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Effect of Inoculation on Population Numbers of *Azospirillum* Bacteria under Winter Wheat, Oat and Maize

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Abstract

Changes in the numbers of *Azospirillum* bacteria were studied in soil under winter wheat, oat and maize crops. The cereal seeds were treated with fungicides and inoculated with *Azospirillum brasilense* actively fixing dinitrogen. Plants were grown without nitrogen fertiliser and with nitrogen application in the amount of 60 and 120 kg N ha^{-1} for wheat, 50 and 100 kg N ha^{-1} for oats and 100 and 200 kg N ha^{-1} for maize. The population number of bacteria was estimated at different developmental stages of plants. Inoculation of cereals with *Azospirillum brasilense* bacteria contributed to the increase of their numbers in soil. No significant influence of fungicidal seed dressings on the numbers of *Azospirillum* bacteria has been noted. The application of mineral nitrogen to the crops was favourable to multiplication of *Azospirillum* bacteria under plants.

Keywords! *Azospirillum brasilense,* diazotrophs, endophyts, inoculation, oats, wheat, maize, fungicides.

Introduction

In connection with numerous manifestations of a beneficial action of *Azospirillum* bacteria on plants, since the beginning of studies on bacterial species of this genus attempts have been made to use them practically in agriculture through inoculation of crops with these bacteria. The effect of inoculation with *Azospirillum* strains on the yield of cultivated plants was demonstrated many times, particularly under conditions of tropical climate. It is reported, that *Azospirillum* occurs in about 90% of tropical soils and in nearly 60% of soils of temperate climate [29]. The two species - *Azospirillum brasilense* and *Azospirillum lipoferum* are found in soils of the temperate zone [9, 15] and even in the zone of the cold climate of Finland [13].

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Azospirillum brasilense is attributed to have affiliation with plants with photosynthesis of type C3 (wheat), while *Azospirillum lipoferum* is considered to have affiliation with plants of type C4 (maize). Investigations of microorganisms isolated from under grasses on the area of Poland and other countries showed that this relationship is not unbiased, since the occurrence of *Azospirillum brasilense* has been found in the rhizosphere of maize and many other plant species [7, 19]. In view of a positive influence of bacteria from the genus *Azospirillum* on plants, attempts have been made to inoculate crops with these bacteria. The effect of inoculation to a large degree dependent on the ability of microorganisms to survive in the soil. Their survival is determined by such factors, as chemical and granulometric composition of soil, temperature, pH, presence of pesticides, plants and other microorganisms [6].

One of the factors determining the size of soil microorganism populations are bacteriophages, which *Azos-*

pirillum bacteria compete with [4]. Antagonistic action of other soil microorganisms against *Azospirillum* was described in the case of *Pseudomonas* sp. [11] as well as in the case of actinomycetes and some fungi [20], which have a negative action on many strains of *Azospirillum brasilense* and *Azospirillum lipoferum* through exretion of components having antibiotic properties. B otna [8] showed that under favourable conditions actinomycetes occurring in soil may reduce the size of *Azospirillum brasilense* population even by 25%.

Another factor having its influence on *Azospirillum* bacteria in cultivated soils may be agrochemicals applied for plant protection, particularly fungicidal seed dressings, which in case of a high toxicity can make impossible root colonization by bactertia. No available literature on that subject has been found. There are only results of *in vitro* experiments, which have proved the influence of herbicides on nitrogenase activity [14] and development of *Azospirillum* [12].

The aim of the present paper was to study the effect of inoculation of winter wheat, oats and maize with *Azospirillum* strain on the numbers of bacteria from the genus *Azospirillum* at different developmental stages of plants on the background of varying nitrogen application and plant protection with fungicidal seed dressings.

Material and Methods

Field experiments were carried out in the years 1996 and 1997 in Ztotniki on the field belonging to the Experimental and Didactic Station of August Cieszkowski Agriculture University of Poznan. The studies were conducted in winter wheat crops *(Triticum aestivum* L.) of the cv. Roma, in oats *(Avena sativa* L.) of the cv. Santor and in maize *(Zea mays* L.) of the cv. Gama. The primary criterion of plant selection for the experiments was their ability to create associative relations with bacteria from the genus *Azospirillum,* their economic importance in our country, and in case of oats - also lower nitrogen requirements.

In the experiments conducted in randomized block design the following three factors were used: inoculation with *Azospirillum brasilense* active strain (strain 65B) originating from the Department of Microbiology, Institute of Soil Science and Plant Cultivation, Pulawy; three levels of nitrogen fertilizer; seed treatment with fungicidal seed dressing.

Inoculation was performed just before sowing: treated and untreated seeds of cereals were mixed with a mixture of bacteria and sprayed over the field (soil in rows was mixed with coulters of a grain drill). Additionally, the field was sprayed with the suspension of *Azospirillum brasilense* bacteria after the emergences, whereas in the case of maize the suspension was applied individually under each plant as a strong stream in the vicinity of roots at the stage of 2-3 leaves. The number of *Azospirillum* cells in the suspension amounted to 10^8 - 10^9 (c.f.u.). per 1 ml. Such methods were used many times by other authors in their field experiments [1, 11, 24, 28, 29, 32].

The applied levels of nitrogen fertlizer are: Nl - no fertilization (control), N2 - half the full dose, N3 - full dose, which in terms of a pure ingredient was 120 kg N ha^{-1} for wheat, 100 kg N ha^{-1} for oats and 200 kg N ha^{-1} Nitrogen was applied in the form of ammonium nitrate.

The applied seed dressings were: Baytan Universal 19.5 DS (active ingredients: 2% fuberidazole, 2.5% imazalil, 15% triadimenol) for wheat, Oxafun T (active ingredients: 37.5% carboxine, 37.5% thiuram) for oats, Vitavax 200FS (active ingredients: 20.0% carboxine, 20.0% thiuram) for corn.

The cereal crops were grown on the soils developed from loamy sand glacial material with a medium content of potassium, phosphorus and magnesium, and with a slightly acid reaction.

The numbers of *Azospirillum* bacteria under wheat and oat crops in the vegetation season were estimated five times at the tillering, shooting, heading, flowering and milk-dough stage. In soil under corn these analyses were carried out four times - at the stages of: 3-4 leaves, 7-9 leaves, tasselling and flowering. The obtained mean number of bacteria was counted per 1 g of dry weight of soil. To estimate the population of *Azospirillum* the method of subsequent dilutions, with five replications was used. In each soil sample bacterial counts were estimated as index for bacterial growth by MPN method using tubes containing 5 cm^3 semisolid nitrogen-free medium [30]. The most probable number of microorganisms was obtained from McCrady's tables.

Results and Discussion

The number of *Azospirillum* bacteria was analysed from the point of view of the action of seed dressing, nitrogen fertilizer and developmental plant stage on these bacteria in crops inoculated and non-inoculated with *Azospirillum brasilense.* Results of the studies concerning this question are presented in Table 1 and Figures 1-3.

Table 1. The numbers *of Azospirillum* bacteria under cereals in inoculated and non-inoculated combinations.

Plant	*Azospirillum* in 1 g dry wt of soil (n • 10^3)			LSD	
	inoculated	non--inoculation	different	$\alpha_{0.05}$	$\alpha_{0.01}$
1996					
Wheat	91	99	8	45	61
Oats	186	109	77*	**62**	83
Maize	279	84	195**	**107**	**145**
1997					
Wheat	858	177	681**	**444**	**646**
Oats	720	502	218*	**171**	249
Maize	194	58	136	240	301

*Significant difference at the level of $\alpha_{0,05}$

** Significant difference at the level of $\alpha_{0,01}$

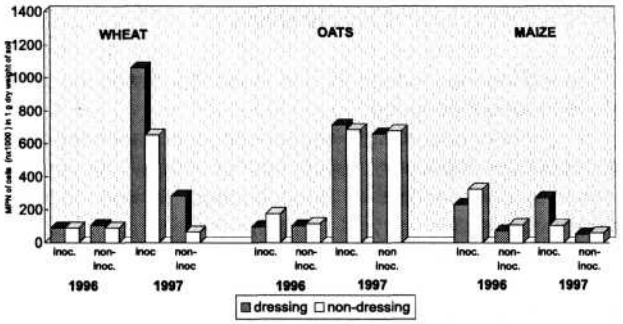
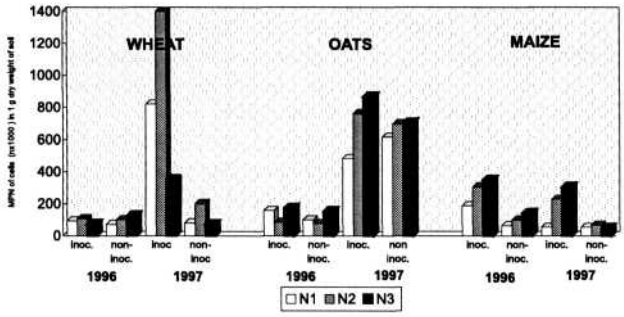
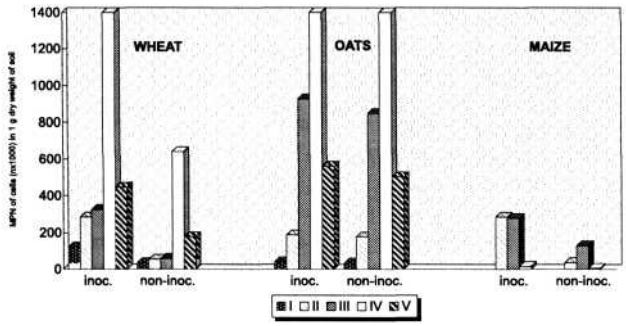


Fig. 1. Effect of seed dressing on *Azospirillum* population under cereals.

Fig. 2. Effect of nitrogen fertilizer on *Azospirillum* numbers under cereals.





Explanation:
I tillering (3-4 leaves in maize)
II shooting (7-9 leaves in maize)
III heading (tasselling in maize)
IV flowering
V milk-dough maturation

Fig. 3. Effect of cereal developmental stage on the number of *Azospirillum* in soil under cereal crops grown in 1997.

As follows from our studies, inoculation of crops with *Azospirillum* contributed to soil enrichment in these bacteria. The number of *Azospirillum* was nearly always higher in cereal crops inoculated with these bacteria (Table 1). Species and developmental stage of a cultivated plant had their influence on multiplication of bacteria (Table 1, Fig. 3). A factor determining *Azospirillum* numbers may also be weather conditions, which is indicated by varying numbers of bacteria under the same crops in different years of the experiment. The year 1997 was considerably more favourable to the development of *Azospirillum* under wheat and oats. The mean number of these bacteria was several times higher than in 1996.

In wheat, during the vegetation season of 1996, no significant differences were found in the numbers of *Azospirillum* bacteria between inoculated and non-inoculated combinations. However, in 1997, inoculation contributed to a highly significant degree to the population increase *of Azospirillum* bacteria under wheat. At all dates of analyses a higher cell number of these microorganisms was found on the inoculated plots (Table 1).

In soil under oats the mean numbers of *Azospirillum* in the inoculated combinations were significantly higher in both years of the studies (Table 1).

Under maize in 1996 as well as in 1997, a markedly larger (over threefold) cell number per 1 g dry weight of soil was found in the inoculated plots. However, a statistical analysis proved the significance of these differences only in 1996 (Table 1).

The numbers of *Azospirillum* bacteria found in the radical zone of the studied crops were high compared with literature data, being on the level of 10^4 - 10^5 cells per 1 g dry weight of soil. Okon [27] reports that bacteria from the genus *Azospirillum* constitute 1-10% of the population of all rhizosphere microorganisms, though some authors consider this number too high, especially under conditions of a temperate climate [26]. A record number of these bacteria was found in the rhizosphere of spring wheat in Brazil (10^8 cells/g of soil) [3]. Usually, however, the detected numbers of bacteria were smaller and in the case of wheat they were on the level of 10^3 - 10^6 cells/g of soil [2, 5, 25]. The largest divergences concern soils of temperate or winter climate. Under these conditions from 0 to 10^7 *Azospirillum* cells were found per 1 g dry weight of soil [9, 15, 19, 22, 31].

The applied seed dressings (Baytan, Vitavax, Oxafun) did not largerly effect *Azospirillum* bacterium population under wheat, oat and maize crops (Fig. 1).

The lack of significant differences between treated and untreated combinations is also confirmed by statistic analysis ($\alpha = 0.05$) (not attached here for that reason). Only in 1997, in the case of both inoculated and noninoculated wheat crops as well as inoculated maize a markedly larger, but statistically also an insignificant number of *Azospirillum* bacteria was observed under treated plants (Fig. 1).

The influence of mineral nitrogen fertilizer on the number of *Azospirillum* bacteria in soil (Fig. 2) became evident most clearly under corn - both in 1996 and 1997 and under oats in 1997 as well as under wheat in the both years of the studies. In these cases the number of *Azospirillum* bacteria increased with the increase of the applied mineral fertilizer. In the case of wheat in 1997, the

cause of decline in the number of *Azospirillum* in combinations with full nitrogen fertilizer was most probably a heavy plant infestation with fungal diseases, and in this connection - a lower plant vigour and as a consequence - a smaller amount of root exudates. As known from literature, dinitrogen fixing bacteria develop successfully in the presence of mineral nitrogen, if they have an ensured source of carbon. Sawicka [30] obtained results similar to ours from the present experiment concerning *Azotobacter* bacteria, the number of which increased with an increase of mineral nitrogen concentration in the soil.

It follows from the studies of many authors [16, 18, 23] that application of high rates of mineral nitrogen decreases the number of diazotrophs or eliminates them from the radical zone of plants, but according to the Kalininskaja [17] the most sensitive appeared to be *Azotobacter* and *Azospirillum* bacteria These relationships are not supported by results of the experimental works, in which cereal inoculation with *Azospirillum* bacteria and, therefore, their presence, contributed to a significant increase of plant yield even at the application of nitrogen fertilizer at 80 kg N ha^{-1} [28] and at a higher rate [29].

Results of the conducted studies concerning the influence of developmental plant stages on the numbers of *Azospirillum* bacteria are presented in Fig. 3. In 1996, no relationships were found between the developmental stage of cereals and occurrence of *Azospirillum* bacteria in soil under these plants. However, the number of *Azospirillum* in soil under wheat and oats grown in 1997 increased with plant growth and development achieving its maximum value at the moment of flowering, after which it decreased with seed setting and maturation (Fig. 3). That is probably related with the development of root system, photosynthetic activity and the amount of exudatives associated with this and produced by plants. This relationship was supported by an earlier decline in the numbers *of Azospirillum* bacteria in untreated combinations, which is caused by their faster drying induced by a stronger infestation with fungal diseases. The dependence of bacterium number on plant developmental stage was also recorded by Sawicka [30]. The numbers of *Azospirillum* bacteria under corn developed in a different way. In the case of that plant no expected increase in the numbers of *Azospirillum* bacteria was recorded as they passed to subsequent developmental stages, whereas a decline in *Azospirillum* numbers was recorded at the flowering stage. A similar relationship with regard to oats and spring barley was reported by Krol [19] and Liljeroth et al. [21]. Maize is a species favourable to the occurrence and development of *Azospirillum* and other microorganisms on account of a large amount of radical exudatives, characteristic of plants with a photosynthesis cycle of the type C-4. These bacteria numerously colonise root surfaces and intercellular spaces, finding there very suitable association conditions [10]. In the present experimental work the numbers of *Azospirillum* bacteria were estimated in soil near plant roots, but not directly on roots, and may be for that reason the influence of plant developmental stage on the number of studied bacteria has not been perceived, particularly because with plant growth, the main root mass and rhizosphere microorganisms together with it, move into the depth of soil profile.

Conclusions

Based on the results of this work the following conclusions could be given:

1. Inoculation of soil and cereal seed material with *Azospirillum brasilense* strain can contribute to an in crease in the numbers of these bacteria in soils of tem perate climate.
2. Species and developmental stage of a cultivated plant are factors, which determine, to a sufficient degree, the number of bacteria from the genus *Azospirillum* oc curring in a crop.
3. The presence of mineral nitrogen in soil can favour multiplication of *Azospirillum* bacteria under cereal crops.

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