993^{a}	6.065^{a}	0.609^{a}	5.175^{b}	10.199^{a}	22.157^{a}
	2nd	3.189^{a}	5.869^{b}	6.279^{a}	0.620^{a}	3.888^{a}	11.823^{b}	23.503^{a}

a, b, c – means with different letters in the columns are significantly different

middle of the plant or on old leaves, which turn yellow-green in colour (Hamlin 2007). Plant B concentrations of 20 to 50 mg kg^{-1} DM are considered to be optimum for the growth of forage legumes. The level of this element at 6-8 mg kg^{-1} DM is regarded as very low. The content of the elements as found in the current study in all the experimental treatments seems to be optimal with respect to the plants' requirements.

The non-soil-related factors with a significant effect on the absorption of boron by plants include the transpiration rate, which depends on relative air humidity (lower relative air humidity accelerates the absorption of boron by plants) and temperature, namely an increase in air temperature increases the boron absorption rate by plant roots even when relative air humidity does not change (Hu, Brown 1997). Neither the level of boron nor its amount absorbed by yellow lupine in the year with a slightly higher average temperature (2011) were higher than in a colder year (2008).

In a study conducted on soybeans at nodule maturity, nodules had the highest iron concentration, as approximately 44% of the iron within soybean plants occurs in nodules compared to 31% in leaves, 7% in seeds and 5% in roots (Burton et al. 1998). At seed maturity, seeds had the highest iron concentration of all organs, approximately 35% compared to 27% in nodules, 23% in leaves, 9% in roots and 3% in the stem (Burton et al. 1998). The yellow lupine presented in this study, harvested both during the blossoming phase and during the full maturity phase, contained the highest concentration of iron in roots, and the greatest amount of this element accumulated in leaves.

Molybdenum, boron and iron are closely associated with the transformation of nitrogen, especially with the mechanisms of nitrogen reactions catalysed by the nitrogenase enzymatic complex (Vieira et al. 1998*a,b*, Brown et al. 2002, Brear et al. 2013). The activity of nitrogenase and, consequently, the

effectiveness of atmospheric nitrogen fixation are sensitive to any deficit of these elements (Seefeldt et al. 2009, Symanowicz, Kalembasa 2012, Liu at al. 2016). A sufficient supply of these micronutrients to legume plants is very important in regard to their self-sufficiency in terms of nitrogen nutrition. Literature data show that the rate of nitrogen fixation in plants' nodules is positively correlated with increasing nodule iron concentrations (Slatni et al. 2008). The correlation coefficients calculated in this experiment (Table 9) did

Table 9

Values of the correlation coefficient between the content, uptake of Mo, B and Fe and amount and percentage of nitrogen taken up by yellow lupine from the atmosphere $p \leq 0.05$

The parameters between which the correlation coefficient values were calculated		The amount of N derived by yellow lupine from atmosphere	Percentage of N derived by yellow lupine from atmosphere
Content of Mo	in roots	-0.171	0.242
	mean for a plant	0.295	0.361
Content of B	in roots	-0.745*	-0.770*
	mean for a plant	-0.202	-0.874*
Content of Fe	in roots	0.403	0.618
	mean for a plant	0.358	-0.123
Uptake (accumulation) of Mo	by roots	-0.217	0.163
	by whole plant	0.124	-0.260
Uptake (accumulation) of B	by roots	-0.474	-0.251
	by whole plant	-0.179	-0.595
Uptake (accumulation) of Fe	by roots	-0.814*	-0.731*
	by whole plant	-0.321	-0.475

* the value of the correlation coefficient is significant $p \leq 0.05$

not reveal any positive relationships between the amount of molybdenum, boron or iron accumulated in roots and whole biomass of yellow lupine and the content and the amount of nitrogen taken up by this plant from the atmosphere (fixed in the process of biological reduction), the findings which were presented by Wysokiński (2013). In this study, the percent shares of nitrogen taken up from air, fertiliser and soil at the flowering stage of yellow lupine were: 71.2%, 6.0% and 22.8%, respectively, whereas at full maturity they were: 65.3%, 5.6% and 29.1%, respectively. The amounts of nitrogen taken up from air, fertiliser and soil at the flowering stage of yellow lupine were 77.2, 6.7, 25.5 kg N ha^{-1}, respectively, whereas at full maturity they were: 129.2, 11.8 and 60.6 kg N ha^{-1}, respectively.

CONCLUSIONS

1. The application of nitrogen at 120 kg N ha^{-1} decreased the content of molybdenum and iron in biomass of yellow lupine without having a significant impact on the concentration of boron. Fertilisation with nitrogen (at 30 and 120 kg N ha^{-1}) decreased the amount of molybdenum taken up by lupine, but it did not have any significant effect on the amount of boron or iron accumulated in biomass.

2. Yellow lupine harvested during the blooming phase contained less boron and iron than in the full ripeness phase, with the concentration of molybdenum at both harvesting times being similar. The amounts of these micronutrients accumulated in lupine harvested during the full ripeness phase were nearly twice as high as during the blooming phase.

3. The content of molybdenum, boron or iron in roots and in whole biomass of yellow lupine was not positively correlated with the amount and percentage of nitrogen taken up by this plant from the atmosphere (derived from fixation N_2).

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