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Biological nitrogen fixation in acid soils of Lithuania

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The results of this study indicate that *Rhizobium leguminosarum* bv. *trifolii* is widely distributed in slightly acid soils with pH_{KCl} 5.6–6.0. The average content of rhizobia was $540.0 \cdot 10^3$ cfu g^{-1} of soil. Less *Rhizobium leguminosarum* bv. *viciae* and significantly less *Sinorhizobium meliloti* and *Rhizobium galegae* were found. *Rhizobium* significantly declined in acid soils (pH_{KCl} 4.1–5.0).

Most of biological nitrogen was fixed at soil pH_{KCl} 6.1–7.0. In this case, *Rhizobium galegae* accumulated 196 to 289 kg N ha^{-1} of nitrogen, whereas rhizobia of alfalfa and clover were less, and it depended on strain efficiency and soil pH. Soil liming had a positive effect on nitogenase activity in red clover. The soil liming ($CaCO_3$ rate 6.2 t ha^{-1}) in combination with inoculation have increased biological nitrogen fixation by red clover at 106 kg N ha^{-1}. Associative diazotrophes in non–legume rhizoplane have fixing the biological nitrogen too. The effective strains of *Rhizobium* spp., *Agrobacter radiobacter* and *Arthrobacter mycorens* have made up an active association with barley, timothy and spring rape and accumulated 11.0 to 20.4 kg N ha^{-1} of biological nitrogen.

Key words: *Rhizobium*, distribution in the soil, nitrogen fixation, associative diazotrophes

INTRODUCTION

From the atmosphere, biological nitrogen is accumulated by symbiotic and non-symbiotic microorganisms which are called diazotrophs. The total value of biological nitrogen depends on the genetic and physiological properties of a symbiotic system. Perennial legumes fix 40–470 kg N ha^{-1} (Umarov, 1986; Коць, 2001) and annual legumes 30–200 kg N ha^{-1} (Carranca et al., 1999). Significantly less (14–50 kg N ha^{-1}) of biological nitrogen was accumulated by associative and free nitrogen fixing soil microorganisms (Umarov, 1986).

The biological nitrogen fixing process depends on the occurrence and survival of *Rhizobium* in soils and also on their efficiency (Adamovich, Klasens, 2001; Hamdi, 1982). Soil reaction is one of the most important factors influencing legume and *Rhizobium* symbiosis. A higher concentration of H^+ ions increases the solubility of Al, Mn and Fe, and these elements may be toxic. *Sinorhizobium meliloti* and *Rhizobium galegae* are highly sensitive to acid pH and soluble Al when the critical soil pH is 4.8–5.0 (Kelner et al., 1997; Lapinskas, 2004). *Rhizobium leguminosarum* bv. *trifolii* and *Rhizobium leguminosarum* bv. *viciae* in comparison with alfalfa rhizobia are more tolerant to soil acidity. However, pH less than 4.6 inhibits their activity. Legumes and *Rhizobium* have form an efficient symbiosis and fix high amounts of biological nitrogen when soil pH is no less than 5.6–6.1 (Hamdi, 1982; Lapinskas, 1998). Soil acidification inhibited the root-hair infection process and nodulation (Ambrazaitienė, 2003; Hartwig, Soussana, 2001).

The optimal soil reaction for a large majority of rhizobia species is close to neutral. However, the value of soil pH may change depending on soil conditions, especially on Al toxicity (Шильников и др., 1996). According to many publications on soil liming, fertilization with PK fertilizers and organic manure, as well as legume inoculation with effective strains of *Rhizobium* are important factors for increasing the efficiency of symbiosis (Vassileva, Kostov, 2001).

One of the central topics in increasing nitrogen fixation is legume inoculation with effective and ecologically adapted rhizobia strains that have become more tolerant to soil acidic pH and especially to mobile Al at a concentration of 400 mM (Ozawa et al., 1999). In the presence of low pH and Ca deficit in the soil, rhizobia cannot form an efficient symbiosis (Čiuberkis et al., 2005). Even small rates of Ca fertilizers (3.0 t ha^{-1}) decrease the toxicity of mobile Al and negative impacts of heavy metals more than by half, improve the metabolism of nitrogen and phosphorus in the soil, because the activity of soil microorganisms and nitrogen fixation increase (Шильников и др., 1996). From average Ca rates (7 t ha^{-1}) nitrogen fixation increases to 30% (Парахин, Петрова, 2001). Large Ca fertilizer rates (20 t ha^{-1}) eliminate Al toxicity entirely. The first indication of their efficiency was visible at day 5 after introducing Ca fertilizers (Андреюк та ін., 2001). The liming of acidic soil has improved both the inoculation process of legumes and nitrogen fixation (Delavechia et al., 2003).

Our object was to determine and estimate the symbiotic and non-symbiotic nitrogen fixation depending on soil acidity and liming.

MATERIALS AND METHODS

The distribution of the main species of rhizobia (*Rhizobium leguminosarum* bv. *trifolii* F., *Rhizobium leguminosarum* bv. *viciae* F., *Sinorhizobium meliloti* D. and *Rhizobium galegae* L.) was established in 400 different soil samples from Lithuania. The dilution method of legume inoculation in sterile conditions was employed (Андреюк та ін., 2001). The red clover, alfalfa and goat's rue were grown in large biological tubes (200 × 20 mm), vetches in 500 ml glasses in 5 repetitions. Nodule formation was determined by the number of *Rhizobium* in the soils (Lapinskas, 1998). Biological nitrogen fixation in field experiments was established by growing legumes and cereal plants (Трепачев, Ягодина, 1991). The soil, according to the FAO-UNESCO modified classification, was *Dystri-Endohypogleyic Albeluvisols* (Abg-n-w-dy), pH_{KCl} 4.1–7.1, organic C 1.06–1.80%, available P_2O_5 and K_2O 85–220 to 137–322 mg kg^{-1} soil.

The colony size was measured in a nutrient yeast agar medium (pH 4.2 to 7.0) after 6 days of growth at 25 °C. Tolerance (percentage) of rhizobia species to the acidity of medium was calculated by measuring the colony size in an acid (pH 5.4) medium and dividing it by the size in a neutral (pH 7.0) medium and multipling by 100.

Field trials were carried out in both *Dystri-Endohypogleyic Albeluvisols* and *Orthi-Haplic Luvisols* with pH_{KCl} 4.7–6.4, organic C 1.17–3.14%, available phosphorus (P_2O_5) 64–139 and potassium (K_2O) 92–118 mg kg^{-1}. Legumes were sown in 28 m^2 (2.0 × 14.0 m) plots. Their inoculation was carried out with ecologically adapted and non-adapted strains of rhizobia in soils of different acidity (Lapinskas, 2002). For establishing biological nitrogen fixation, darnel (*Lolium perenne* L.) was grown in parallel plots. The experimental area was fertilized with $P_{60}K_{60}$. Just before legume sowing, the seeds were inoculated with rhizobia bacterial suspension at 600 × 10^9 cfu (colony forming units) ha^{-1}. After cutting legumes and cereal plants (2–3 times every year), 0.5 kg herbage samples from all plots were taken for dry matter content analysis. All samples were dried and weighed to a constant weight in an oven controlled at 105 °C, and the amount of dry matter harvested was determined. The root samples were taken from soil monoliths (200 × 200 × 200 mm) dug out at bound from each plot.

For establishing biological nitrogen fixation, ryegrass (*Lolium perenne* L.) was grown in parallel plots. The experimental area was fertilized with $P_{60}K_{60}$. Just before legume sowing, the seeds were inoculated with rhizobia bacterial suspension at 600 × 10^9 cfu ha^{-1}. After cutting legumes and cereal plants (2–3 times every year), 0.5 kg herbage samples from all plots were taken for dry matter content analysis. All samples were dried and weighed to a constant weight in an oven controlled at 105 °C, and the amount of dry matter harvested was determined. The root samples were taken from soil monoliths (20 × 20 × 20 cm). The content of nitrogen was calculated according to the equation:

$$F_n = (U_n + R_n) - (U_s + R_s),$$

where F_n – fixed nitrogen, U_n is total nitrogen in the overground part of legume, R_n is total nitrogen in legume roots, U_s is total nitrogen in the overground part of cereal plants, R_s is total nitrogen of cereal roots. In all cases the dimension was kg N ha^{-1}.

The efficiency of associative nitrogen-fixing bacteria was examined under field and pot experiments. The soil was *Dystri-Endohypogleyic Albeluvisols* (pH_{KCl} 5.6–5.9). By fertilizing with $P_{60}K_{60}$, the background was formed. In field trials, barley and timothy seeds were inoculated with preparations from sequential bacteria: *Azospirillum lipofera* (strain 137), *Agrobacterium radiobacter* 10, *Arthrobacter mycorens* 7 and *Rhizobium leguminosarum* bv. In pot experiments, the efficiency *of Rhizobium leguminosarum* bv. trifolii 348a, *Rhizobium galegae* and *Bradyrhizobium japonicum* was examined on barley and spring rape. Biological nitrogen was calculated from total nitrogen differences in inoculated and uninoculated plants (Umarov, 1987).

The efficiency of soil liming, red clover inoculation and leaching of Ca and nitrates from soil were measured in lysimetric experiments. The soil pH_{KCl} was 5.2 before liming and 6.2 after liming (6.2 t ha^{-1} $CaCO_3$). In the lysimetric trial, the soil was *Dystri Albeluvisol* packed with natural layers. The area of the lysimetric trial was 1.0 m^2 and depth 1.1 m. Experiments had three replicates. The seed of red clover before sowing was inoculated with *Rhizobium leguminosarum* bv. *trifolii* strain 348a.

Pattern analysis was processed using ANOVA and STAT programs (Tarakanovas, Raudonius, 2003).

RESULTS AND DISCUSSION

Rhizobium distribution and symbiotic efficiency. The results have shown that *Rhizobium leguminosarum* bv. *trifolii* is widely distributed in acid soils with pH_{KCl} 5.6–6.0. The amount of rhizobia was 540.0 · 10^3 g^{-1} of soil (Fig. 1). Less *Rhizobium leguminosarum* bv. *viciae* and significantly less *Sinorhizobium meliloti* and *Rhizobium galegae* were found. *Rhizobium* significantly declined in acid soils (pH_{KCl} 4.1–5.0).

The optimal soil pH for different species of *Rhizobium* was different. Rhizobia of clover grew best at pH 5.6–6.0, rhizobia of viciae and alfalfa at pH 6.6–7.0, and rhizobia of fodder galega at pH above 7.0.

The highest level of biological nitrogen was found at soil pH 6.1 to 7.0. *Rhizobium galegae* fixed 196 to 289 kg N ha^{-1} of biological nitrogen, while rhizobia of alfalfa and clover were less, and it depended on strain efficiency and soil pH (Fig. 2).

The minimal soil acidity on biological nitrogen fixation was pH_{KCl} 4.5. At the same time, other species of this bacterium did not fix the atmospheric nitrogen. When soil pH_{KCl} was 5.0, all

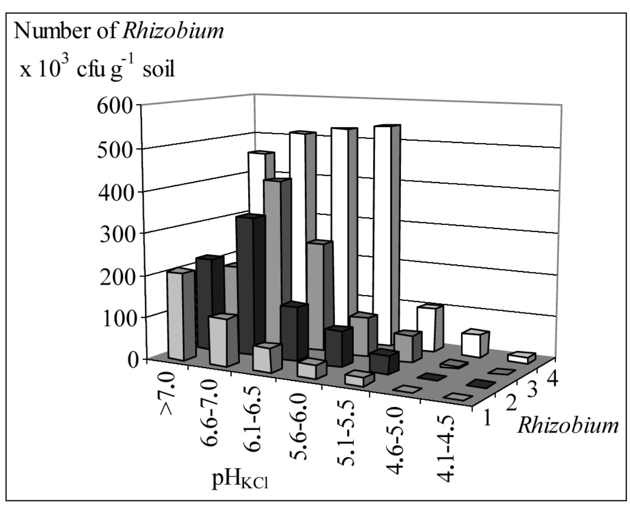

Fig. 1. The impact of soil pH_{KCl} on the distribution of different *Rhizobium* species: *1 – Rhizobium galegae* (R_{05} 12.6), *2 – Sinorhizobium meliloti* (R_{05} 27.4), *3 – Rhizobium leguminosarum* bv. *viciae* (R_{05} 23.5), *4 – Rhizobium leguminosarum* bv. *trifolii* (R_{05} 37.8)

rhizobia species fixed nitrogen (123 to 205 kg N ha^{-1}). The inhibitory effect of a low soil pH has been indicated in references (Lapinskas, 2002; Каминскій, Голодна, 2000). The optimal reaction for various species was different. *Rhizobium leguminosarum* bv. *viciae*, *Sinorhizobium meliloti* and *Rhizobium leguminosarum* bv. *trifolii* fixed biological nitrogen most intensively at soil pH_{KCl} 6.5, whereas *Rhizobium galegae* formed an efficient symbiosis at pH_{KCl} 7.0. Until now, it has been known that *Sinorhizobium meliloti* but not *Rhizobium galegae* has higher requirements for soil pH (Lindström, Milliniemi, 1987). According to literature data, *Sinorhizobium meliloti* shows a higher symbiotic efficiency than other *Rhizobia* (Hamdi, 1982; Kelner et al., 1997). The prevailing acid soils in West Lithuania do not offer optimal conditions for cultivating alfalfa and their rhizobia. When soil acidity decreased from pH 5.5 to 6.5, the amount of fixed nitrogen increased from 40 to 88 kg N ha^{-1}. When liming was not applied, legume inoculation with effective strains adapted to ecological conditions was an effective means (Fig. 3).

Rhizobium* spp. *tolerance of acid reaction. Results of laboratory analyses showed that of all rhizobia species *Sinorhizobium meliloti* was most sensitive to acid reaction. The index of rhizobia tolerance to acid medium was 67%, while in the other species it reached 79–87%. In the acid medium (pH 4.2), no strains of this bacteria grew, while other bacteria species grew satisfactorily and formed colonies 2.1 to 5.9 mm in size (Table 1). The other rhizobia species were more tolerant to medium acidity as compared with *Sinorhizobium meliloti*. In a neutral medium (pH 7.0), all rhizobia species grew very intensively and formed colonies 6.9 to 10.3 mm in diameter.

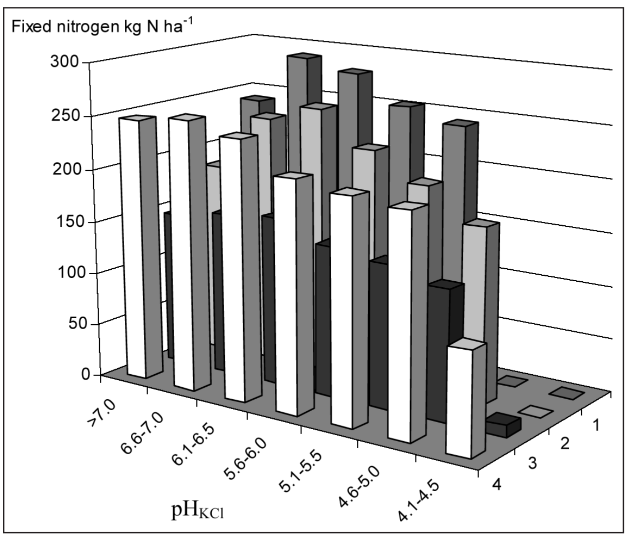

Fig. 2. The impact of soil pH_{KCl} on symbiotic nitrogen fixation by different *Rhizobium* spp.: *1 – Rhizobium galegae*, *2 – Sinorhizobium meliloti*, *3 – Rhizobium leguminosarum* bv. *viciae*, *4 – Rhizobium leguminosarum* bv. *trifolii*

Rhizobium ecological adaptation. In other series of field experiments, we estimated the efficiency of different species and strains of *Rhizobium* depending on their ecological adaptation. The results showed that depending on rhizobia species and soil pH, legume symbiosis fixed 160–264 kg N ha^{-1} on average (Table 2). The highest amount of nitrogen was accumulated by *Rhizobium leguminosarum* bv. *trifolii* and the lowest by *Rhizobium leguminosarum* bv. *viciae.* From adapted strains, only *Rhizobium galegae* and *Rhizobium leguminosarum* bv. *viciae* had an advantage as compared to non-adapted, whereas *Sinorhizobium meliloti* and *Rhizobium leguminosarum* bv. *trifolii* strains from the standpoint of nitrogen fixation were of the same efficiency.

The content of crude protein depended on symbiosis. In all cases, legume inoculation had a positive effect on crude protein accumulation in legume yield. The inoculation of goat's rue and field bean with adapted rhizobia strains increased the efficiency of their symbiosis and had a positive effect on legume yield and biological nitrogen fixation.

Soil liming was an effective measure of improving the symbiosis and nitrogen fixation. Acid soil liming (6.2 t ha^{-1} of $CaCO_3$) enriched the soil with biological nitrogen by 57 kg N ha^{-1} (Table 3). Liming in combination with inoculation of red clover fixed the highest amount of nitrogen – 275 kg N ha^{-1}. Agricultural means effectively increased DM yield (by 2.4 t ha^{-1}) and crude protein content by 365 kg ha^{-1}.

Data of nutrient leaching analysis showed that a combination of soil liming with legume seed inoculation had an effect on the leaching of Ca^{+}. However, somewhat more nitrates (NO_3^- 18 kg ha^{-1}) leached in the plots where soil was unlimed and uninoculated clover grew. The explanation can be that the same measures intensified clover growth because there

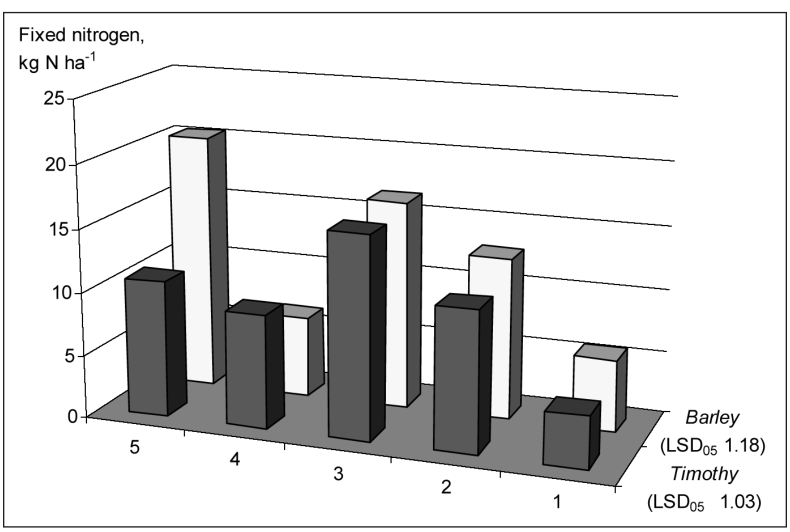

Fig. 3. The impact of associative diazotrophs on biological nitrogen fixation by barley and timothy: *1 – Flavobacterium* spp., *2 – Arthrobacter mycorens*, *3 – Agrobacterium radiobacter*, *4 – Azospirillum lipoferum*, *5 – Rhizobium leguminosarum* bv. *trifolii*

Table 1. **The impact of nutrient medium pH on *Rhizobium* spp. biomass growth**

Rhizobium spp.	Nutrient medium pH and colony size					LSD_{05}	Index of rhizobia tolerance to acid (pH 5.4) reaction, %
	4.2	5.4	6.0	6.3	7.0		
S. meliloti	0	4.6	6.4	6.5	6.9	0.9	67
R. leguminosarum bv. *trifolii*	2.1	8.1	10.2	10.6	10.3	1.3	79
R. galegae	5.5	7.5	8.5	8.4	8.4	1.1	89
R. leguminosarum bv. *viciae*	5.9	8.0	8.8	8.9	9.2	1.3	87

was less nutrients leached from the area under clover crop (Hamdi, 1982).

Associative nitrogen fixation. Different species of diazotrophs fixed unequal amounts of biological nitrogen in the rhizoplane of barley and timothy (Fig. 3). The highest amount of nitrogen was accumulated by *Agrobacterium radiobacter*, *Rhizobium leguminosarum* bv. *trifolii* and *Arthrobacter mycorens*. They fixed 11.0–20.4 kg N ha^{-1} from the atmosphere. It is known that in non-acid and rich in phosphorus and potassium soils, associative nitrogen fixers accumulate 50 kg N ha^{-1} and more (Elgersma, Schlepers, 2001). It is obvious that slightly acid soils inhibit nitrogen fixation by diazotrophs.

Table 2. **Symbiotic efficiency of different *Rhizobium* spp. depending on their ecological adaptation**

Rhizobium strains	Fixed nitrogen kg N ha^{-1}	Number of nodules per $plant^{-1}$	DM yield t ha^{-1}	Crude protein	
				%	kg ha^{-1}
Rhizobium leguminosarum bv. *trifolii*					
Uninoculated	222	139	6.39	16.74	1070
Not adapted	264	184	6.94	18.33	1272
Adapted	261	202	7.39	17.00	1256
LSD_{05}	9.3	16.6	0.2		42
Rhizobium galegae					
Uninoculated	43.1	48.2	2.56	14.65	375
Not adapted	167.1	89.8	4.89	15.60	763
Adapted	213.3	129.0	5.73	16.47	944
LSD_{05}	22.5	19.3	0.30		24
Sinorhizobium meliloti					
Uninoculated	184	23	5.62	15.78	887
Not adapted	205	38	6.26	15.80	989
Adapted	213	41	6.35	16.12	1024
LSD_{05}	12.8	4.2	0.30		57
Rhizobium leguminosarum bv. *viciae*					
Uninoculated	160	62	3.05	29.81	772
Not adapted	171	78	3.25	29.86	824
Adapted	183	119	3.51	29.46	878
LSD_{05}	8.6	17.7	0.29		29

CONCLUSIONS

Our study has shown that soil acidity is the decisive factor in the distribution and symbiotic efficiency of *Rhizobium leguminosarum* bv. *trifolii* F., *Rhizobium leguminosarum* bv. *viciae* F., *Sinorhizobium meliloti* D. and *Rhizobium galegae* L.

Rhizobium leguminosarum bv. *trifolii* was mostly distributed and tolerant on acid soils. In a slighty acid soil, the average amount of rhizobia was $540.0 \cdot 10^3$ cfu g^{-1} soil. In highly acid and medium acid soils (pH_{KCl} 4.1–5.0), *Rhizobium galegae* and *Synorhizobium meliloti* were not found.

Under field conditions, rhizobia, depending on their species and soil pH_{KCl}, have fixed biological nitrogen from 0 to 289 kg N ha^{-1}. The most active fixer of atmospheric nitrogen was *Rhizobium galegae* and the worst *Rhizobium leguminosarum* bv. *viciae*. The minimal soil acidity for all studied species was pH_{KCl} 5.0 and optimal pH_{KCl} 6.5–7.0.

The results of this study indicated that different *Rhizobium* species were unequally tolerant to soil acidity. The index of tolerance of *Sinorhizobium meliloti* was 67%; in more tolerant *Rhizobium galegae* and *Rhizobium leguminosarum* bv. *viciae* it was 79 and 89%, respectively, comparing their growth in acid (pH 5.4) and neutral (pH 7.0) media.

Effective and ecologically adapted strains had an advantage if compared to non-adapted ones only in forming symbiosis and nitrogen fixation with *Rhizobium galegae* and *Rhizobium leguminosarum* bv. *viciae*. However, adapted strains formed markedly more nodules in legume roots than did non-adapted ones.

The most important factors to activate nitrogen fixation were acid soil liming and legume seed inoculation with effective and in some cases ecologically adapted rhizobia strains. Liming ($CaCO_3$ rate 6.2 t ha^{-1}) in combination with inoculation of red clover increased the content of fixed biological nitrogen from 169 to 275 kg N ha^{-1}.

The application of associative nitrogen fixing bacteria on non-legumes is highly promising. Our experiment data have shown that barley and timothy fixed 18.2–20.4 kg ha^{-1} of biological nitrogen when their seeds were inoculated with

Table 3. **Efficiency of inoculation and liming on red clover symbiosis and relation with leaching of nutrients**

Treatment	Fixed nitrogen kg N ha^{-1}	Leached nutrients kg ha^{-1}		DM yield t ha^{-1}	Crude protein	
		Ca^{+}	NO_3^{-}		%	kg ha^{-1}
Uninoculated, unlimed	169	134	18	4.60	13.63	627
Inoculated, unlimed	202	126	13	5.41	13.49	730
Uninoculated, limed	226	138	11	5.92	14.17	839
Inoculated, limed	275	128	15	7.00	14.17	992
LSD_{05}	43	14.2	2.54	0.60	1.67	86

diazotrophs (*Agrobacterium radiobacter, Rhizobium leguminosarum* bv. *trifolii* and *Arthrobacter mycorens*). *Rhizobium leguminosarum* bv. *trifolii* and *Rhizobium galegae* have been the most effective associates on spring rape.

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References

1. Adamovich A., Klasens V. Symbiotically fixed nitrogen in forage legume–grass mixture // Grassland Science in Europe. 2001. P. 12.
2. Ambrazaitiene D. Activity of symbiotic nitrogen fixation in the *Dystric albeluvisols* differing in acidity and fertilization // Agriculture. Scientific Articles. 2003. Vol. 83. N 3. P. 173–186.
3. Carranca A., Varennes de A., Rolston D. Biological nitrogen fixation by faba been, pea and chickpea under field conditions, estimated by the N–15 isotope dilution technique // Europian Journal of Agronomy. 1999. Vol. 10. Iss. 1. P. 49–56.
4. Čiuberkis S., Čiuberkienė D., Končius D. ir kt. Kalkinimo bei tręšimo sistemų poveikis dirvožemio savybėms ir agrocenozėms // Žemės ūkio mokslai. 2005. Nr. 2. P. 1–12.
5. Delavechia C., Hampp E., Fabia A. et al. Influence of pH and calcium on growth, polysaccharide production and symbiotic association of *Sinorhizobium meliloti* SEMIA 116 with alfalfa roots // Biology and Fertility of Soils. 2003. Vol. 38. Iss. 2. P. 110–114.
6. Elgersma A., Schlepers H. N-use efficiency in grass–clover mixtures // Grassland Science in Europe. 2001. Vol. 6. P. 73–75.
7. Hamdi Y. A. Application of nitrogen-fixing systems in soil improvement and management // Soil's Bulletin. Rome, 1982. Vol. 49. 188 p.
8. Hartwig U. A., Soussana J. F. Ecophysiology of symbiotic N_2 fixation in grassland legumes // Grassland Science in Europe. 2001. Vol. 6. P. 1–10.
9. Kelner D. J., Vessey J. K., Antz M. H. The nitrogen of 1,-2- and 3-year stands of alfalfa in a cropping system // Agriculture Ecosystems and Environment. 1997. Vol. 64. Iss. 1. P. 1–10.
10. Lapinskas E. Biologinio azoto fiksavimas ir nitraginas. Akademija, 1998. 218 p.
11. Lapinskas E. Effectiveness of *Sinorhizobium meliloti* strains adapted to soil acidity. In: Advances of Agricultural Sciences Problem Issues. Warsaw, 2002. Iss. 482. P. 323–328.
12. Lapinskas E. Liucernų inokuliavimo efektyvumas, priklausomai nuo gumbelinių bakterijų štamų adaptacijos dirvožemio rūgštingumui // Vagos. LŽŪU mokslo darbai. 2004. T. 63(16). P. 20–25.
13. Lindstrom K., Myllyniemi H. Sensitivity of red clover rhizobia to soil acidity factors in pure culture and in symbiosis // Plant and Soil. 1987. Vol. 98. N 3. P. 353–362.
14. Ozawa T., Imai Y., Sukiman H. L. et al. Low pH and aluminium tolerance of *Bradyrhizobium* strains isolated from acid soils in Indonesia // Soil Science and Plant Nutrition. 1999. Vol. 45. Iss. 4. P. 987–992.
15. Tarakanovas P., Raudonius S. Agronominių tyrimų duomenų statistinė analizė taikant kompiuterines programas ANOVA, STAT, SPLIT, PLOT iš paketo „Selekcija ir Irristat“. Akademija, 2003. 56 p.
16. Umarov M. M. Nitrogen in the modern biological farming // Proceedings of 13 Congress International Society of Soil Science, 1986. Hamburg, 1987. Vol. 6. P. 950–953.
17. Vassileva V., Kostov O. Effect of mineral and organic nitrogen on nodulation and production of lucerne *Medicago sativa* L. under imposed drought conditions // Grassland Science in Europe. 2001. Vol. 6. P. 23–26.
18. Андреюк К. І., Иутінска Г. А., Антіпчук А. Ф. Функціонування мікробних ценозів грунту в умовах антропогенного навантаження. Київ: Наукова думка, 2001. Ч. 1–2. 240 с.
19. Каминскій В. Ф., Голодна А. В. Зернобобові культури – джерело біологічного азоту // Вісник аграрної науки. 2000. Спец. вид. С. 45–47.
20. Коць С. Я. Фізіологічні особлівості формування та функціонування сімбіотичних систем бобових рослин бульбучков // Онтогенез рослин, біологічна фіксація молекулярного азоту та азотний метаболізм. Тернопіль, 2001. С. 86–90.
21. Парахин Н. В., Петрова С. Н. Сравнительная симбиотическая активность, урожайность и белковая продуктивность многолетних бобовых трав в условиях Орловской области // Известия ТСХА. 2001. Вып. 3. С. 18–33.
22. Трепачев Е. П., Ягодина М. С. Симбиотический азот и органическое вещество бобовых в земледелии нечерноземной зоны РСФСР: вклад в плодородие почвы и урожайность озимой пшеницы // Сельскохозяйственная биология. Сер. биология растений. 1991. Т. 1. С. 18–34.
23. Шильников И. А., Кирпичникова Н. А., Удалова Л. П. Проблемы известкования почв // Химия в сельском хозяйстве. 1996. № 5. С. 18–21.

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BIOLOGINIO AZOTO FIKSACIJA LIETUVOS RŪGŠČIUOSE DIRVOŽEMIUOSE

Santrauka

Daugiamečių ekspedicinių, lauko, vegetacinių ir laboratorinių tyrimų rezultatais, įvairių rūšių gumbelinių bakterijų paplitimui ir simbioziniam efektyvumui lemiamos reikšmės turi dirvožemio pH. Dobilų gumbelinės bakterijos (*Rhizobium leguminosarum* bv. *trifolii*) daugiausia paplitusios ir tolerantiškiausios dirvožemio rūgščiai reakcijai. Vidutinis jų skaičius siekia 540,0 · 10^3 KSV g^{-1} dirvožemio. Rūgščiuose dirvožemiuose, kurių pH_{KCl} 4,1–5,0, ožiarūčių ir liucernų gumbelinių bakterijų (*Rhizobium galegae* ir *Sinorhizobium meliloti*) nerasta. Skirtingų rūšių gumbelinės bakterijos dėl dirvožemio pH_{KCl} gali sukaupti atmosferos azoto nuo 0 iki 289 kg N ha^{-1}. Daugiausia azoto fiksuoja rytinių ožiarūčių gumbelinės bakterijos ir mažiausiai – vikių grupės bakterijos. Visų rūšių *Rhizobium* azoto fiksacijos kritinis dirvožemio pH_{KCl} yra 5,0 ir optimalios sąlygos – pH_{KCl} 6,5–7,0.

Tyrimų duomenimis, skirtingų rūšių gumbelinės bakterijos nevienodai tolerantiškos dirvožemio rūgščiai reakcijai. Liucernų bakterijų tolerantiškumo indeksas, lyginant jų augimą rūgščioje (pH 5,0) ir neutralioje (pH 7,0) mitybos terpėse, buvo 67%, tuo tarpu ožiarūčių ir vikių grupių bakterijų – atitinkamai 89 ir 87%.

Efektyvūs ir ekologiškai pritaikyti ožiarūčių ir vikių gumbelinių bakterijų kamienai, formuojant simbiozę, buvo pranašesni už nepritaikytus: sudarė daugiau gumbelių ir aktyviau fiksavo atmosferos azotą. Dėl dirvožemio kalkinimo (6,2 t ha^{-1} $CaCO_3$), derinant su inokuliavimu, raudonųjų dobilų azoto fiksavimas padidėjo nuo 169 iki 275 kg N ha^{-1}.

Asociatyvių azotą fiksuojančių bakterijų naudojimas neankštiniams augalams turi dideles perspektyvas, ypač ekologinėje žemdirbystėje. Dėl miežių ir motiejukų inokuliavimo veiksmingais diazotrofų *Agrobacterium radiobacter*, *Arthrobacter mycorens* ir *Rhizobium leguminosarum* bv. *trifolii* kamienais aktyvėjo azoto fiksacija augalų rizoplanoje vidutiniškai 18,2–20,4 kg N ha^{-1}. Vasarinių rapsų atmosferos azoto asimiliaciją stimuliavo dobilų ir ožiarūčių gumbelinės bakterijos.

Raktažodžiai: *Rhizobium*, paplitimas dirvožemyje, azoto fiksacija, asociatyvūs diazotrofai