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Contribution of *Azospirillum brasilense* to nitrogen fertilization for maize crops planted successively to soybean crops

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Abstract

Maize is very important in Brazilian agribusiness: it is the second most produced grain in the country. Crops need to be fertilized, especially with nitrogen, to achieve high yields. This study aimed to evaluate the contribution of *Azospirillum brasilense* in maximizing the use of nitrogen by maize crops after soybean cultivation in the same soil. This study was carried out at the Experimental Farm of the State University of Minas Gerais (UEMG), Passos unit. The experiment consisted of planting maize in the same soil where soybeans had previously been grown, with a strip- and split-plot design. The seeds in one strip were inoculated with *Azospirillum brasilense*, and those in the other strip were not. Each strip was divided into 24 plots measuring 18 m^2, which received nitrogen doses as urea (0, 10, 35, 70, 140 and 210 kg ha^{-1} of N), in four replications. Seed inoculation did not result in statistical differences. The plots that received the treatment with chemical nitrogen fertilization exhibited the same results as those that did not, which can be explained by the previous soybean cultivation letting nitrogen in the soil and/or by the effect of *Azospirillum brasilense*.

Keywords: *Azospirillum brasilense*. Grass. Nitrogen.

Introduction

Maize is one of the most cultivated plants worldwide: it has high versatility, numerous uses, and many possible by-products. Maize production bears an extreme socio-economic importance, since this cereal is present both in animal and human food, represents raw material for the industry, and is widely used in Brazilian gastronomy (RODRIGUES et al., 2018).

According to the National Supply Company (CONAB, 2021), in the 2020/2021 harvest, Brazil produced 85.7 million tons of maize, that is, 16.4% less than in the 2019/2020 harvest, in which 102.5 million tons were produced. Data evince the importance of maize as a second crop, usually planted after the soybean harvest.

To achieve high yields, it is necessary to enhance soil fertility with macro- and micronutrients, essential to both the vegetative and reproductive cycles of crops. Nature follows Liebig's law of the minimum, which was proposed the 19th century and states that the development of a plant can be limited by the lack of a single nutrient, even if all the others are balanced. Nitrogen (N) deficiency is often a limiting factor in grain production, as N is the most required nutrient by most crops (SBCS, 2017).

This nutrient is responsible for numerous physiological processes in plants, such as the ionic absorption of distinct nutrients, root and aerial growth and development, respiration, and photosynthesis. Because N is a constituent part of amino acids and proteins, it is found in essential compounds such as nucleic acids (DNA and RNA) and nitrogenous bases, which are also known as nucleobases (pyrimidines and purines), chlorophyll, and cytochromes (LEMOS et al., 2013).

According to Cruz et al. (2008), N is the nutrient most exported by the maize crop during its cycle. The deficiency of this nutrient is reflected in old leaves, which become more yellow when they lack nitrogen, losing their intense green color, which is characteristic of vigorous plants (NOVAIS et al., 2007).

Malavolta et al. (2002) state that plants are chemically supplied with N through mineral fertilizers, which can be synthetic, natural, or inorganic. Its absorption occurs during almost the entire life cycle of plants, only reducing at the end of grain filling and when leaf senescence begins (RIVERA, 2006). On the other hand, producers can choose to reduce the use of chemical fertilizers and switch to organic fertilizers or green manure. These alternatives not only provide nutrients for plants: they promote an increase in organic matter which, in turn, increases soil fertility (MALAVOLTA et al., 2002). Santos et al. (2007) add that the continuous use of alternative fertilizers improves soil fertility and can even replace chemical fertilization.

Plants can also be supplied with N through associative bacteria belonging to the *Azotobacter* and *Azospirillum* genera, which promote N_2 fixation. However, part of the N fixed by these bacteria is not made available to plants, due to the fact that the association is not very close and that these bacteria are free-living, which causes part of the fixed N to transform and/or become unavailable during its process from the soil to the roots. Thus, these bacteria can contribute to plants nutrition by making N available, but not to fully supply their demands (HUNGRIA, 2011).

When in the rhizosphere of maize, the *Azospirillum brasilense* bacterium can transform N_2 into mineral nitrogen and release compounds that stimulate root growth, increasing the volume of soil explored by the root, consequently, absorbing more water and nutrients, as well as inducing plant resistance (LÓPEZ; HERRERA, 2017).

Given that nitrogen fertilizers are essential for agricultural production, this study aimed to evaluate the use of *Azospirillum brasilense* with the potential to make N_2 available from atmospheric air in a natural and effective way for maize crops.

Material and methods

This study was carried out at the Experimental Farm of the State University of Minas Gerais (UEMG), Passos Unit, in the southwest of Minas Gerais (latitude: −20.7453°; longitude: −46.6339°) and at an altitude of 780 m. According to the Köppen system, the climate of the region is Cwa—a subtropical/tropical monsoon climate, with dry winter and rainy summer (ALVARES et al., 2013).

This experiment is related to the 2019/2020 and 2020/2021 harvests. Each harvest began with the cultivation of soybeans and, in succession, of maize. The soil used was classified as a dystrophic oxisol with a medium texture (PIRES et al., 2012). Before the crops were planted, the soil was sampled at a depth of 0 to 20 cm for the first conventional crop and 0 to 10 cm for the no-till crops, for chemical analysis.

Soil samples were collected from 10 random points (single samples) of the planting area arranged in a zigzag pattern. At the end of the collection, a composite sample was formed.

Cultivation I: Biological nitrogen fixation and straw production for no-till farming

Two harvests were evaluated. The first crop, a soybean one (*Glycine max* L.), was grown on an area of 1,008 m², under a conventional system. The seeds were inoculated with bacteria of the species *Bradyrhizobium japonicum* before sowing. The aim of this cultivation was to allow the biological fixation of N by the crop and the production of straw for subsequent maize cultivation in a no-till system. The productivity of the area was also calculated.

At the start of cultivation, the soil was prepared by deep plowing, from 25 to 35 cm, then by leveling harrows, until it was suitable for sowing.

The soil was limed before plowing began, which allowed the limestone to be incorporated into the soil. This process aimed to raise the base saturation percentage to 60%. Before the last grading harrow was implemented, sowing fertilization was carried out with the amounts of fertilizer recommended for soybean crops (RIBEIRO et al., 1999), which were equivalent to: 153.8 kg ha^{-1} of monoammonium phosphate (MAP) and 133.3 kg ha^{-1} of potassium chloride (KCl).

Sowing was carried out with a seven-row pneumatic seeder with 0.5 m row spacing and regulated to distribute 13 seeds per linear meter for the Monsoy 7739 cultivar. The soybean was harvested 115 days after sowing, the moisture content of the grain reached values of 13 to 15%.

Cultivation II: Effect of nitrogen doses and of *Azospirillum brasilense* inoculation on maize yields

In the two harvests evaluated, the second crop was grown on an area of 810 m^2 and sown in the no-till system, under the residues of the soybean cultivation. A strip design with subdivided plots was used. Within the plots, there were planting strips with and without seeds inoculated with the *Azospirillum brasilense* bacterium. Six different doses of nitrogen fertilizer (0, 10, 35, 70, 140 and 210 kg ha^{-1} of N) were applied to the subdivisions, with four repetitions. The bacterial inoculum *A. brasilense* was added to the seeds before sowing, following the manufacturer's recommendation.

Each plot consisted of seven planting rows 4.5 m long and 0.5 m apart from each other. The three central rows were used for evaluation, excluding 0.75 m from the ends of the plot, which left, in total, 9 linear m of useful plot.

As recommended by Ribeiro et al. (1999), sowing fertilization and soil correction were manually applied before the planting with a mechanical seeder.

Topdressing was carried out twice with urea (45% N), following the recommendations of Gott et al. (2014). First, half of the recommended nitrogen fertilizer dose was applied to the leaves during the V3 stage (the vegetative stage of three leaves). Then, during stage V8, the remaining dose was applied.

In the first year of cultivation, sowing of the AG 8740 PRO3 hybrid maize seeds was carried out with a seven-row pneumatic seeder, which left a 0.5 m row spacing and was set to distribute four viable seeds per linear meter. In the second year, SHS 7990 hybrid maize was sown using the same method.

In order to ensure balanced nutrition for the plant, a foliar application of the organo-mineral fertilizer Torped Gold at a dose of 1.5 L ha^{-1} was made. This application was divided: the first was carried out about 30 days after emergence and the second was conducted before the male inflorescence (tassel) appeared.

Developmental evaluations, such as assessing plant height and stem diameter, were carried out from the first nitrogen fertilizers were applied to the moment of male inflorescence (tasseling). Plant height was measured using a millimeter tape measure extended from ground level to the insertion of the flag leaf, while the diameter of the stem was measured close to the ground using a caliper.

The maize cob insertion height was measured at the time of harvest using a millimeter tape measure, and the number of cobs per plant was also counted.

The leaf N content was monitored both when 50 to 75% of the plants had produced the female inflorescence (cob) and when the style-stigmas were exposed. With scissors, the leaf below and opposite to the insertion of the cob was removed, and only the central third of the leaf was used, discarding its vein.

The harvest began when the grains reached physiological maturity and an average moisture content of 18 to 20%. After the cobs were harvested, the following factors were evaluated: cob diameter, length, number of rows, number of grains per cob, weight of 100 grains and yield.

To take these measurements, five cobs were sampled and, at the end, the average for each plot was calculated. The diameter of the cobs was determined using a caliper and its length was measured using a millimeter ruler. Before threshing, the number of rows of the cobs was counted.

In total, 100 maize grains were weighed and the yield achieved by each plot at 13% moisture was calculated and extrapolated to the hectare.

In the second harvest (2020/2021), the soil sample was collected at two points in each crop plot, which, after being homogenized, were identified and sent to the laboratory to undergo a chemical analysis of soil fertility. This analysis was made to assess the effect of the residues of the first crop on soil fertility.

The means were evaluated using the Shapiro-Wilk test for error normality. The data were then subjected to analysis of variance (ANOVA) and the means were compared using the F test at a 5% significance level. Significant values were then subjected to the Scott-Knott test, applied using the AgroEstat program (BARBOSA; MALDONADO, 2014).

Results and discussion

For all the variables evaluated, there were no significant interactions between the inoculation of the seeds with *Azospirillum brasilense* and the different nitrogen fertilizer doses applied during topdressing. However, the doses of nitrogen fertilization and/or the inoculation with *Azospirillum brasilense* significantly affected the vast majority of the characteristics of the plants, which were then analyzed and discussed separately.

Table 1 shows the vegetative development as a function of plant height (PH), stem diameter (SDM) and cob insertion height (CIH). There

Table 1. Effects of seed inoculation with *Azospirillum brasilense* on the variables stem diameter (SDM), plant height (PH) and cob insertion height (CIH) in two different years. Passos, MG.

2019/2020	SDM (cm)	PH (cm)	CIH (cm)	2020/2021	SDM (cm)	PH (cm)	CIH (cm)
Not inoculated	2.8 a	171.3 a	87.8 a	Not inoculated	2.4 a	167.6 a	98.3 a
Inoculated	2.9 a	169.6 a	85.4 a	Inoculated	2.3 a	183.8 a	94.5 a
	SDM	PH	CIH		SDM	PH	CIH
Dose 0	3.0 a	183.4 a	84.7 a	Dose 0	2.4 a	183.6 a	96.6 a
Dose 1	2.6 a	154.4 a	74.7 a	Dose 1	2.2 a	166.6 a	96.4 a
Dose 2	2.7 a	146.7 a	84.2 a	Dose 2	2.3 a	162.2 a	90.1 a
Dose 3	2.9 a	176.5 a	87.7 a	Dose 3	2.3 a	179.2 a	97.9 a
Dose 4	2.7 a	182.4 a	90.7 a	Dose 4	2.5 a	186.0 a	98.7 a
Dose 5	2.9 a	179.4 a	97.2 a	Dose 5	2.3 a	176.5 a	98.6 a
CV (%)	10.2	23.8	17.7		9.9	27.7	7.8

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a significance level of 5%.

were no statistical differences for these variables, nor for the N rates evaluated in the two years of cultivation. Bassetto Junior et al. (2020) and Araújo (2014) also found no statistical differences for the SDM, but Bassetto Junior et al. (2020) and Debastiani (2016) found significant differences for maize PH and CIH, obtaining results that differed from those found in this study. Picazevicz (2017), working with maize in a greenhouse, mentioned that *Azospirillum brasilense* increases vegetative growth as a result of the release of phytohormones such as gibberellins, auxins and cytokinins—a finding that this study did not confirm.

Regarding the variable CIH, the lack of effects of the inoculation and doses can be considered a positive aspect. Kappes et al. (2013) reported that plants with taller CIH are more prone to falling over: at the end of the cycle, plants that reach a high mass value in their upper part, added to that of the cobs, can fall over more easily. These authors support the idea that the upper part of the maize plant reaches a ratio of approximately 50% of its mass by the end of its cycle.

Our SDM values were similar to those found by Besen et al. (2020), who did not observe a statistical difference between plants subjected or not to the inoculation treatment with *Azospirillum brasilense*. This supports the theory of Mauri, Bellaver, and Richart (2020), who reported that these factors are directly related to the genetics of the material used and do not depend much on the fertilizer applied during topdressing. Kappes et al. (2013) correlated SDM with lodging and stated that larger diameters are found in plants that are more vigorous and resistant to lodging.

In both years of cultivation, the number of rows (NR) per cob did not differ significantly depending on the inoculation or on which dose it was applied (Table 2), which corroborates the studies by Bassetto Junior et al. (2020) and Tomazela (2005). Pereira et al. (2021) pointed out that this variable may be directly related to the genetics of the material used for cultivation, which means that this lack of statistical differences could be due to the fact that the environment was the same for all treatments.

Table 3 shows the mean clustering test applied to the post-harvest variables in the two years of cultivation: cob length (CL), cob diameter (CD), mass of 100 grains (MOG) and grains per ear (GPC). In most variables, statistical differences were only observed in the first year of the study—this occurred both for the inoculation with *Azospirillum* and for the nitrogen doses applied during topdressing.

Table 2. Effect of seed inoculation with *Azospirillum brasilense* on the variable number of rows (NR) in two different years. Passos, MG.

2019/2020	NR	2020/2021	NR
Not inoculated	14.7	Not inoculated	14.6
Inoculated	14.6	Inoculated	14.5
2019/2020	**NR**		**NR**
Dose 0	14.1	Dose 0	14.5
Dose 1	15.1	Dose 1	14.4
Dose 2	14.7	Dose 2	14.4
Dose 3	14.5	Dose 3	14.9
Dose 4	14.6	Dose 4	14.5
Dose 5	14.7	Dose 5	14.7
CV (%)	5.3		8.4

Table 3. Effect of seed inoculation with *Azospirillum brasilense* on the variables cob length (CL), cob diameter (CD), mass of 100 grains (MOG) and grains per cob (GPC). Passos, MG.

2019/2020	CL	CD	MOG	GPC	2020/2021	CL	CD	MOG	GPC
Not inoculated	14.1 b	4.4 a	30.7 b	307.8 a	Not inoculated	12.3 a	4.2 a	22.4 a	284.0 a
Inoculated	15.0 a	4.5 a	37.1 a	303.7 a	Inoculated	12.3 a	4.2 a	21.8 a	285.2 a
	CL	**CD**	**MOG**	**GPC**		**CL**	**CD**	**MOG**	**GPC**
Dose 0	13.6 b	4.3 b	29.2 c	283.8 b	Dose 0	11.8 a	4.1 a	21.4 a	296.0 a
Dose 1	15.1 a	4.5 a	41.7 a	257.8 b	Dose 1	11.4 a	4.0 a	21.2 a	252.9 a
Dose 2	13.5 b	4.3 b	29.2 c	277.9 b	Dose 2	12.5 a	4.2 a	22.7 a	268.1 a
Dose 3	15.3 a	4.5 a	35.0 b	324.5 a	Dose 3	12.0 a	4.2 a	21.0 a	282.9 a
Dose 4	14.7 a	4.6 a	30.8 c	351.2 a	Dose 4	13.0 a	4.3 a	23.2 a	293.1 a
Dose 5	15.2 a	4.6 a	37.5 b	339.0 a	Dose 5	13.0 a	4.3 a	22.5 a	314.5 a
CV (%)	5.4	3.4	13.1	12.8		10.7	5.0	8.6	16.2

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% significance level.

During the first year of evaluation, plants that were inoculated with *Azospirillum* did not show significantly different CD and GPC values from the ones that were not. These results partially contradict those found by Silva Junior, Freitas and Resende (2021), and Silva et al. (2020), who found no significant differences representing an increase due to the inoculation treatment with *Azospirillum* bacteria.

However, during the second year of cultivation, no statistical differences were linked to the inoculation or doses, nor to the interaction between these two factors. These results partially corroborate what Mauri, Bellaver and Richart (2020) found when evaluating CL and NR: they did not observe significant differences.

Due to the findings of Kotowski (2015), it was expected that the plots that received higher doses of nitrogen fertilizer would reach higher MOG values, but this did not happen. Souza and Pires (2013) pointed out that this variable may be directly tied to nitrogen fertilization: nitrogen fertilizers help increase the productive potential of plants, as long as no distinct factors are negatively influencing them.

Table 4 presents the maize yields obtained in the two different years. In the 2019/2020 harvest, statistical differences were observed both for inoculation and N doses. The plots that received the inoculation achieved higher yields than those that did not, just as Kappes et al. (2013) observe in their work. Albuquerque et al. (2013) stated that bacteria have a positive effect on plants, acting as stimulants in the root system, which increases nutrient absorption to their maximum productive capacity. The yield gains observed after the inoculation with *Azospirillum brasilense* bacteria differ from those reported by Silva Junior, Freitas and Rezende (2021) and Moreira, Valadão and Valadão Junior (2018), who found that this inoculation process, made via seed, did not cause statistical differences on any variables. However, these authors' results do corroborate those obtained in the second year of the present study.

In relation to the doses in the two years of cultivation, the results obtained were similar to those of the study by Mumbach et al. (2017), in which higher nitrogen fertilizer doses, applied via soil, generated greater yields, which reinforces the fact that maize plants respond to N by increasing productivity (MALAVOLTA, 2006; DINIZ, 2009).

These results partially contradict Besen et al. (2019), who suggested that productivity was directly related to CL, as higher values of this

Table 4. Effect of seed inoculation with *Azospirillum brasilense* on crop yields in two different years. Passos, MG.

2019/2020	Productivity (kg/ha)	2020/2021	Productivity (kg/ha)
Not inoculated	5,690.2 b	Not inoculated	3,806.6 a
Inoculated	6,660.8 a	Inoculated	3,715.3 a
2019/2020	**Productivity (kg/ha)**	**2020/2021**	**Productivity (kg/ha)**
Dose 0	4,968.2 c	Dose 0	3,821.5 a
Dose 1	6,391.9 b	Dose 1	3,168.8 b
Dose 2	4,771.2 c	Dose 2	3,624.8 b
Dose 3	6,794.5 b	Dose 3	3,583.3 b
Dose 4	6,474.5 b	Dose 4	4,106.8 a
Dose 5	7,652.6 a	Dose 5	4,260.4 a
CV (%)	15.6		16.5

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% significance level.

variable would represent higher yields: this did not occur in the second year of this experiment, when there was a statistical difference in productivity (Table 4) even though CL values remained the same (Table 3).

In the second year of cultivation, the control plot (dose 0) yielded the same as the plots that received the highest doses of nitrogen (Table 4). This demonstrates that the plants may have been affected by the high levels of some nutrients in the soil—a statistical analysis of the elements in the soil found that the amounts of phosphorus (P) and magnesium (Mg) were higher in the control plots (Table 5).

According to Karam, Melhorança and Oliveira (2006), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) are the nutrients extracted in greater amounts by maize crops. Coincidentally, the values of all these nutrients, except for Ca, were found to be higher in the control plot, which confirms the hypothesis that they helped increase the productivity of the soil that did not undergo the topdressing treatment. Another hypothesis referred to the low extraction/export that the control plots promoted in the first year of cultivation due to lower productivity (Table 4), which left their fertility higher for the subsequent year.

Table 5. Effects of chemicals in the soil and of the maize yields in the second crop as a function of the inoculation with *Azospirillum brasilense* and of different N doses. Passos, MG.

2019/2020	Productivity (kg/ha)	P (mg/dm³)	K (mmolc/dm³)	Mg (mg/dm³)	S (mmolc/dm³)
Dose 0	3,821.5 a	6.2 a	53.5 a	36.9 a	69.5 a
Dose 1	3,168.8 b	4.9 b	25.7 b	13.0 b	42.6 b
Dose 2	3,624.8 b	5.1 b	37.0 b	17.4 b	52.6 b
Dose 3	3,583.3 b	5.0 b	35.6 b	16.2 b	49.9 b
Dose 4	4,106.8 a	5.5 b	47.6 a	23.7 b	62.9 a
Dose 5	4,260.4 a	5.4 b	45.5 a	24.0 b	59.5 a
CV (%)	16.5	11.5	42.8	54.1	29.8

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% significance level.

The contents of potassium (K), sulfur (S), boron (B), iron (Fe), and zinc (Zn) in the leaves were not statistically different (Table 6). On the other hand, the contents of nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), copper (Cu), and manganese (Mn) in the different plots did reach a significant difference (Table 7).

The leaf N contents (Table 7) were found to have a direct relationship with the different nitrogen fertilizer doses applied to the soil: as the fertilizer doses increased, so did the N content in the leaves, which is in line with the findings by Muller (2013). Ferreira (2017) also found a relationship between nitrogen fertilizer doses in the soil and N contents extracted from leaf tissues. Despite being statistically different, the N levels found in the leaves (Table 7) did not interact with the yield rates (Table 4), since the latter did not present statistical differences (SOUZA et al., 2011).

This proves Liebig's law of the minimum, which indicates the importance of balance in plant nutrition, stating that productivity can be limited by the lack of a single nutrient in a plant, even if another (which can be a micronutrient) is available in high concentrations.

It was found that the seed inoculation with *Azospirillum brasilense* did not cause statistical differences in leaf N contents, which corroborates the results obtained by Picazevicz, Kusdra, and Moreno (2019).

P concentration was higher in plants that received the inoculation treatment with *Azospirillum brasilense* (Table 7). This occurred because nitrogen fertilization and microorganisms promote enzymatic activities, which help solubilize phosphates, in the rhizosphere. Moreover, inoculation helps roots develop and increase in volume (MORAES, 2016), and P is absorbed by diffusion, a process that requires the roots to come directly into contact with the nutrient (MIKKELSEN, 2015). Pires et al. (2015) found that P levels increased as a result of higher N doses, which was not confirmed in this study.

Both the inoculation with *Azospirillum brasilense* and the high doses of N increased Cu values in leaf tissues. Miranda et al. (2015) found similar results, obtaining a linear increase in Cu as they applied higher nitrogen fertilizer doses, which demonstrates a synergistic interaction between these nutrients: the nitrogen fertilization influenced the Cu absorption. Besen et al. (2020) also obtained results similar to those observed in

Table 6. Effect of seed inoculation with *Azospirillum brasilense* on the analysis of leaves in two different years. Passos, MG.

2019/2020	S (g/kg)	K (g/kg)	B (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
Not inoculated	1.2 a	23.0 a	7.4 a	53.5 a	208.1 a
Inoculated	1.2 a	21.2 a	6.9 a	47.7 a	197.9 a
2019/2020					
Dose 0	1.2 a	22.1 a	7.0 a	48.4 a	181.6 a
Dose 1	1.1 a	22.1 a	7.7 a	49.3 a	201.6 a
Dose 2	1.2 a	22.9 a	6.7 a	53.8 a	198.5 a
Dose 3	1.1 a	20.4 a	7.6 a	49.8 a	224.9 a
Dose 4	1.1 a	21.8 a	7.6 a	52.7 a	217.6 a
Dose 5	1.3 a	23.1 a	6.2 a	49.6 a	193.8 a
CV (%)	15.0	13.8	20.8	17.2	18.6

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% significance level.

Table 7. Effect of seed inoculation with *Azospirillum brasilense* on the analysis of leaves in two different years. Passos, MG.

2020/2021	N (g/kg)	P (g/kg)	Ca (g/kg)	Mg (g/kg)	Cu (mg/kg)	Mn (mg/kg)
Not inoculated	23.2 a	1.3 b	2.2 b	0.9 a	5.4 b	32.3 a
Inoculated	22.4 a	1.5 a	2.6 a	1.0 a	8.4 a	33.9 a
2020/2021	**N**	**P**	**Ca**	**Mg**	**Cu**	**Mn**
Dose 0	19.2 c	1.3 a	2.0 b	0.8 b	4.9 b	28.5 b
Dose 1	18.1 c	1.4 a	2.1 b	0.7 b	6.6 a	30.2 b
Dose 2	22.0 b	1.4 a	2.4 a	0.8 b	7.4 a	31.7 b
Dose 3	24.1 a	1.4 a	2.5 a	1.0 a	6.9 a	31.3 b
Dose 4	26.2 a	1.4 a	2.9 a	1.2 a	8.2 a	36.6 a
Dose 5	27.4 a	1.4 a	2.6 a	0.9 b	7.5 a	40.5 a
CV (%)	12.6	15.9	17.5	25.2	18.2	16.3

Means in columns followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% significance level.

this study, but in a wheat crop: they found that increased N doses generated higher Cu and Mn values in leaves.

Conclusions

In both harvests, seed inoculation with *Azospirillum brasilense* did not cause consolidated statistical differences in the maize crops planted successively to soybean crops.

The N content in the leaf tissues increased along with the dose of nitrogen fertilizer applied as topdressing, but this increase was not reflected in the yield.

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