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THE EFFECT OF INOCULATION WITH *AZOSPIRILLUM BRASILENSE* STRAIN 410 ON SPRING BARLEY CV. NOSIVSKY DEVELOPMENT AND YIELD

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Aim. To study the efficiency of inoculating spring barley with *Azospirillum brasilense* 410. **Methods**. 1) A field experiment on turf-podzolic sandy soil with different mineral fertilization regimes; 2) A greenhouse experiment on sand as substrate, with isotope dilution analysis using ^{15}N; 3) A lysimetric experiment in a stationary lysimetric installation. Furthermore, chromatography to determine nitrogenase activity of bacteria in the root zone of plants, agrochemical, and statistical methods. **Results.** Under field conditions, the inoculation with *A. brasilense* 410 promoted a significant increase (37–103 %) in the nitrogenase activity in the “soil-plant” system without any mineral fertilization and (especially) where $N_{60}P_{60}K_{60}$ was used. A high fertilizer level ($N_{120}P_{120}K_{120}$) lead to a long-term inhibition of the nitrogenase activity. In both cases (plants with and without inoculation with *A. brasilense* 410) this fertilization level showed an increase in the nitrogenase activity only at the end of the vegetation period. The highest increase in yield (0.7 t/ha, 27 %) in yield following *A. brasilense* strain 410 inoculation, occurred in plots with $N_{60}P_{60}K_{60}$ fertilization; the least increase in yield (0.33 t/ha, 16.5 %) was observed in plots receiving no fertilizers. The pre-sowing inoculation led to an increase in the protein content of 0.3–1.0 % in the barley grain, especially when receiving high fertilization levels, enhancing its value for the use in cereals and feeds, but decreasing its value for its use in brewing. The greenhouse experiment with ^{15}N established an increase 77.1 % in the nitrogen intake into the plants due to the activation of the nitrogen-fixation process and enhanced 29.5 % nitrogen consumption from fertilizers. The lysimetric studies demonstrated that inoculation of spring barley cv Nosivsky with *A. brasilense* 410 limited the vertical migration and leaching of nitrogen by 27–30 %, potassium by 13–30, calcium by 32–51 %, manganese by 33–100 %, and water-soluble organic matter by 46–75 %. **Conclusions.** The pre-sowing inoculation of spring barley cv. Nosivsky seeds with *A. brasilense* 410 intensifies nitrogen consumption by plants within 29.5 % due to active nitrogen-fixation and a better utilization of N from mineral fertilizers. The barley yield increase with 0.7 t/ha was in our limited experiment, using one cultivar roughly equivalent to the increase after mineral fertilization with $N_{60}P_{60}K_{60}$. Thus, pre-sowing inoculation with *A. brasilense* 410 may lead to substantial reduction of the use of mineral fertilisers in practice.

Key words: microbial inoculant, biofertilizer, isotope dilution of ^{15}N, lysimeters.

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INTRODUCTION

Among plant growth-promoting rhizobacteria (PGPR) (Kloepper et al, 1980), some of the most studied and promising ones are the representatives of *Azospirillum genus* (Florio et al, 2017; Le et al, 2019; Zeffa et al, 2019). Numerous studies demonstrate the increase in the productivity of crops after the inoculation with *Azospirillum* (Martins et al, 2017; Garsia et al, 2017; Di Salvo et al, 2018; Scott et al, 2020). At the initial stage of the investigation of *Azospirillum* bacteria, it was thought that their prospect as a potential basis of biofertilizers was related to their nitrogen-fixation ability. And though the contribution of *Azospirillum* into the nitrogen nutrition of the inoculated plants due to N_2-fixation often raised doubts (Boddey et al, 1986;

Bashan et al, 1989), different studies confirmed possible activation of the associative nitrogen-fixation process in case of inoculation (Saubidet, Barneix, 1998; Rodrigues et al, 2008). The intake of atmospheric nitrogen into plants due to the inoculation with *Azospirillum* is estimated within the range of 10 kg N/ha^{-1}/year. (Dobbelaere, Okon, 2007). Other researchers also pay attention to the low levels of atmospheric nitrogen supply of plants inoculated with azospirilla (Fukami et al, 2018; Cassan et al, 2020). However, this rather modest index does not exclude the contribution of *Azospirillum* to the enhanced yield of crops, since the effect of these bacteria on plant development is explained not only by the biological fixation of nitrogen, but also by other mechanisms, including the production of phytohormones and other biologically active substances, and higher assimilation of nutrients, including nitrogen by plants (Bashan et al, 2014; Souza et al, 2014; O'Callaghan, 2016; Fukami et al, 2018; Zeffa et al, 2019). Both the increase in the activity of N_2-fixation, and higher consumption of the active substance from mineral fertilizers by the inoculated plants may be used to reduce the use of mineral fertilizers, limit the emission of N_2O (Calvo et al, 2016; Florio et al, 2019; Volkogon et al, 2021) and facilitate global food production (Kenneth, 2017; Bargaz et al, 2018; Fasusi et al, 2021). Research in this direction is therefore relevant and urgent.

The current study was aimed at investigating the effect of *Azospirillum brasilense* under the temperate climate conditions of Ukraine at one location on a) the nitrogen nutrition of barley plants, b) the yield of the crop, and c) the quality of the product. We also conducted research with different varieties of barley, on different soils, under different agro-backgrounds, in different zones of Ukraine. These results are reported here, but will be subject of a further article.

MATERIALS AND METHODS

Field experiment. The study was conducted from 2018–2020 at the Institute of Agricultural Microbiology and Agro-industrial Manufacture, the National Academy of Agrarian Sciences of Ukraine in the Polissia zone of Ukraine (Chernihiv). The soil was turf-podzolic, pulverescent-sandy ($pH_{sal.}$ – 5.8; hydrolytic soil acidity−2.5 mg-eq./100 g; humus content – 1.02 %; easily hydrolyzed nitrogen − 45 mg/kg of soil; P_2O_5 – 170 mg/kg; K_2O – 65 mg/kg). The climate in the research area is moderately continental. The average temperature in July is from 18.4 to 19.9 °C, in January – from minus 6 °C to minus 8 °C. The period with a temperature above 10 °C is 150–160 days a year. The amount of atmospheric precipitation was 500 mm with minor differences by year. Weather conditions were not substantial different over the three years.

The plots of 86.4 m^2 (7.2 × 12.0 m) were arranged in a randomized block design. The experiment had four repetitions within three blocks of variants with different dosages of mineral fertilizers: 1) control – without fertilizers, 2) $N_{60}P_{60}K_{60,}$ and 3) $N_{120}P_{120}K_{120}$. Each block contained the variants of spring barley, cv Nosivsky, without inoculation and pre-sowing inoculation with A. brasilense 410.

The Azospirillum brasilense strain 410 (IMV V-7222) was originally isolated by Volkogon et al (1991) from the rhizoplane of meadow ryegrass as an active associative nitrogen fixer and producer of phytohormones. and deposited in the Depository of the Institute of Microbiology and Virology (IMV), NAAS of Ukraine. The strain is also stored at 4 °C in the laboratory collection on a semi-solid agar medium with malate. The strain actively progresses in the root zones of meadow ryegrass, spring barley, potatoes, and oats and enhances the productivity of these crops (Volkogon et al, 2006). To obtain inoculum, strain 410 was grown on a microbiological shaker at 180 rpm at 28 °C within 72 hours in the liquid culture medium, the following composition (g/l of water): L-malic acid – 5; K_2HPO_4 – 0.5; $MgSO_4 \times 7H_2O$ – 0.2; NaCl – 0.1; $FeSO_4$ – 0.01; $CaCl_2$ – 0.02; KOH – 3.0; $(NH_4)_2SO_4$ – 3.0; yeast extract – 0.5; pH = 6.8.

Prior to sowing, the barley seeds of Nosivsky cultivar were treated with the bacterial suspension (the number of bacterial cells in 1 ml of suspension – 3.0 × × 10^9 in a dose of 100 ml/200 kg of the seeding material. The titer of the bacterial suspension was determined before use by direct microscopic counting with a hemocytometer and by plating dilutions of the suspension on a semi-liquid medium with malate (Baldani et al, 2014) using the acetylene test (Villemin et al, 1974). Seed inoculation was carried out manually in the laboratory by adding water (2.0 % of the seed weight) to the bacterial suspensions to evenly distribute the bacterial cells on the seeds. Bacterized seeds were planted immediately after inoculation.The mineral fertilizers (nitrogen – ammonia nitrate, phosphorus – superphosphate, potassium – potassium chloride) were introduced in the estimated amounts prior to spring cultivation according to the scheme of the field experiment. During all the study years, the predecessor crop in the field was the potato.

Nitrogenase activity of the "soil-plant" system was determined in the field in different phases of plant development using the acetylene reduction method (Hardy et al, 1968) with field chambers (Zviagintsev, 1991; Kusa et al, 2008). Cylindrical stainless steel chambers, 0.3 m in diameter and 0.35 m high were used. The chambers were installed in soil at the depth of 5 cm. A water plug was made around the chamber for better isolation of the system and prevention of the loss of gases, accumulated in the chambers. A weighing cup with water was put inside the chamber with the addition of 20 g of calcium acetylide prior to the exposition (the reactions of calcium acetylide with water result in the formation of acetylene). The research was carried out in four-fold repetition. The period of the exposition with acetylene was 3 h. After the exposition, the gas samples were taken from the chambers using syringes, placed into the medical vacuum flasks with rubber membranes, and delivered to the laboratory. The gas samples were analyzed on a gas chromatograph "Chrom-5" (Czech Republic) with a flame ionization detector and a 3m steel column, filled with sorbent Parapak Q 60–80 mesh (Waters Corporation, USA); the thermostat temperature – 40 °C; the use of gases: hydrogen – 15 cc/min, nitrogen – 100 cc/min, air – 500 cc/min).

The nitrogenase activity in μmol of C_2H_4 per 1 sq.m. an hour was evaluated using formula 1:

$$HA = E \cdot V_1 \cdot V_2 \cdot 10000/S \cdot t \qquad (1)$$

where: E – the amount of ethylene in a gas sample, placed into the chromatograph; V_1 – the volume of a gas phase in the flask, cc; V_2 – the volume of the chamber, cc; 10,000 – the area in cm^2; S – the area of the chamber, in cm^2; t – the time of exposure to acetylene in h.

The barley yield was determined by measuring the grain weights at a standardized moisture content of 14 % from each plot.

The nitrogen content in grain was determined by Kjeldahl method with the subsequent calculation as per protein using the coefficient of 6.25 Preparation for the analysis included the formation of average grain samples and subsequent determination of the nitrogen content according to the protocol (Rhee, 2001). Protein content was determined in three analytical replicates.

Greenhouse experiment with ^{15}N. The study of the specificities of nitrogen nutrition for spring barley plants under the inoculation was conducted using the method of isotope dilution (with ^{15}N) (Chalk, 1985; Hardarson, Danso, 1993). The experiment was conducted using well-washed river sand, heated at 900 °C. The scheme of the experiment envisaged the following variants: 1 – control, no inoculation; 2 – pre-sowing inoculation with *A. brasilense* 410. Each experiment variant included the application of $^{15}NH_4{}^{15}NO_3$ enriched with ^{15}N – 99.5 % (at.). The substrate (1,000 g of sand/glass vial) was introduced 13 mg N. A share of labeled nitrogen was 1/3. In addition to nitrogen compounds, other salts were introduced into the substrate, according to Pryanishnikov (g.kg^{-1}): $CaHP0_4 \times 2H_20$ – 0,172; KCI – 0,16; $MgSO_4$ – 0,06; $CaSO_4 \times 2H_2O$ – 0,344; $Fe_2Cl_3 \times 6H_2O$ – 0,025 (Zhurbytskyi, 1986). Demineralized water was used to fill the weight of the vial up to 1,250 g and it was kept during the period of plant cultivation (60 days), controlling it by daily weighing. Each vial had 5 spring barley plants, Nosivsky cultivar. The experiment had five repeats. The inoculation of the seeds with *Azospirillum* in the corresponding variant was conducted as described above. The plants were grown in a luminostate. The illumination mode – 20 thousand lux, the illumination period – 16 h per 24 h, the air temperature – 26 ± 2 °C.

The weight of the dry aboveground part of plants and the weight of dry roots was measured; the nitrogen content in the aboveground part, the roots, and sand was determined by Kjeldahl method (Rhee, 2001). The titrated solutions were oxidized with hydrochloric acid and evaporated down to a small volume. Nitrogen in the samples was transformed into N_2 by ammonia oxidization using sodium hypobromite. The isotope composition of samples was determined using a mass spectrometer MI-1305 (Ukraine, Sumy).

Lysimetric experiment. The stationary lysimetric installation of the Institute of Agricultural Microbiology and Agroindustrial Manufacture, the NAAS, has 48 lysimeter sections, located in two parallel lines of 24 lysimeters each. The vessels for infiltrate collection are placed under them. The lysimeters have a concrete structure of the bulk type. They are filled with soil, starting with the parent material, with the consideration of the capacity of genetic horizons. The soil in the lysimeters was turf-podzolic sandy. The agrochemical indices of the arable layer (0–23 cm) of soil: pH_{salt} – 5.0; humus content – 1.1 %; the content of P_2O_5 – 170.0; K_2O – 62.0 mg/kg of soil, were determined with the methods described above. The planting area of each lysimeter is 3.8 m2, the soil depth is 155 cm, the soil weight is 10.5 tons/lysimeter.

Spring barley, Nosivsky cultivar, was grown in the lysimeters.

The scheme of the experiment:

I. No inoculation:
1. No fertilizers; 2. $N_{60}P_{60}K_{60}$.
II. Inoculation with *A. brasilense* 410:
1. No fertilizers; 2. $N_{60}P_{60}K_{60}$.

The inoculation of the grain was conducted as described above. The high dose of nitrogen fertilizers was not included in this experiment, since preliminary results indicated that these high doses were suboptimal.

During the vegetation period, the washing (filtration) waters were collected to determine the content of the compounds of biogenic elements (NO_3, NH_4, P_2O_5, K_2O, CaO, MgO) and soluble organic matter in them using the relevant methods (Fomin, Fomin, 2000; Horodniy et al, 2005). The crop performance in the experiment was determined by direct weighing of grain from each plot.

Statistical analysis. The differences between the variant indices depending on the investigated factors were analyzed using ANOVA at a 5 % level of significance using Statistica 6.0 (Stat Soft Inc., USA) software.

RESULTS

Field studies. The determination of the field nitrogenase activity in the spring barley fields demonstrated that the inoculation with *A. brasilense* ensured a significant increase in the indices as compared to the variant without fertilizers for an extended period of time **(Table 1).**

The observed effect was greatly eliminated only at the end of the vegetation period. The highest indices of the nitrogenase activity were registered while growing the inoculated plants with an average mineral fertilizer dose of $N_{60}P_{60}K_{60}$. And the increase in the activity in this variant was observed, starting with the tillering phase and continuing throughout the vegetation period. However, the highest dose of mineral fertilizers at the beginning of plant vegetation led to a decrease in the nitrogenase activity and stimulated the process of associative nitrogen-fixing only at the end of the vegetation period. It also stimulated the nitrogenase activity only in the phase of milky grain ripeness. These results were confirmed in the following two years of study. There were differences in absolute values in different years, but the specificities of the effect of the investigated factors on nitrogenase activity were the same.

The inoculation with *A. brasilense* resulted in a fair increase of yield over all three years, but they depended on the mineral fertilization level. For instance, the least inoculation-induced increase in performance was registered when the barley was grown without mineral fertilizers (**Table 2**). The highest inoculation-induced increase in yield was obtained after the introduction of mineral fertilizers in the dose of $N_{60}P_{60}K_{60}$ into the soil. When the inoculated plants were grown with the highest mineral fertilization variant in the experiment ($N_{120}P_{120}K_{120}$), a substantially smaller yield increase (0.39 t/ha) was obtained, which was confirmed over the three-year study.

Inoculation promoted the increase in protein content in the grain (**Table 3**).

Table 1. The effect of seed inoculation with *A. brasilense* 410 on the nitrogenase activity in the root zone of spring barley cv. Nosivsky plants in a field experiment with or without mineral fertilization in 2019 (data representative for the three years of the experiments)

Treatments	Nitrogenase activity, µmol of $C_2H_4/m^2/h$		
	tillering stage	stem elongation stage	kernel milk stage
Variant I – no fertilizers			
No inoculation	8.13	2.90	2.96
Inoculation with *A. brasilense* 410	13.5^{ab}	5.91^{ab}	4.06
Variant II – $N_{60}P_{60}K_{60}$			
No inoculation	8.50	3.50	6.28^{ab}
Inoculation with *A. brasilense* 410	11.83^{ac}	15.89^{abc}	9.50^{abc}
Variant III – $N_{120}P_{120}K_{120}$			
No inoculation	4.43	4.06	8.13^{b}
Inoculation with *A. brasilense* 410	3.72	4.62	15.12^{abc}

Note. The changes are statistically significant at 95 % significance level: (^{a}) in general for the experiment, (^{b}) for agricultural variants, (^{c}) for inoculation with *A. brasilense*, and the interaction with fertilizers.

Table 2. The effect of inoculation of spring barley cv. Nosivsky with *A. brasilense* 410, with or without the use of mineral fertilizers on yield in a three-year field experiment

Treatments	Yield, tons/ha				Inoculation-induced increase of yield, t/ha
	2018	2019	2020	average	
Variant I – no fertilizers					
No inoculation	2.21	1.96	1.84	2.00	–
Inoculation with *A. brasilense* 410	2.55^{abc}	2.23^{abc}	2.20^{abc}	2.33^{abc}	0.33
Variant II – $N_{60}P_{60}K_{60}$					
No inoculation	2.74^{ab}	2.61^{ab}	2.42^{ab}	2.59^{ab}	–
Inoculation with *A. brasilense* 410	3.26^{abc}	3.43^{abc}	3.17^{abc}	3.29^{abc}	0.70
Variant III – $N_{120}P_{120}K_{120}$					
No inoculation	2.93^{b}	3.43^{ab}	2.81^{ab}	3.06^{ab}	–
Inoculation with *A. brasilense* 410	3.40^{abc}	3.7^{abc}	3.23^{abc}	3.45^{abc}	0.39

Note. The changes are statistically significant at 95 % significance level: (a) in general for the experiment, (b) for agricultural variants, (c) for inoculation with *A. brasilense*, and the interaction with fertilizers.

Table 3. The effect of inoculation of spring barley cv. Nosivsky with *A. brasilense* 410, with or without the use of mineral fertilizers on protein content in a three-year field experiment

Treatments	Protein content, %				Inoculation-induced increase of protein, %
	2018	2019	2020	average	
Variant I – no fertilizers					
No inoculation	8.3	8.1	8.5	8.3	–
Inoculation with *A. brasilense* 410	8.6	8.6	8.9	8.7	0.4
Variant II – $N_{60}P_{60}K_{60}$					
No inoculation	10.3^{ab}	10.7^{ab}	10.5^{ab}	10.5^{ab}	–
Inoculation with *A. brasilense* 410	10.9^{ab}	11.3^{ab}	11.0^{ab}	11.1^{ab}	0.6
Variant III – $N_{120}P_{120}K_{120}$					
No inoculation	13.7^{ab}	13.4^{ab}	14.0^{ab}	13.7^{ab}	–
Inoculation with *A. brasilense* 410	14.8^{abc}	14.3^{abc}	14.9^{abc}	14.7^{abc}	1.0

Note. The changes are statistically significant at 95 % significance level: (a) in general for the experiment, (b) for agricultural variants, (c) for inoculation with *A. brasilense*, and the interaction with fertilizers.

It should be noted that the pre-sowing inoculation of grain while growing the crop without mineral fertilizers and also when grown with low doses of mineral fertilizers led only to a slight increase in protein content. When growing plants with a high dose of mineral fertilizers, there was a substantial (1.0 %) increase in protein content of the grain (Table. 3)

Greenhouse experiment with ^{15}N. Because effect of fertilizers and inoculation with *A. brasilense* 410 on the nitrogenase activity in the root zone of barley plants under field conditions was studied using the indirect acetylene method, we additionally studied the effect on associative nitrogen-fixation using the method of isotope (^{15}N) dilution. The obtained results demonstrate a substantial effect of *A. brasilense* 410 on the formation of both the weight of the aboveground part (by 53.9 %) and the roots (by 78,5 %) (**Table 4**).

The effect of the investigated factors was especially vivid in nitrogen uptake by plants (Table 4). The indicators of nitrogen content in the experimental plants were lower than the control, because the dilution of nitrogen concentration due to the increase in biomass was observed. However, the total assimilation of nitrogen in the experimental version significantly exceeded the control indicators.

The analysis of the isotope composition of samples demonstrated that in case of the inoculation with *A. brasilense* there was a substantial (77.1 %) increase in the atmospheric nitrogen intake into plants. *Azospirillum* also promoted the activated intake of nitrogen from fertilizers by plants (29,5 %) (**Table 5**).

The determination of the residual nitrogen in sand demonstrated low indices since the absence of colloid parts in the sandy substrate and the development of the introduced bacteria only within the root spheres did not provide for the element fixation in the substrate.

The obtained data demonstrated that the application of *Azospirillum* facilitated the decrease from 9.66 to 8.68 mg/vessel in the loss of mineral nitrogen, which may be explained by its consumption by plants (**Table 6**).

Lysimetric experiment. The results of examination of the biogenic element compounds content in the filtration lysimetric waters demonstrated the decrease in the content of practically all the investigated compounds due to inoculation. Special contrasts between the corresponding controls and experimental variants were traced in the study of the content of nitrates, ammonia, calcium, and manganese. Thus, losses of nitrates as a result of inoculation of *A. brasilense* 410 decreased by 21.3–33.4 %, K_2O – by 11.1–23.1 %, CaO – by 24.7–33.8 %, MgO – by 25–50 %, depending on the

Table 4. The effect of inoculation with *A.brasilense* 410 on the biomass of spring barley cv. Nosivsky and the content of total nitrogen (mineral salts, including nitrogen, were applied in all variants, specified under Material and Methods

Treatments	Stems and leaves		Roots		Total (stems, leaves and roots)	
	weight*	content of N**	weight*	content of N**	weight*	content of N**
No inoculation	0.985	5.92	0.330	1.05	1.315	6.97
Inoculation with *A. brasilense* 410	1.516^{ac}	7.17^{ac}	0.589^{ac}	1.26^{ac}	2.105^{ac}	9.03^{ac}

Note. *) g/5 plants; **) mg/5 plants. The changes are statistically significant at 95 % significance level: (^{a}) in general for the experiment, (^{b}) for agricultural variants, (^{c}) for inoculation with *A. brasilense*, and the interaction with fertilizers.

Table 5. The effect of inoculation with *A. brasilense* 410 on nitrogen uptake for spring barley cv. Nosivsky plants

Variants of experiment	Nitrogen, mg/5 plants			
	total (A)	fertilizers (B)	grain (C)	biological (A-B-C)
No inoculation	6.97	3.32	2.25	1.40
Inoculation with *A. brasilense* 410	9.03^{ac}	4.30^{ac}	2.25	2.48^{ac}

Note. The changes are statistically significant at 95 % significance level: (^{a}) in general for the experiment, (^{b}) for agricultural variants, (^{c}) for inoculation with *A. brasilense,* and the interaction with fertilizers.

Table 6. The nitrogen losses in the "plant-substrate" system with spring barley cv. Nosivsky after inoculation with *A. brasilense* 410

Treatment	Consumed nitrogen from fertilizers by the plants, mg/vessel	Nitrogen residue in the substrate, mg/vessel	Nitrogen losses, mg/vessel
Control (no inoculation)	3.32	0.02	9.66
Inoculation with *A. brasilense* 410	4.30^{ac}	0.02	8.68^{ac}

Note: the changes are statistically significant at 95 % significance level: (^{a}) in general for the experiment, (^{b}) for agricultural variants, (^{c}) for inoculation with *A. brasilense,* and the interaction with fertilizers.

Table 7. The losses of nutrients, removed with the filtration water in a lysimetric experiment while growing spring barley cv. Nosivsky, kg/ha

Experiment variants	NO_3	NH_4	P_2O_5	K_2O	CaO	MgO	Water-soluble organic matter
No inoculation							
No fertilizers	27.0	0.6	2.7	3.9	37.2	8.0	10.5
$N_{60}P_{60}K_{60}$	30.5	1.4	2.7	4.5	40.5	12.0	12.0
Inoculation with A. brasilense 410							
No fertilizers	18.0[a]	not found	2.7	3.0[a]	28.0[a]	6.0[a]	6.0[a]
$N_{60}P_{60}K_{60}$	24.0[a]	not found	2.7	4.0[a]	26.8[a]	6.0[a]	8.2[a]

Note. Index [a] demonstrates a statistically significant decrease in the indices after inoculation, compared to the corresponding controls (without inoculation).

Table 8. The performance of spring barley cv. Nosivsky after inoculation of seed with *A. brasilense* 410, with or without the use of mineral fertilizers (2018–2020) (*Lysimetric experiment*)

Experiment variants	Yield, tons/ha	Yield increase due to inoculation, t/ha	Protein content in grain, %
No inoculation			
No fertilizers	2.56	–	8.7
$N_{60}P_{60}K_{60}$	3.55[ab]	–	10.4[ab]
Inoculation with A. brasilense 410			
No fertilizers	3.03[ac]	0.47	9.0
$N_{60}P_{60}K_{60}$	4.08[abc]	0.53	11.2[abc]

Note: the changes are statistically significant at 95 % significance level: ([a]) in general for the experiment, ([b]) for agricultural variants, ([c]) for inoculation with *A. brasilense,* and the interaction with fertilizers.

background of mineral fertilizers. No ammonia was detected in the washing waters of the variants with inoculation. There is also a significant reduction in the loss of water-soluble organic matter (by 31.7–42.9 % compared to the corresponding controls) (**Table 7**).

The barley grain yield, obtained in the lysimeters, expressed t/ha^{-1} demonstrated that the increase in yield, induced by fertilizers, is substantial, amounting to 0.99 (**Table 8**). The combination of mineral fertilizers and pre-sowing inoculation led to an increase in yield up to 1.52 t/ha^{-1} (63.2 %).

DISCUSSION

The results, obtained in the field experiments, demonstrated significant perspectives of *A. brasilense* 410 as a biofertilizer while growing spring barley in conditions of Ukrainian moderate climate. Although the inoculation in case of growing barley without mineral fertilizers promoted the increase with an average of 60 % in the nitrogenase activity, the effect of *A. brasilense* 410 under these conditions was much lower than in combination with $N_{60}P_{60}K_{60}$. Our previous studies with *Azospirillum* demonstrated that while growing perennial ryegrass*, Lolium perenne*, the application of mineral nitrogen in small doses promoted a better colonization rate of bacteria in the root spheres of plants and the manifestation of their nitrogenase activity (Volkogon, 2013). It is quite possible this also applies for *A. brasilense* – barley interaction.

The favorableness of the dose of mineral fertilizers $N_{60}P_{60}K_{60}$ for the manifestation of the efficiency of *Azospirillum* is also seen in the formation of spring barley yield. In particular, the highest inoculation-induced gains in crop productivity were noted against this agricultural variant. A high agricultural variant did not realize the potential of *Azospirillum* in the interaction with barley plants. Our results confirm those of Ozturk et al (2003), using wheat and barley,and those of Sharooma et al, 2008 and Gallart et al, 2021, using other plant-microbe associations).

The combination of inoculation with *Azospirillum* with an average N dose ($N_{60}P_{60}K_{60}$) led to a yield slightly higher to that of a single high dose ($N_{120}P_{120}K_{120}$) without *Azospirillum* (3.29 and 3.06 t/ha, respectively), which could lead to less depency on mineral fertilisers in practice. The possibility of decreasing the doses of mineral nitrogen fertilizers by the use of microbial inoculants to achieve high yields of crops was also reported by Hungria et al, 2010, Fukami et al, 2016; Santos et al, 2019 and Boleta et al, 2020.

The inoculation with *Azospirillum* had a positive effect (0.4 to 1.0 %) on the protein content, which demonstrate an improved nitrogen nutrition of plants. It should be noted that the increase in protein content in grain is certainly a positive feature for the products, intended for the production of cereals, flakes and fodder. Płaza et al (2022) also drew attention to this aspect. However, this effect is undesired for brewer's barley cultivars (Horash, Klymyshena, 2008). Thus, we consider that while growing brewer's barley on turf-podzolic soil using *A. brasilense* for inoculation, mineral fertilizers should be applied in low to prevent excessive protein synthesis in the grain.

For instance, the ^{15}N nitrogen-dilution experiment showed that the pre-sowing inoculation with *A. brasilense* 410 promoted both a higher intake of atmospheric nitrogen into plants (by 77.1 %) and higher consumption of mineral nitrogen (by 29.5 %). This is in agreement with results of Fukami et al (2017) studying maize. Higher consumption of nitrogen from the fertilizers by the inoculated plants may be induced by the effect of physiologically active substances of *Azospirillum*, not only on the rhizogenesis processes as described by e.g. Adesemoye, Kloepper, 2009 and Pii et al, 2018, but also on the additional synthesis and the activation of the enzymes of nitrogen exchange of the plants. For instance, there are data about the effect of phytohormones of *A. brasilense* on the activation of plant enzyme systems of the nitrogen cycle, including the ones, produced by rhizobacteria (Pereira-Defilippi et al, 2017; Fonseca Breda et al, 2019).

It should be noted that it is not only nitrogen nutrition of barley plants that is improved due to the inoculation with *Azospirillum*. Our lysimetric experiment indirectly demonstrated the improvement in phosphorus and potassium nutrition as well. The inoculation resulted in a decrease in the removal of micro-elements along the soil profile out of the root-containing layer. Noteworthy is a substantial decrease in leaching of calcium and manganese compounds. A reduced loss of water-soluble organic matter of 31.7–42.9 % was also noted.

Certainly, the reduction of nutrient losses from soil under inoculation may be explained by their better consumption by the plants which is also shown above with nitrogen, using the method of isotope (with ^{15}N) dilution. In addition, these reduced losses apparently also resulted in higher grain yield, from 0.47 to 0.53 t/ha.

The decrease in the nutrients losses from soil under inoculation may also hypothetically be explained by an increase in root size. An enlarged root system is capable of temporarily keeping substantial amounts of nutrients in the form of organic compounds. After the mineralization of plant residues, these nutrients may serve as a source of nutrients for the following crops in the rotation (Menichetti et al, 2015).

The reduction of the leaching of calcium and manganese compounds under inoculation is an interesting and significant aspect of effects of inoculation of *Azospirillum* on the performance of crops and the fertility of soils. For instance, calcium plays an important role in metabolism regulation of both eukaryotes and prokaryotes (Paradelo et al, 2015) and in the aggregation and structure-formation of soil (Rowley et al, 2018). Manganese is actively involved in the chloroplast biogenesis of plants and promotes the active metabolism of phosphorus in the plant organism (Kwon et al, 2019; Alejandro et al, 2020). Manganese deficiency in plants leads to chlorosis in leaves, and severe cases result in growth delay (Bang et al, 2021). However, the observed substantial decrease in the losses of calcium and manganese compounds after inoculation with *Azospirillum* by the inoculated plants remains unclear and requires further studies.

The decrease in leaching of the water-soluble was surprising. It may be assumed that some part of water-soluble organic matter is consumed after its mineralization by the inoculated plants. In addition, there is a known ability of plants to consume low-molecular organic substances and fragments of humic acids (Farzadfar et al, 2021). Thus, under the increased consumption ability of the root system, there may be an increase in the consumption of water-soluble organic matter by plants.

The increase in the consumption of nutrients by plants after inoculation with *Azospirillum* was also demonstrated in other results, obtained in the lysimetric experiment. For instance, in addition to the increase in the barley performance rate, an increase in protein content of 0.3–0.8 % in grain was registered. It is an additional demonstration of the fact that in addition to the passive nutrient uptake, including nitrogen, by plants due to the enlarged area of the consumption surface of the roots, the pre-sowing inoculation also promotes changes in the nitrogen metabolism of plants. This assumption is confirmed by other authors (Pereira-Defilippi et al, 2017; Fonseca Breda et al, 2019).

We did not investigate in this field experiment the effect of inoculation on the emission of nitrous oxide, as suggested by Klimasmith and Kent (2022) (this will be a next stage of our research). However, we think that reducing the doses of nitrogen fertilizers for barley when using *Azospirillum* will reduce the amount of substrate for the activity of nitrifying and denitrifying microorganisms.

CONCLUSIONS

In our three-year field experiment, inoculation of spring barley cv. Nosivsky seeds, planted in poor turf-podzolic soil, with *Azospirillum brasilense* strain 410 stimulated the nitrogen nutrition of plants. This manifested itself in an increased uptake of atmospheric nitrogen and a higher rate of nitrogen consumption from the fertilizers, an increase of 16.5 % in yield and of 0.4–1.0 % in protein synthesis. Furthermore nitrogen losses were diminished by 21.3–33.3 % and leaching of some microelemewnts, more in particular Calcium and Magensium were also reduced with 24.7–33.8 % and 25.0–50.0 %, respectivelt.

The efficiency of the inoculation with *Azospirillum* of springbarley on poor peat-podzolic soil was highest when average doses of mineral fertilisers were applied. No fertilization or high mineral fertilization rate led to less efficient results (crop growth 16.5 and 12.7 % respectively).

Adherence to ethical principles. This article does not relate to any studies using humans and animals as investigation subjects.

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Особливості живлення ячменю ярого за впливу *Azospirillum brasilense* та мінеральних добрив

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Мета. Дослідити ефективність інокуляції ячменю ярого за використання *Azospirillum brasilense* 410. **Методи**. Вегетаційний дослід за ізотопного розбавлення з ^{15}N (піщана культура), польовий дослід на дерново-підзолистому супіщаному ґрунті, лізиметричний дослід у стаціонарній лізиметричній установці, хроматографічні – для визначення нітрогеназної активності бактерій у кореневій зоні рослин, агрохімічні, статистичні. **Результати**. У польових умовах інокуляція *A. brasilense* 410 сприяла достовірному зростанню нітрогеназної активності в системі «ґрунт-рослина» при вирощуванні культури по фону без добрив і (особливо!) за внесення $N_{60}P_{60}K_{60}$. Високий агрофон ($N_{120}P_{120}K_{120}$) забезпечував тривале пригнічення нітрогеназної активності. Як без інокуляції, так і за використання *A. brasilense* 410 зростання нітрогеназної активності по цьому фону спостерігали лише наприкінці вегетації рослин. Найбільші прирости врожайності ячменю ярого від інокуляції відмічено при вирощуванні культури по фону $N_{60}P_{60}K_{60}$, найменші – по фону без добрив. Передпосівна інокуляція забезпечувала зростання вмісту білка у зерні ячменю, особливо при вирощуванні культури по високому агрофону, що підвищує цінність продукції, призначеної для виробництва круп і кормів для худоби. У той же час, це є небажаним при вирощуванні пивоварних сортів ячменю. У досліді з ^{15}N встановлено зростання надходження азоту в рослини за інокуляції як наслідок активізації процесу азотфіксації, так і за рахунок підсилення засвоєння азоту з добрив. Лізиметричними дослідженнями показано, що інокуляція ячменю ярого азоспірилами сприяє обмеженню вертикальної міграції сполук азоту, фосфору, калію, кальцію, мангану та водорозчинної органічної речовини. **Висновки**. Передпосівна інокуляція насіння ячменю ярого *A. brasilense* 410 сприяє інтенсифікації засвоєння рослинами азоту внаслідок активізації процесу азотфіксації та засвоєння діючої речовини з добрив. Вплив інокуляції на урожайність ячменю ярого еквівалентний дії мінеральних добрив у дозі, не меншій за $N_{60}P_{60}K_{60}$. Отже, за використання передпосівної інокуляції запланований рівень урожайності культури можна отримати, суттєво зменшивши агрохімічний фон.

Ключові слова: інокуляція, *Azospirillum brasilense,* добрива, ячмінь, ізотопне розбавлення ^{15}N, лізиметри.

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ПРАВИЛА ДЛЯ АВТОРІВ

У журналі «Agricultural Science and Practice» публікуються результати фундаментальних досліджень з питань агрономії, біології, біотехнології та біоінженерії, ветеринарної медицини.

Друкуються фундаментальні статті, огляди літератури, короткі повідомлення, які раніше не видавалися. Особливо просимо уникати технічного плагіату та самоплагіату. Редакція дуже уважно відслідковує ці моменти.

Рукописи надсилаються на **конфіденційне** рецензування спеціалістам відповідної галузі.

За умови позитивної рецензії з рукописом знайомиться науковий редактор – **конфіденційно.**

Статті надсилаються українською мовою, перекладаються в редакції безкоштовно і публікуються лише англійською мовою; резюме – українською мовою. В електронній версії журналу (http://www.agrisp.com) з 2014 р. розміщуються резюме, список літератури і повний текст статей англійською мовою (окрім поточного року).

Кожній статті присвоюється цифровий ідентифікатор (DOI), що сприяє коректному розповсюдженню матеріалу статті в мережі Інтернет.

Комплект документів, необхідних для реєстрації статті.

Електронною поштою надсилаються:

- лист – направлення від організації (pdf);
- договір про передачу авторських прав (pdf), оформлений та підписаний окремо кожним із співавторів, наприклад, 4 автори – 4 договори (зразок договору на сайті журналу);
- звертаємо Вашу увагу на те, що договір про передачу авторських прав набуває чинності після прийняття статті до публікації. У разі відхилення Вашої статті редколегією журналу договір автоматично втрачає силу. Підписання договору автором означає, що він ознайомлений та згоден з умовами договору;
- рукопис подається у форматах: текст (doc, docx); ілюстрації (.tif, .jpg, .cdr, excel); таблиці.

Статті обов’язково супроводжуються українсько-англійським словником специфічних термінів (не менше 30), використаних у статті.

Рукопис має містити:

- індекс УДК;
- назву статті англійською й українською мовами;
- прізвища та ініціали всіх авторів двома мовами;
- назву і поштову адресу(и) з індексами установ(ви), де працює(ють) автор(и), двома мовами;
- електронну пошту всіх авторів, автора для листування позначити зірочкою.