Fragm. Agron. 30(3) 2013, 172–182

EFFICIENCY OF SIMULTANEOUS INOCULATION OF ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM* L.) SEEDLINGS WITH BACTERIAL STRAINS OF *AZOSPIRILLUM* AND *RHIZOBIUM* GENERA

Dorota Swędrzyńska[1], Arkadiusz Swędrzyński[2], Alicja Niewiadomska[1], Justyna Starzyk[1]

[1]*Katedra Mikrobiologii Ogólnej i Środowiskowej,* [2]*Katedra Łąkarstwa i Krajobrazu Przyrodniczego, Uniwersytet Przyrodniczy w Poznaniu*

dorotas@up.poznan.pl

Abstract. The recognition and practical application of rhizosphere microorganisms stimulating plant growth and development (plant growth promoting rhizobacteria – PGPR) refers increasingly frequently to a simultaneous action of different groups of these organisms. One of such combinations is a simultaneous inoculation of leguminous plants with symbiotic (*Rhizobium*) and non-symbiotic bacteria. The latter ones, even though they participate in nitrogen fixing process only slightly, can stimulate the development of both symbiotic microorganisms as well as plants. It turns out that such combinations of microorganisms can also affect growth and development of non-legume plants. The presented study makes an attempt to evaluate the effectiveness of a simultaneous inoculation of Italian ryegrass (*Lolium multiflorum*) seedlings with bacteria from *Rhizobium* and *Azospirillum* genera. Italian ryegrass was selected for the experiment because this intensively growing and yielding grass constitutes a frequent component of grass-legume mixtures cultivated for fodder. The experiment involved individual or simultaneous inoculation of Italian ryegrass seedlings growing *in vitro* with strains of *Azospirillum lipoferum* or *Azospirillum brasilense* and *Rhizobium leguminosarum* bacteria. The following parameters were assessed in individual experimental treatments: numbers of microorganisms, nitrogenase activity as well as selected parameters of *Lolium multiflorum* seedlings – mass of shoots and roots. The effects of the applied simultaneous inoculation depended on *Azospirillum* species and, in many instances, turned out to be ambiguous. The employed co-inoculation with the assistance of the *A. lipoferum* strain supported, for example, increasing numbers of *Rhizobium*, although higher nitrogenase activity was determined in the treatment with *A. brasilense*. The mass of Italian ryegrass shoots was higher in combinations with the simultaneous inoculation irrespective of the applied *Azospirillum* strain. It is clear that the problem is interesting but it requires more comprehensive studies with the use of a greater pool of strains of microorganisms and a greater population of plants accompanied by longer observations of their growth and development in conditions of simultaneous inoculations by diazotrophs from *Azospirillum* and *Rhizobium* genera.

Key words: *Azospirillum*, *Rhizobium*, simultaneous inoculation, *Lolium multiflorum*

INTRODUCTION

One of the elements which exert a positive impact on plant growth and development is the activity of soil microorganisms situated in the zone directly adjacent to plant roots, i.e. rhizosphere, most commonly referred to as *plant growth promoting rhizobacteria* (PGPR) [Bashan et al. 2004, Swędrzyńska and Sawicka 2000]. These bacteria, due to their phytohormone synthesis, increase and facilitation of nutrient availability (among others, by biological fixation of

molecular nitrogen and mineralisation of organic phosphorus compounds), reduction of heavy metal toxicity against plants, antagonisation of plant pathogens or stimulation of plant induced systemic resistance (ISR) to pathogens are treated as microorganisms quite welcome in agrocenoses [De Bellis and Ercolani 2001, Krey et al. 2011, Król 2003]. Good recognition as well as practical utilisation of these microorganisms refer increasingly frequently to the simultaneous action of their different groups [Bashan et al. 2004].

Numerous articles indicate that simultaneous inoculation of different plants by various bacterial strains enhance their biological activity by exerting a favourable influence on their own development as well as on the development of the inoculated cultivations [Krey et al. 2011]. One of such combinations is a simultaneous inoculation of leguminous plants by symbiotic (*Rhizobium*) and non-symbiotic bacteria. The latter ones, even though are only slightly engaged in nitrogen fixation, can, nevertheless, stimulate the development of symbiotic microorganisms as well as plants thanks to production of phytohormones and other mechanisms. Barea et al. [2005], Burdman et al. [1996], Niewiadomska and Swędrzyńska [2011] as well as Tchebotar et al. [1998] reported a very promising co-inoculation of leguminous plants with bacteria from the *Rhizobium* and *Azospirillum* genera. Bacteria from the *Azospirillum* genus, due to their indirect action, affected the infection efficiency of plant roots with *Rhizobium* or *Bradyrhizobium*. Increased numbers of root nodules, more abundant growth of *Rhizobium* population as well as increased activity of N_2 fixation were observed and, consequently, increased yields of leguminous plants in comparison with the control plants which were not inoculated with *Azospirillum* ssp. A similar effect of a simultaneous application of *Sinorhizobium meliloti* and *Herbaspirillum frisingense* strains to the inoculation of alfalfa was observed in experiments carried out by Niewiadomska and Swędrzyńska [2011]. Alfalfa coinoculation with the above-mentioned strains exerted a positive impact on the process of symbiosis and yielding of seed alfalfa, as evidenced by increased numbers of root hair, elevated nitrogenase activity and higher weight of plants. However, there are also research results confirming a negative influence of *Azospirillum* on the process of nodulation [Plazinski and Rolf 1985].

Both international and domestic literature provides instances of experiments in which nodulation bacteria and endophytes were applied to inoculate non-leguminous plants to achieve a positive, synergistic impact on plant growth. It was found, for example, that the simultaneous inoculation of oat seedlings with *Azospirillum brasilense* and *Sinorizobium meliloti* bacteria exerted a significant influence on plant growth [Rozconi et al. 2002]. Other investigations [Swędrzyńska and Niewiadomska 2006] examined the effect of inoculation of maize seedlings with strains of *Azospirillum* and *Rhizobium* bacteria in *in vitro* conditions. Results of this study indicate that simultaneous inoculation leads to considerably greater numbers of these bacteria in the rhizosphere in comparison with the inoculation with a single strain, contributes very distinctly to increased nitrogenase activity and – in the case of plants – to more abundant mass of shoots. This kind of experiments are based on well known examples of natural associations occurring between non-leguminous plants and bacteria from the *Rhizobium* genus, e.g. *Rhizobium etli* and maize [Gutiérrez-Zamora and Martínez-Romero 2001] or *Rhizobium leguminosarum* and rice [Yanni et al. 1997, 2000].

The objective of this study was an attempt to evaluate the effectiveness of a simultaneous inoculation of Italian ryegrass (*Lolium multiflorum*) seedlings with bacterium strains from *Rhizobium* and *Azospirillum* genera. The choice of the Italian ryegrass was due to the fact that this intensively growing and high-yielding grass is a frequent component of grass-legume mixtures grown for fodder.

MATERIALS AND METHODS

The experiment was established in laboratory conditions in 2007–2008 at the Department of General and Environmental Microbiology of Poznań University of Life Science. This paper presents results of two parallel *in vitro* experiments carried out on plants growing in test tubes on Schenke & Hildebrandt Medium (SHM) slants containing appropriate minerals and vitamins sealed with cotton corks ensuring sufficient gas exchange for plants [Someregarn and Hoben 1994]. In the first experiment, the effect of inoculation of Italian ryegrass seedlings with strains of *Rhizobium leguminosarum* and *Azospirillum brasilense* bacteria was examined, whereas in the second experiment – Italian ryegrass seedlings were inoculated with *Rhizobium leguminosarum* and *Azospirillum lipoferum* bacteria.

Strains of *Rhizobium leguminosarumr* bv. *trifolii* C37 and *Azospirillum lipoferum* Br17 bacteria derived from the Section of Microbiology, IUNG in Puławy, while *Azospirillum brasilense* sp T60 – from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH w Braunschweig, Germany. Kernels of the tetraploid Italian ryegrass cv. "Lotos" derived from the collection of the Department of Grass Science and Natural Landscape of Poznań University of Life Sciences.

Italian ryegrass is one of the most important and valuable fodder grasses. It is a fast-growing, profusely-yielding grass which develops very strong root systems and which is characterised by high nutritional value resulting, among others, from high sugar content. It is the main grass species cultivated on short-term grasslands in clear-sowing or in mixtures with other grasses or legumes [Kozłowski and Swędrzyński 2006].

The following experimental combinations were applied:

- control (without inoculation),
- inoculation of plants using a bacterial strain from the *Rhizobium leguminosarum* genus,
- inoculation of plants with a bacterial strain from the *Azospirillum brasilense* genus (experiment 1) or *Azospirillum lipoferum* genus (experiment 2),
- simultaneous inoculation (coinoculation) with *Rhizobium leguminosarum* and *Azospirillum brasilense* bacteria (experiment 1) or *A. lipoferum* bacteria (experiment 2).

Kernels of Italian ryegrass employed in the discussed trials were sterilised according to the method described by [Chebotar et al. 2001], i.e. 5 minutes in 70% alcohol solution, followed by 2-minute treatment in 5% hydrogen peroxide and, finally, rinsing in sterile water (5 times for 2 minutes). Kernels prepared in this way were pre-germinated and placed in test-tubes on slants (20 kernels for each combination). After 24 hours, they were inoculated with a bacterial culture of *Azospirillum* incubated earlier for 24 h at the temperature of 28°C on a liquid DAS medium [Döbereiner 1980] or with a *Rhizobium* culture incubated in identical conditions on a liquid YMB medium [Someregarn and Hoben 1994]. The suspension was introduced in the amount of 0.1 ml per each replication. The number of bacterial cells in 1 ml suspension ranged from 10^8 to 10^9 CFU.

In the course of three weeks following the inoculation (after 7, 14 and 21 days), the effectiveness of the applied association system: plants – diazotrophs operating in conditions of a single or double inoculation was assessed. The above assessment was carried out on the basis of the following:

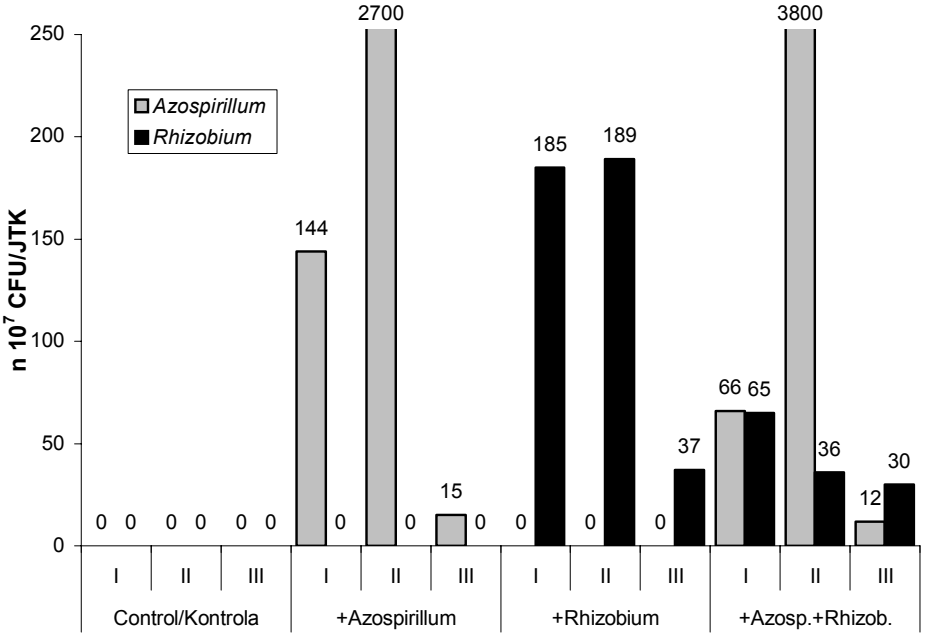
- numbers of bacteria in plant roots – method of flooded plates using the DAS medium [Döbereiner 1980] for *A. brasilense* and *A. lipoferum* and YMB medium for *Rhizobium* [Someregarn and Hoben 1994].
- nitrogenase activity of the examined strains determined by acetylene method [Sawicka 1983].

• weight of seedlings (shoot and roots after removal from the test tube, rinsing the medium and filtering off water).

The obtained research results were subjected to statistical analysis. The significance of differences between objects obtained in the course of the analysis of variance was estimated using Snedecor's F test establishing least significant differences (LSD) at the level of $\alpha = 0.05$.

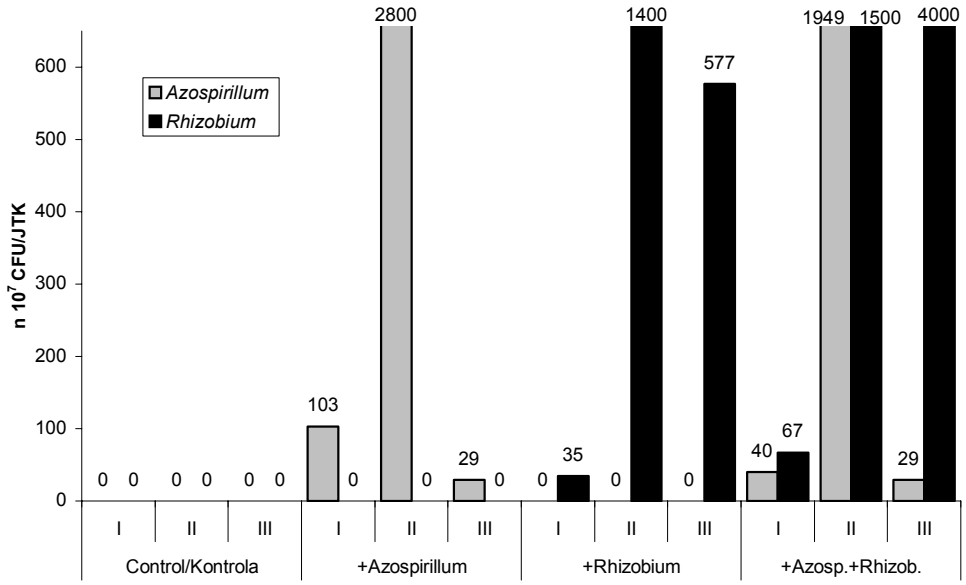
RESULTS

During the performed *in vitro* trial, in the course of a three-week period (after 7, 14 and 21 days) of development, following the inoculation of Italian ryegrass seedlings with strains of *Azospirillum brasilense* (experiment 1) or *Azospirillum lipoferum* (experiment 2) and *Rhizobium leguminasorum*, the effectiveness of the association system: plants – bacteria was evaluated. This evaluation was performed on the basis of: numbers of bacteria determined on plant roots, nitrogenase activity as well as the mass of seedlings. The occurrence of bacteria was determined only in inoculated combinations but their quantities in individual experimental treatments as well as in the two investigated experiments differed. The most consistent behaviour in the case of both experiments was determined in the case of bacteria from the *Azospirillum* genus which always attained maximal counts on the second date of analysis (14 days from the establishment of the experiment) and these counts were many times higher in comparison with the remaining dates. Simultaneous *Rhizobium leguminosarum* inoculation affected relatively slightly the size of *Azospirillum* population in comparison with the applied single inoculation (Fig. 1 and 2).



I – after 7 days, II – after 14 days, III – after 21 days

Fig. 1. Average number of *Azospirillum brasilense* ($LSD_{0.05}$ – 63) and *Rhizobium leguminosarum* ($LSD_{0.05}$ – 42) bacteria in 1 g of italian ryegrass roots in 3 terms of analyses

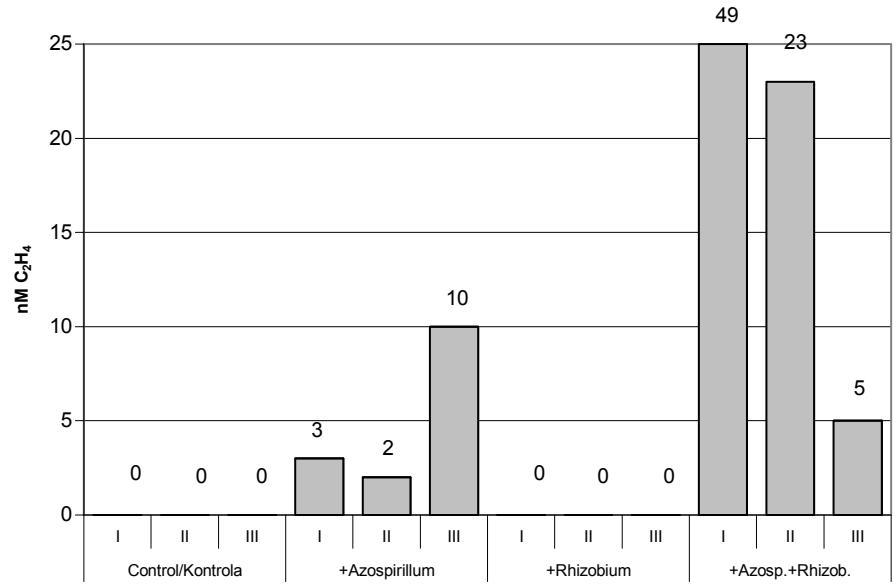


I – after 7 days, II – after 14 days, III – after 21 days

Fig. 2. Average number of *Azospirillum lipoferum* ($LSD_{0.05}$ – 98) and *Rhizobium leguminosarum* ($LSD_{0.05}$ – 55) bacteria in 1 g of italian ryegrass roots in 3 terms of analyses

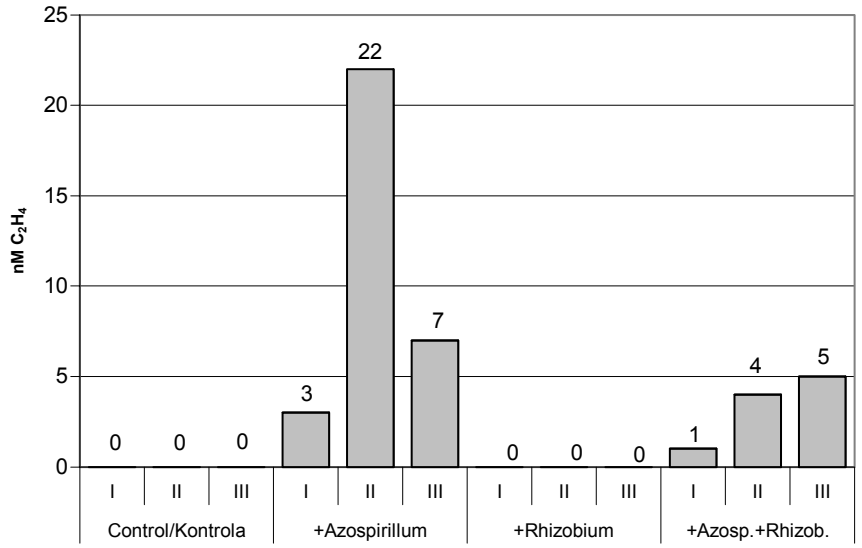
The examined *Rhizobium leguminosarum* bacteria behaved differently in the discussed experiments and their behaviour was difficult to interpret unequivocally. When analysing only the combinations with dual inoculation, it was observed that in the case of coinoculation with *Azospirillum brasilense*, bacteria from the *Rhizobium* genus were considerably less numerous in comparison with conditions of a single inoculation, while a simultaneous inoculation with *Azospirillum lipoferum* resulted in higher numbers of *Rhizobium*. However, it should be mentioned that the development of bacteria from the *Rhizobium* genus analysed on the basis of changes in population numbers of these bacteria followed a slightly different course also in the combination with a single inoculation.

Bacterial counts failed to translate directly into the process of diazotrophy (Fig. 3 and 4). The highest nitrogenase activity was recorded in experiment 1 in which the *Azospirillum brasilense* strain was used for inoculation in combination with *Rhizobium leguminasorum*. It amounted to 49 nM C_2H_4 per 1 plant/h after 7 days of culture and to 23 nM C_2H_4 per 1 plant/h after successive 7 days and was several times higher in comparison with nitrogenase activity in plants inoculated only with the *Azospirillum brasilense* strain. In the case of experiment 2 in which the *Azospirillum lipoferum* strain was used for inoculation, the highest nitrogenase activity was determined in the combination with *Azospirillum lipoferum* alone 14 days after inoculation – 22 nM C_2H_4 per 1 plant/h. The application of the *Rhizobium leguminasorum* strain in combination with *Azospirillum lipoferum* resulted in a several times smaller nitrogenase activity in comparison with the treatment in which the *Azospirillum lipoferum* strain alone was used. Nevertheless, this level remained unchanged until the end of the experiment, i.e. until day 21.



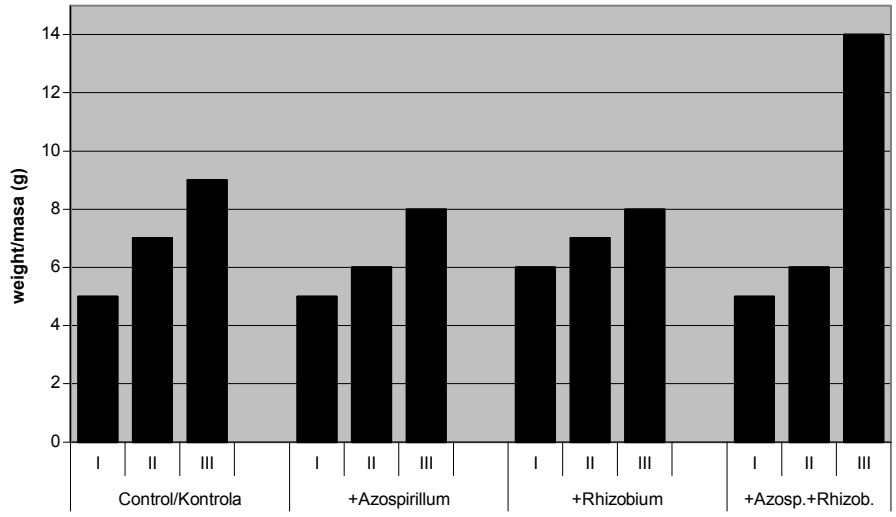
I – after 7 days, II – after 14 days, III – after 21 days

Fig. 3. Nitrogenase activity (nM $C_2H_4 \cdot h^{-1} \cdot 1$ plant^{-1}) in inoculation with *Azospirillum brasilense* and *Rhizobium leguminosarum* conditions in 3 terms of analyses ($LSD_{0.05}$ – 3)



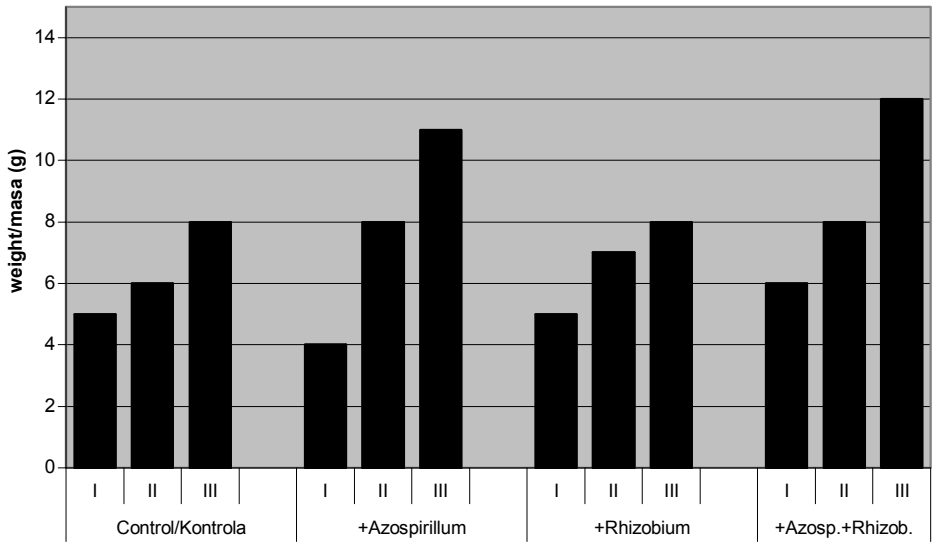
I – after 7 days, II – after 14 days, III – after 21 days

Fig. 4. Nitrogenase activity (nM $C_2H_4 \cdot h^{-1} \cdot 1$ plant^{-1}) in inoculation with *Azospirillum lipoferum* and *Rhizobium leguminosarum* conditions in 3 terms of analyses ($LSD_{0.05}$ – 4)



I – after 7 days, II – after 14 days, III – after 21 days

Fig. 5. Average weight ($g \cdot 10^{-3}$) of seedlings shoots of Italian ryegrass inoculated with *Azospirillum brasilense* and *Rhizobium leguminosarum* strains in 3 terms of analyses ($LSD_{0.05}$ – 3)



I – after 7 days, II – after 14 days, III – after 21 days

Fig. 6. Average weight ($g \cdot 10^{-3}$) of seedlings shoots of Italian ryegrass inoculated with *Azospirillum lipoferum* and *Rhizobium leguminosarum* strains in 3 terms of analyses ($LSD_{0.05}$ – non significant)

At least from practical point of view, the most important measure of functional efficiency of the diazotrophs – plant system is plant growth and development. It turned out that the response of the examined seedling traits of Italian ryegrass to the applied experimental combinations was not as clear as expected; it was confined only to shoots and it was statistically significant only in experiment 1. Nevertheless, it should be emphasised that plants subjected to a simultaneous inoculation in both experiments were characterised by greater shoot weight not only in comparison with the control plants but also with respect to plants inoculated with *Azospirillum* alone. This dependence was particularly apparent on the third date of analysis (Fig. 5 and 6). Single inoculation contributed to greater weight of shoots – in comparison with the control – only in the case of *Azospirillum lipoferum*.

DISCUSSION

Plants are widely considered as the most important factors affecting populations of rhizosphere microorganisms. Root secretions can stimulate or inhibit the activity of soil organisms causing them to release nutritive substances, infection of roots or modification of plant growth by means of appropriate signals. Common cohabitation of microorganisms in the same niche – rhizosphere – and their contact with plant roots forced a number of interdependences to develop among them which can assume either antagonistic or mutualistic character [Micallef et al. 2009, Paungfoo-Lonhienne et al. 2010, Watt et al. 2006]. In the experiments presented here, an attempt was made to evaluate the effectiveness of a simultaneous inoculation of Italian ryegrass (*Lolium multiflorum*) seedlings with strains of endophytic bacteria from the *Azospirillum* genus forming association systems with grasses and symbiotic bacteria – from the *Rhizobium* genus which are characteristic for leguminous plants. Since grasses occur frequently in communities together with legumes, the relationship analysis of such combinations of microorganisms and grass plants appeared justified.

Simultaneous inoculation of Italian ryegrass seedlings with strains of *Azospirillum brasilense* or *Azospirillum lipoferum* and *Rhizobium leguminosarum* bacteria in *in vitro* conditions caused a varied response of the latter ones. The application of *Azospirillum brasilense* bacteria was found to reduce numbers of bacteria from the *Rhizobium* genus in the rhizosphere of the Italian ryegrass in comparison with the inoculation by a single bacterial strain, contrary to the coinoculation with *Azospirillum lipoferum* which stimulated the development of *Rhizobium*. Very similar results were recorded in earlier investigations [Swędrzyńska and Niewiadomska 2006] during which the same strains of bacteria were employed for the inoculation of maize seedlings. Numbers of *Azospirillum* in coinoculation remained at the similar level as in the case of single inoculation which also corresponds with the research results of the above-mentioned study.

There are numerous examples in literature of mutual toxic interactions of microorganisms. For example, 2,4-diacetylphloroglucinol (DAPG) production by *Pseudomonas fluorescens* or manufacture of peptide antibiotics known as trifolitoxins (TFX) by *Rhizobium etli* cause reduction of biodiversity among rhizosphere bacteria [Burdman et al. 1996]. In the case of the experiment described in this study, the cause could have also been nutritional competition. In turn, the observed stimulating impact of *Azospirillum lipoferum* on *Rhizobium* could have resulted from synthesising and secretion by these bacteria of biologically active compounds: indolil acetic acid (IAA), gibberellins, cytokinins or others [Bashan et al. 2004].

The activity of nitrogenase in coinoculated combinations also depended on the species of *Azospirillum*. Simultaneous inoculation using *Azospirillum brasilense* exerted a very distinct

impact on the growth of nitrogenase activity in comparison with the single inoculation, while in the case of *Azospirillum lipoferum*, greater nitrogenase activity was recorded when single inoculation was applied. This indicates the need to carry out further investigations in order to verify if the observed differences in the response can be attributed to the specifics of the bacterium species from the *Azospirillum* genus or, perhaps, to the applied strain and what mechanisms are involved.

A positive response of Italian ryegrass to dual inoculation was apparent with respect to a greater weight of shoots and this concerned both experiments, i.e. both *Azospirillum* species. Therefore, it is difficult to conclude unequivocally whether the factor stimulating seedling growth was nitrogen or, perhaps, some other kind of influence. Also in this case, it would be interesting to continue investigations allowing longer observation of the system: diazotrophs – plants.

The results of the performed investigations remain in keeping with a series of reports published in recent years regarding the occurrence of bacteria from the *Rhizobium* genus in the rhizosphere of non-leguminous plants, especially cereals and fodder grasses [Gutiérrez-Zamora and Martínez-Romero 2001, Yanni et al. 1997, 2000]. Such situations were reported frequently in regions where these plants were cultivated in the employed rotation system interchangeably or simultaneously with leguminous plants. The research results obtained in this study as well as the results of earlier experiments [Rozconi et al. 2002, Swędrzyńska and Niewiadomska 2006] are sufficiently encouraging to undertake further investigations on the simultaneous application of bacterial strains from *Rhizobium* and *Azospirillum* genera because they indicate appropriateness of the assumption that the combined inoculation with bacteria from *Rhizobium* and *Azospirillum* genera may support effectively grass growth and development.

REFERENCES

Barea J.M., Pozo M.J., Azcón R., Azcón-Aguilar C. 2005. Microbial co-operation in the rhizosphere. J. Exp. Bot. 56: 1761–1778.

Bashan Y., Holguin G., Bashan L.E. 2004. Azospirillum- plant relationshpis: physiological, molecular, agricultural, and environmental advances (1997–2003). Can. J. Microbiol. 50: 521–577.

Burdman S., Volpin H., Kigel J., Kapulnik Y., Okon Y. 1996. Promotion of nod gene inducers and nodulation in common bean (*Phaseolus vulgaris*) roots inoculated with *Azospirillum brasilense* Cd. Appl. Environ. Microbiol. 62: 3030–3033.

Chebotar V.K., Asis C.A Jr, Akao S. 2001. Production of growth-promoting substances and high colonization ability of rhizobacteria enhance the nitrogen fixation of soybean when coinoculated with *Bradyrhizobium japonicum*. Biol. Fert. Soils 34: 427−432.

De Bellis P., Ercolani G. 2001. Growth interactions during bacterial colonization of seedling rootlets. Appl. Environ. Microbiol. 67: 1945–1948

Döbereiner J. 1980. Forage grasses and grain crops. In: Methods for Evaluating Biological Nitrogen Fixation. Bergsen F.J. (ed.) Wiley and Sons, New York: 535–555.

Gutiérrez-Zamora M.L., Martínez-Romero E. 2001. Natural endophytic association betveen *Rhizobium etli* and *Zea mays* L. J. Biotechnol. 91: 117–126.

Kozłowski S., Swędrzyński A. 2006. Implikacje ploidalności w sferze jakości odmian *Lolium perenne* L. wielokrotnie defoliowanych. Pr. Kom. Nauk Rol. Kom. Nauk Leśn. PTPN 100: 63–76.

Krey T., Caus M., Baum C., Ruppel S., Eichler-Löbermann B. 2011. Interactive effects of plant growth-promoting rhizobacteria and organic fertilization on P nutrition of *Zea mays* L. and *Brassica napus* L. J. Plant Nut. Soil Sci. 174: 602–613.

Król M.J. 2003. Interakcje *Azospirillum* spp z mikroorganizmami glebowymi. Post. Nauk Rol. 5:137–145.

Micallef S.A., Shiaris M.P., Colon-Carmona A. 2009. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J. Exp. Bot. 60: 1729–1742.

Niewiadomska A. Swędrzyńska D. 2011. Effect of coinoculation of lucerne (*Medicago sativa* L.) with *Sinorhizobium meliltoi* and *Herbaspirillum frisingense* in relation to the interactions between bacterial strains. Arch. Environ. Prot. 37(4): 37–48.

Paungfoo-Lonhienne C., Rentsch D., Robatzek S., Webb R.I., Sagulenko E., Näsholm T., Schmidt S., Lonhienne T.G.A. 2010. Turning the table: plants consume microbes as a source of nutrients. PLoS ON. 5(7): e11915.

Plazinski J., Rolf B.G. 1985. Influence of *Azospirillum* strains on nodulation of clovers y *Rhizobium* strains. Appl. Environ. Microb. 49: 984–989.

Rozconi F., Cabró A., Castro-Sowinski S., Martinez-Drets G. 2002. Growth promotion of *Avena sativa* by *Azospirillum* and *Sinorhizobium*. 9th International Symposium on Nitrogen Fixation with Non-legumes. Leuven, Belgium: 125.

Sawicka A. 1983. Ekologiczne aspekty wiązania azotu atmosferycznego, Rocz. AR Poznań, Rozpr. Nauk. 134.

Someregarn P., Hoben H.J. 1994. Handbook for Rhizobia. Methods in legume-Rhizobium technology. Springer-Verlag, New York: ss. 450.

Swędrzyńska D., Niewiadomska A. 2006. Czy jednoczesna inokulacja siewek kukurydzy (*Zea mays* L.) szczepami bakterii z rodzaju *Rhizobium* i *Azospirillum* może być efektywna? Acta Agr. Silv., Ser. Agraria 49: 461–470.

Swędrzyńska D., Sawicka A. 2000. Effect of inoculation with *Azospirillum brasilense* on development and yielding of maize (*Zea mays* spp. *saccarata* L.) under different cultivation conditions. Pol. J. Environ. Stud. 9: 505–509.

Tchebotar V.K., Kang U.G., Asis C.A., Akao Jr. S. 1998. The use of GUS-reporter gene to study the effect of *Azospirillum-Rhizobium* coinoculation on nodulation of white clover. Biol. Fertility Soils 27: 349–352.

Watt M., Silk W.K., Passioura J. B. 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. Ann. Botany 97: 839–855.

Yanni Y.G., Rizk R.Y., Corich V., Squartini A., Ninke K., Philip-Hollingsworth S., Orgambide G., De Bruijn F., Stoltzfus J., Buckley D., Schmidt T.M., Mateos P.F., Ladha J.K., Dazzo F.B. 1997. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194: 99–114.

Yanni Y.G., Rizk R.Y., Maya-Flores J., Dazzo F. 2000. Potential of the rice root occupant *Rhizobium leguminosarum* bv. *trifolii* as plant growth-promoting biofertilizer for rice. 17th North American Conf. on Symbiotic Nitrogen Fixation. University Laval, Quebec: 79.

D. Swędrzyńska, A. Swędrzyński, A. Niewiadomska, J. Starzyk

EFEKTYWNOŚĆ JEDNOCZESNEJ INOKULACJI SIEWEK ŻYCICY WIELOKWIATOWEJ (*LOLIUM MULTIFLORUM* L.) SZCZEPAMI BAKTERII Z RODZAJÓW *AZOSPIRILLUM* I *RHIZOBIUM*

Synopsis. Poznawanie i praktyczne wykorzystanie drobnoustrojów ryzosferowych stymulujących wzrost i rozwój roślin (PGPR) coraz częściej odwołuje się do symultanicznego działania różnych ich grup. Jedną z takich kombinacji jest jednoczesna inokulacja roślin motylkowatych bakteriami symbiotycznymi (*Rhizobium*) i niesymbiotycznymi. Te drugie, choć mają niewielki udział w wiązaniu azotu, to przez produkcję fitochormonów i inne mechanizmy, mogą stymulować rozwój drobnoustrojów symbiotycznych jak i roślin. Okazuje się, że takie kombinacje drobnoustrojów mogą mieć również wpływ na wzrost i rozwój roślin niemotylkowatych. Rzadziej stosuje się inokulację roślin niemotylkowatych w kombinacjiach z *Rhizobium.* Niniejsza praca jest próbą oceny efektywności jednoczesnej inokulacji siewek życicy wielokwiatowej

(*Lolium multiflorum*) szczepami bakterii z rodzaju *Rhizobium* i *Azospirillum*. Wybór życicy wielokwiatowej podyktowany był tym, że ta intensywnie rosnąca i plonująca trawa jest częstym komponentem mieszanek trawiasto-motylkowatych uprawianych na paszę. Doświadczenie polegało na indywidualnej lub jednoczesnej inokulacji siewek *Lolium multiflorum*, rosnących *in vitro*, szczepami bakterii *Azospirillum lipoferum* albo *Azospirillum brazilense* i *Rhizobium leguminosarum*. W poszczególnych kombinacjach doświadczalnych oceniano liczebność drobnoustrojów, aktywność nitrogenazy oraz wybrane parametry siewek życicy – masa pędów i korzeni. Efekty jednoczesnej inokulacji zależały od gatunku *Azospirillum* i w wielu momentach okazały się niejednoznaczne. Koinokulacja z użyciem szczepu *A. lipoferum* sprzyjała np. wzrostowi liczebności *Rhizobium*, ale większą aktywność nitrogenazy wykazano w wariancie z *A. brasilense*. Masa pędów życicy była większa w kombinacjach z jednoczesną inokulacją, niezależnie od użytego szczepu *Azospirillum*. Zagadnienie okazuje się zatem interesujące jednak wymaga głębszego poznania z zastosowaniem większej puli szczepów drobnoustrojów i większą populacją roślin i przy dłuższym śledzeniu ich wzrostu i rozwoju w warunkach jednoczesnej inokulacji diazotrofami z rodzajów *Azospirillum* i *Rhizobium*.

Słowa kluczowe: *Azospirillum*, *Rhizobium*, jednoczesna inokulacja, *Lolium multiflorum*