THE EFFECT OF LUCERNE SEED INOCULATION ON YIELD AND QUALITY OF TWO DIFFERENT VARIETIES*

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Seed inoculation belongs among factors improving lucerne N_2 fixation. The aim of this study was to investigate the effect of seed inoculation on forage yield and nutrient concentration in respect to different types of lucerne varieties. The field plot experiment with lucerne monoculture was conducted in rich-nutrient Luvisol in 2011–2012. There were two varieties (Jarka, Oslava) and three variants of seed inoculation (control without inoculation, Rizobin LF, Nitrazon + N) in completely randomized design with four replicates. Differences in mineral concentration between treatments were detected in roots, while no differences were observed in lucerne forage. Oslava variety bred for higher N_2 fixation reached higher nodulation intensity, root N concentration, and uptake. This variety also increased yield and root N uptake where seed inoculation was used, mainly by Nitrazon. The cv. Jarka did not provide constant positive reaction on seed inoculation in all evaluated traits with slightly better response to Rizobin inoculation. In the field condition, it seems that seed inoculation does not need to have a general positive effect on lucerne yield and quality under rich-nutrient soil. However, our results support the idea that specific interaction between seed inoculants and lucerne variety could have a potential for improving forage yield.

Medicago; alfalfa; *Rhizobium*; nodulation; Nitrazon

INTRODUCTION

The symbiotic association between certain plants and microorganisms plays an important role in soil fertilization, and improves their growth and mineral nutrition (Diouf et al., 2003). Biological N_2 fixation, especially the symbiotic association between legumes and *Rhizobia*, can provide sufficient amount of N to plants, which reduces the need for industrial fertilizers (Ledgard, Steele, 1992) and the use of nitrogen fertilizers is thus not recommended (Frame et al., 1997; Oliveira et al., 2004). Due to effective N_2 fixation, perennial forage legumes have a great potential to increase sustainability in grassland farming based on livestock production (Carlsson, Huss-Danell, 2003).

Lucerne (*Medicago sativa* L.) is considered as an excellent nitrogen extractor (Oliveira et al., 2004). According to Frame (1997), estimates of N_2 fixation by lucerne vary widely but are generally higher on an annual basis than for other temperate forage legumes. For lucerne monoculture, Carlsson, Huss-Danell (2003) reported rate of N_2 fixation in the range of up to 350 kg N/ha per year while in lucerne-grass mixture a lower amount (82–254 kg N per season) was described by Heichel, Henjum (1991). According to Rasmussen et al. (2012), surprisingly not even the addition of N fertilizer lowered N_2 fixation in lucerne-ryegrass mixture, probably due to competition for available soil N from perennial ryegrass. In the field crop rotation including lucerne as a monoculture, the contribution of nitrogen fixation to nitrogen budget based on average nitrogen uptake could exceed 200 kg N/ha (Kubát et al., 2003).

Generally, the efficiency of N_2 fixation is a function of host genotype, Rhizobium genotype, and environmental factors (Whitehead, 1995). Due to sensitivity of N_2 fixation to environment, wide variation was found from site to site in connection with soil mineral N, deficiency of certain minerals or soil acidity (Frame et al., 1997). The symbiosis between Rhizobia and legume in interaction with a wide range of environment was elaborated by Zahran (1999). As regards lucerne genotype, the ability of N_2 fixation could be improved by a breeding process (Chloupek et al., 1996), however, this effort has met with a limited success (Frame et al., 1997). The selected parameters such as nodulation rate, nitrogenase activity, nitrogen

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content, and others could be improved in controlled and semi-controlled environments but the performance of these selections in the field has been disappointing (Teuber, Phillips, 1988).

Management of legume seed inoculation is considered as a factor affecting N input and stands productivity (Virgona et al., 2012). Inoculation of lucerne seeds with *Rhizobium meliloti* is generally recommended, particularly on soils where lucerne has not been sown for the previous 3 or more years (Frame et al., 1997). The inoculation can be done by coated seeds or as granules in the seed row (Hoflich et al., 1993). The results of Gemell et al. (2005) demonstrated the importance of inoculation timing where average Rhizobia count was significantly higher for freshly inoculated seed in comparison with preinoculated seed. They also reported that results of nodulation test were positively correlated with Rhizobia numbers on seed, confirming the use of Rhizobia count to assess quality of inoculation. In the Czech Republic, the lucerne seed inoculation has had a long tradition until 1949 (Mikanová, Šimon, 2011). However, it is used sporadically at the present, the extent of use being estimated at around 16% (Hakl, unpublished). The more extensive utilization of forage legume inoculation has not been supported by recent research in spite of the fact that new inoculants with various species of microorganisms are available. Moreover, interaction between Czech lucerne variety and a different inoculation type has not been investigated. The aim of this study was to present the results on the interaction of two Czech lucerne varieties with two different inoculants focusing on the nutrient concentration and uptake in connection with forage yield. This investigation could be important for improving N_2 fixation effectiveness in the field conditions.

MATERIAL AND METHODS

In 2011–2012, the plot experiment with lucerne monoculture was conducted at the Research Station of the Czech University of Life Sciences Prague, at Červený Újezd (50°04′N, 14°10′E, 405 m a.s.l., clay-loamy Luvisol, long-term annual temperature 7.7 °C and precipitation 493 mm). The chemical soil characteristics prior to the experiment establishing were: soil pH ($CaCl_2$) 7.26, plant available K, P, Ca, Mg contents were 324, 263, 3937, and 116 mg/kg, respectively. Soil analyses were performed at Eko-Lab Žamberk (http://www.ekolab.zamberk.cz), an accredited national laboratory, using the Mehlich III method (Mehlich, 1984) and the exchangeable acidity measurement (ISO 10390, 1994).

The plot experiment was established by row sowing (row spacing 12.5 cm) on May 2, 2011 at a seeding rate of 700 germinated seeds per m^2. There were three variants of seed inoculation: without inoculation (Control) and inoculated by Rizobin LF (LEGUME Technology Ltd., AGRO-PROFI s.r.o., Prague, Czech Republic) or Nitrazon + N (Farma Žiro s.r.o., Nehvizdy, Czech Republic), both based on peat as the carrier of bacteria. The count of *Rhizobium* sp. (higher number of various strains) is declared as 1×10^9 and 5×10^8/g of inoculants for Rizobin and Nitrazon, respectively. Nitrazon + N also declare 1×10^4 of *Azotobacter* sp. and 1×10^6 of *Bacillus megatherium* per g for improving phosphorus availability. Seeds were coated immediately before seeding. Used lucerne varieties were the early Czech variety cv. Jarka (standard N_2 fixation) and cv. Oslava (improved N_2 fixation). The experiment was arranged in completely randomized blocks with four replications for each treatment which resulted in 24 plots. There were six plots with a size of 7.5 × 2.5 m per block. All measurements were performed once per plot.

Except in the sowing year 2011, when only one autumn harvest was evaluated, the plots were cut (by mower MF-70 with working width 1.2 m) four times in 2012 and fresh matter yield was assessed per plot. In all cuts, the forage samples were taken from 0.5 m of one inside row in each plot. In the clipped sample, the number of all stems (stem density, SD) and stem length of the longest stem (maximal stem length, MSL) were determined. To determine dry matter yields (DMY), forage sample was weighed after oven drying at 60°C to constant weight, and DMY was calculated as t/ha. In the last cuts of the years, root samples were also taken in the same area of forage sampling with average depth of 25 cm. The number of plants (plant density, PD) and root weight (g/m^2) were determined. Intensity of nodulation was evaluated by each plant on the scale from 0 (absence of nodules) to 3 (6 and more nodules). In the forage and root samples, total N content was determined by the Dumas method. P, K, and Ca concentrations were determined by spectrophotometry and emission flame spectrometry after digestion in sulphuric acid at accredited laboratory (Eko-Lab Žamberk). Multifactor analyses of variance (year, cut, variety, inoculation) and linear simple correlation were used. All analyses were carried out using the Statistica software (StatSoft, Version 9.1, 2003).

RESULTS

Throughout the two years of the experiment, lucerne stand survived without any substantial damage by pests, weeds, and water logging of soil or similar. In 2011 and 2012, the average temperature and sum of precipitation over vegetation period achieved 15.7 and 14.6 °C or 412 and 357 mm, respectively. The long term average temperature and sum of precipitation over vegetation period is 13.8 °C and 333 mm.

Table 1. Correlation matrix between mineral concentrations and amount of biomass (n = 48, coefficients significant at $P < 0.05$ are in bold)

Variables	N	P	K	Ca
Root weight	**–0.57**	–0.17	**0.44**	**0.48**
N	1.00	0.23	**–0.79**	**–0.66**
P		1.00	0.10	**–0.38**
K			1.00	**0.70**

Nutrient concentration

The correlation matrix in Table 1 showed significant correlation between root weight and all evaluated mineral nutrients, except for P. In spite of it, using root weight as covariate did not change effect of year, variety, and seed inoculation to nutrient concentration in the root biomass. From this reason, these results are presented without covariate effect in Table 2. Year significantly changed all concentrations, except for

Table 2. The effect of year, variety, and seed inoculation on average concentration of N, P, K, Ca (%) and mineral nutrient ratios in lucerne roots in the autumn sampling

Factor		N (%)	P (%)	K (%)	Ca (%)	N/P	N/K	N/Ca
Year	2011	2.43^{a}	0.36	0.62^{a}	0.21^{a}	7.00^{a}	3.97^{a}	12.19^{a}
	2012	2.07^{b}	0.34	0.89^{b}	0.37^{b}	6.11^{b}	2.35^{b}	5.70^{b}
	*P**	< 0.000	0.166	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Variety	Jarka	2.21^{a}	0.35	0.79^{a}	0.29	6.33	2.97^{a}	9.34
	Oslava	2.28^{b}	0.34	0.72^{b}	0.29	6.70	3.36^{b}	8.56
	P	0.020	0.448	0.004	0.892	0.067	0.001	0.156
Inoculation	Control	2.25	0.34	0.76	0.29	6.63	3.31	9.09
	Rizobin	2.23	0.35	0.77	0.29	6.46	3.08	8.76
	Nitrazon	2.26	0.35	0.74	0.30	6.44	3.19	9.00
	P	0.635	0.644	0.429	0.830	0.685	0.545	0.881

**P*-value for Three-Way ANOVA with interactions, ^{a,b}statistical differences for Tukey's HSD test, α = 0,05

Table 3. The effect of variety and seed inoculation on average dry matter yield in the cut (Cut DMY), total DMY over year, maximal stem length (MSL), stem density (SD), nitrogen concentration (N %), and uptake (N kg/ha) in the forage biomass

Year			Cut DMY (t/ha)	Total DMY (t/ha)	MSL (cm)	SD (pcs/m^2)	N (%)	N (kg/ha)
2011	variety	Jarka	-	3.23	75	438	2.60	84
		Oslava	-	3.50	80	382	2.62	92
		*P**	-	0.196	0.317	0.238	0.879	0.222
	inoculation	Control	-	3.40	80	413	2.98	92
		Rizobin	-	3.33	77	403	2.64	87
		Nitrazon	-	3.36	77	413	2.51	86
		P	-	0.960	0.851	0.977	0.412	0.633
2012	cut	1	6.74^{a}	-	98^{a}	797^{a}	2.49^{a}	168^{a}
		2	4.55^{b}	-	82^{b}	617^{b}	2.90^{b}	132^{b}
		3	3.35^{c}	-	75^{c}	718^{a}	-	-
		4	1.84^{d}	-	37^{d}	797^{a}	-	-
		P	0.000	-	< 0.000	< 0.000	< 0.000	< 0.000
	variety	Jarka	4.09	16.58	74	703	2.68	150
		Oslava	4.15	16.37	72	749	2.70	150
		P	0.478	0.669	0.184	0.053	0.759	0.925
	inoculation	Control	4.10	16.39	73	756	2.66	147
		Rizobin	4.20	16.80	73	702	2.63	149
		Nitrazon	4.06	16.25	72	719	2.78	153
		P	0.299	0.641	0.719	0.166	0.197	0.610

**P*-value for Two- or Three-Way ANOVA with interactions, $^{a–d}$statistical differences for Tukey's HSD test, α = 0,05

Table 4. The effect of year, variety, and seed inoculation on average uptake of N, P, K, Ca in lucerne roots, intensity of nodulation (IN; 0 = plant without nodules, 3 = plants with 6 and more nodules), root weight (RW), and plant density (PD) in the autumn sampling

Factor		N (g/m^2)	P (g/m^2)	K (g/m^2)	Ca (g/m^2)	IN (0–3)	RW (g/m^2)	PD (pcs/m^2)
Year	2011	6.66[a]	0.96[a]	1.67[a]	0.59[a]	0.86[a]	274[a]	249
	2012	9.32[b]	1.55[b]	4.00[b]	1.64[b]	0.53[b]	451[b]	205
	*P**	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	0.133
Variety	Jarka	7.29[a]	1.16	2.74	1.03	0.64	334[a]	247
	Oslava	8.70[b]	1.35	2.93	1.20	0.74	391[b]	206
	P	0.017	0.067	0.384	0.070	0.076	0.022	0.154
Inoculation	control	7.91	1.22	2.85	1.13	0.65	359	212
	Rizobin	7.94	1.25	2.87	1.08	0.70	363	256
	Nitrazon	8.11	1.29	2.79	1.14	0.73	365	212
	P	0.949	0.854	0.951	0.861	0.503	0.983	0.346

**P*-value for Three-Way ANOVA with interactions, [a,b]statistical differences for Tukey's HSD test, α = 0,05

P, where N was decreased while K and Ca were increased over years. These changes were also obvious for mineral ratios. The cv. Oslava reached significantly higher concentration of N and lower of K in comparison with cv. Jarka, however the differences were not substantial. The single effect of seed inoculation was insignificant for all evaluated concentrations. In 2011, the above-ground biomass of lucerne was also analyzed for N, P, K, Ca concentration and uptake, however, any significant differences were not observed between varieties, variants, and their interaction (data not presented). In 2012, from this reason, only N concentration was analyzed in above-ground biomass in the first and second cut. These results are presented together with stand structure, forage, and N yield in the Table 3.

Nutrient uptake and nodulation intensity

In Table 4, root nutrient uptake was in relation to root weight per area unit and mineral concentration from Table 2. Differences between years were given mainly by significantly higher weight in 2012. The cv. Oslava provided significantly higher weight of root as well as uptake of N, however, there were insignificant differences for other nutrients. The seed inoculation did not significantly affect any evaluated parameters. However, the significant interaction (year × variety × inoculation) in Fig. 1 shows that differences in N uptake between varieties were more obvious in 2012 as well as the higher value for Nitrazon in contrast to control by Oslava variety in both years.

Nodulation intensity based on the number of nodules per plant was generally low with average value 2 nodules per plant. It could be connected with higher level of available nutrient in the soil, term of root sampling as well as potential destruction of fine roots with nodules during root sampling in the field conditions. The nodulation intensity was significantly higher in the first year (2011) and almost significant for Oslava variety. Interaction showed ($P = 0.051$, figure not presented) that there was a positive reaction of nodulation intensity on seed inoculation by Oslava variety in 2012 while Jarka variety did not provide

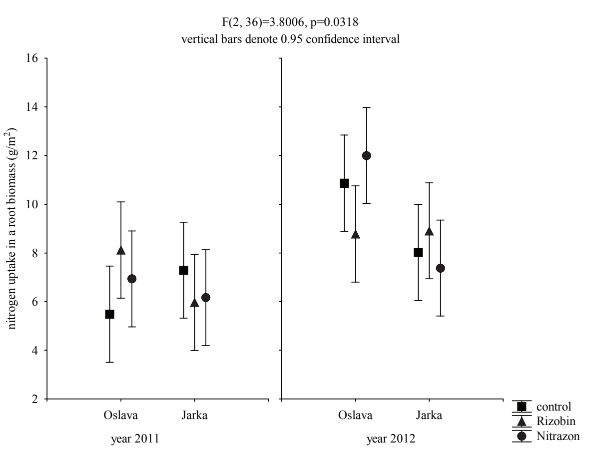

Fig. 1. Average nitrogen uptake in a root biomass at different lucerne varieties in relation to the variant of seed inoculation (control was without inoculation, roots were sampled in October, 2011 is the year of stand establishment)

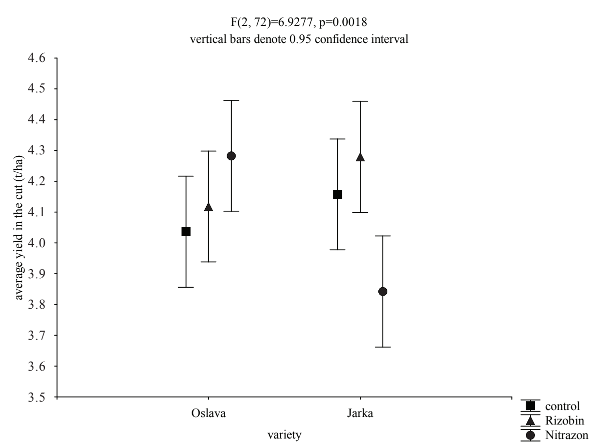

Fig. 2. Average forage yield in the cut at different lucerne varieties in relation to the variant of seed inoculation (second year of vegetation)

consistently positive response on seed inoculation in both years.

Forage yield and quality

In the seeding year, there were no significant differences in DMY, stand structure, and N concentration or uptake between varieties or variants of seed inoculation (Table 3). In 2012, the number of cuts significantly influenced all evaluated traits, however, no significant differences were observed for single effect of variety or seed inoculation, except for almost significantly higher stem density by Oslava variety. The significant interaction variety × inoculation presented in Fig. 2 shows the highest differences in average cut yield between varieties when Nitrazon was used. In this case, the cv. Oslava reached a significantly higher average yield in contrast to cv. Jarka.

DISCUSSION

Based on our results, it is possible to conclude that the single effect of seed inoculation did not significantly influenced nutrients concentrations, uptakes or a forage yield. As was noted by T e u b e r , P h i l l i p s (1988), increases in the N_2 fixation are not often observed in the field conditions. In our study, it could be closely related to high content of plant available nutrients, which is in accordance with O l i v i e r a et al. (2004). They reported that absence, as well as high concentration of N, negatively affected the nodulation because of a reduced plant growth in the N absence and probably no nodules formation in presence of high N concentration. Also L e d g a r d , S t e e l e (1992) documented that N_2 fixation is reduced by increases in soil inorganic N during dry conditions and where N fertilizer is used. It must be added that these relations could be different in lucerne-grass mixture due to competition for available soil N from grass component (R a s m u s s e n et al., 2012). In spite of our results about forage yield, Blažinkov et al. (2012) described significant increase of lucerne yield under seed inoculation management in contrast to untreated variants. However, there was low fertile acid soil in their field experiment which resulted in generally lower yield ranging 6.47–13.57 t/ha in comparison with yields in our study. It seems that rich-nutrient soil could decrease the effect of seed inoculation. However, for poor soil it must be added that necessary soil and environment properties must be kept in accordance with request for N_2 fixation described by Z a h r a n (1999).

In regard to nutrient concentration, it seems that differences between treatments could be detected mainly in roots in comparison with lucerne forage, where no differences were observed. Obtained values of nutrient concentration are in correspondence with results of Vasileva (2013). The higher nodulation intensity, root N concentration and uptake by Oslava variety support the idea that it could be related to its improved N_2 fixation, when breeding for this property was described by C h l o u p e k et al. (1996). In 2012, N uptake by lucerne root (Fig. 1) was in accordance with average yield in the cut for interaction variety × inoculation (Fig. 2). However, these relations did not influence the total forage yield over year. According to S t a n c h e v a et al. (2008), dual inoculation with mycorrhizal fungi (*Glomus intraradices*) and *Rhizobium* ssp. significantly increased P and N concentration in lucerne tissues. In our experiment, the P concentration was very stable across variants of seed inoculations and varieties in spite of using inoculant with bacteria species improving P solubility. It could be connected with high content of plant available P at the level of 263 mg/kg in contrast to 42 mg/kg in the experiment of S t a n c h e v a et al. (2008). Furthermore, the fact that also N_2 fixation naturally enhanced phosphatase activity (H o u l t o n et al., 2008) must be taken into account. It must be also remembered, that the water deficiency stress could changed nutrient concentration and uptake in lucerne forage (Vasileva, 2013)

Forage N concentration showed inverse relation to the DMY in the cuts, indicating a dilution effect in accordance with O l i v i e r a et al. (2004). However, neither variety, seed inoculation, nor their interaction influenced forage N concentration or uptake. Based on estimation of N_2 fixation given by C a r l s s o n , H u s s - D a n e l l (2003), the N_2 fixation derived from forage yield in 2012 was estimated 227 kg N/ha on average. It is in accordance with K u b á t et al. (2003) who reported that the annual contribution of N_2 fixation could exceeded 200 kg N/ha in the Czech Republic. For our study, the higher yield level given by plot experiment should be taken into account.

In spite of insignificant single effect of seed inoculation, our results suggest that interaction of seed inoculation with the variety and also the year could be important in correspondence to W h i t e h e a d (1995). According to D e l i ć et al. (2010), the effectiveness of the N_2 fixation varied widely in different *Rhizobia*-host plant combinations thus it would be possible to identify highly effective *Rhizobia* strains, as commercial strains of microbiological fertilizers for particular lucerne varieties. In conformity with their state, our results documented that cv. Oslava with improved N_2 fixation performed better in interaction with seed inoculation, mainly by Nitrazon. The cv. Jarka did not provide constant positive reaction on seed inoculation in all evaluated traits with slightly better response to Rizobin inoculation.

CONCLUSION

Based on our experiment in rich-nutrient soil, it is possible to conclude that significance of single effect of lucerne seed inoculation was not observed for any of the evaluated traits. However, significant interaction between variety and used inoculate preparation was detected in higher intensity of nodulation, root N uptake, and forage yield in the cut. As regards forage yield, some effects were observed in the yield of each cut but not in total yield over the year. These results suggest the idea that seed inoculation could be effective in spite of rich-nutrient soil, however, positive interaction between variety and preparation used for seed inoculation should be verified before.

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