Research Article

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Influence of *Azospirillum brasilense* associated with silicon and nitrogen fertilization on macronutrient contents in corn

<https://doi.org/10.1515/opag-2020-0013>

received June 30, 2018; accepted November 22, 2018

Abstract: Information regarding the interaction between biological nitrogen fixation (BNF) with *Azospirillum brasilense* inoculation and the use of silicon (Si) is needed. Silicon exerts numerous benefits on grasses, especially when the plants are subjected to biotic and abiotic stresses, affecting plant nutrition. The aim of this research was to determine if there is a synergistic effect between the inoculation with *A. brasilense* and Si use, on macronutrient content in corn shoot and root. The field trial was performed in Selvíria, Brazil, on a Typic Rhodic Hapludox soil under no-till system. The experimental design was a completely randomized block design with four replicates arranged in a $2 \times 5 \times 2$ triple factorial arrangement, consisting of two soil acidity corrective sources (dolomitic limestone and Ca and Mg silicate as sources of Si); five N doses (0, 50, 100, 150 and 200 kg ha⁻¹ applied in topdressing); with and without seed inoculation with *A. brasilense*. The inoculation favored N concentration in shoots and increased the N and S concentration even when associated to high N rates in topdressing. The Si as Ca and Mg silicate

associated with the increment of N rates does not promote an increase in the macronutrients uptake. Although it did not favor the N use, the Si also did not negatively affect the benefits of the *A. brasilense*.

Keywords: Nutritional evaluations; Biological nitrogen fixation in grasses; Plant growth promoting bacteria; Silicon use; Nitrogen fertilization management; *Zea mays* L.

1 Introduction

Despite the technological advances available in corn cropping systems, Brazilian average yield is still low as compared to its potential, which can reach over 10,000 kg ha⁻¹ (Galindo et al. 2016; Conab 2018; Galindo et al. 2018). High nitrogen (N) doses are needed to be applied in order to obtain an increased plant nutrition and corn grain yield. Tropical soils do not supply the higher demand of N during the cropping season (Teixeira Filho et al. 2014; Galindo et al. 2016; Galindo et al. 2017). N fertilization comprises one of the highest production costs of cereal crops (Espindula et al. 2014; Nunes et al. 2015). Also, N fertilizer production and application contribute to the emission of gases such as carbon dioxide and nitrous oxide, which increase the greenhouse effect on the planet (Xu et al. 2012).

The use of inoculants containing plant growth-promoting bacteria (PGPB) represent a new strategy to increase the N use efficiency (NUE), providing increased plant nutrition and corn grain yield. New research investigating beneficial PGPB are being conducted, especially for crops such as corn and wheat (Marks et al. 2015; Fukami et al. 2016; Fukami et al. 2017). Many researchers have reported that *Azospirillum* spp. produces phytohormones that stimulate root growth in several plant

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species (Bashan and de-Bashan 2010; Meza et al. 2015), increase nutrient availability (Hungria et al. 2010), nitric oxide (Fibach-Paldi et al. 2012), and favor biological nitrogen fixation (BNF) (Pankiewicz et al. 2015). It is believed that PGPB might act either in a cumulative or sequential pattern, in a multiple mechanism theory (Bashan and de-Bashan 2010).

Another practice that exerts several benefits on grasses cultivation is the silicon (Si) application in tropical agriculture, mainly when the plants undergo biotic and abiotic stresses, for example pathogens and insect attacks (Bakhat et al. 2018), salt and drought stress (Crusciol et al. 2013a, 2013b), strong rain and wind (Guntzer et al. 2012), common in adverse edaphoclimatic conditions such as Brazilian Savannah region. In addition, Ca and Mg silicate increase the soil pH and contents of P, Ca, Mg and Si and, consequently, base saturation, besides being able to reduce the toxic effect of Fe, Mn, Zn, Al and Cd (Reis et al. 2008; Guntzer et al. 2012; Camargo et al. 2014a, 2014b; Sarto et al. 2015). The better plant architecture which increase photosynthetic rate through the formation of upright leaves can stimulate plant growth and yield with Si use (Ma and Yamaji 2008; Gong and Chen 2012). Also, the transpiration rate and bedding can be reduced due to the greater structural rigidity of the tissues (Reis et al. 2008; Camargo et al. 2014a, 2014b).

Although one or more of the benefits are observed with Si application as Ca and Mg silicate and after seed inoculation with *Azospirillum brasilense*, an increase in corn nutrient uptake may not always be evident. Further studies involving *A. brasilense* associated with Si application are needed to provide an in-depth understanding of its influence on plant nutrition and maximize corn development (Galindo et al. 2018). In addition, studies are still lacking to define how much N fertilizer needs to be applied in combination with *A. brasilense* and Si to achieve a better macronutrient uptake. We believe that it may exist a synergic relation between *A. brasilense* inoculation and Si supply in the soil, thus enabling greater N fertilization efficiency and macronutrient uptake. Therefore, we evaluated the effect of nitrogen rates associated with the inoculation by *A. brasilense* and supply of Si, as a corrective acidity on macronutrients concentrations in the shoot and root of irrigated corn in the region of Brazilian Cerrado.

2 Methods

2.1 Field sites description

The study was conducted in Selvíria, Mato Grosso do Sul state (20°22'S, 51°22'W, altitude of 335 m above sea level), Brazil, during the 2015/16 and 2016/17 growing season (Figure 1). The soil of the experimental area was classified as a Latossolo Vermelho distrófico according to Embrapa (2013), Typic Rhodic Hapludox, according to the USDA (2010). The area has been cultivated with annual crops including corn, wheat, soybean and common beans for more than 28 years, and no-tillage system has been used for the past 10 years. The crop used before corn planting was corn and wheat, respectively (Galindo et al., 2018). The average annual temperature was 23.5°C, the annual average precipitation was 1,370 mm, and the annual average relative air humidity was 70-80%. The climatic condition recorded during the field trial are shown in Figure 2.

2.2 Experimental design

The experimental design was a completely randomized block design with four replicates arranged in a $2 \times 5 \times 2$ factorial arrangement, consisting of two soil corrective sources (silicate of Ca and Mg as Si source with effective neutralizing power (ENP) = 88%, Ca = 25%, Mg = 6% and Si total = 10% and dolomitic limestone with ENP = 80%, CaO = 28% and MgO = 20%); five N rates (0, 50, 100, 150 and 200 kg ha⁻¹, in the form of urea) applied at topdressing; with and without inoculation of the seeds with *A. brasilense*. In both crops, the plots of the corn experiment was 5 m long with 6 lines spaced by 0.45 m, the plot area being the 4 central rows, excluding 0.5 m from the extremities.

2.3 Trial establishment and management

The granulometric analysis and soil chemical properties (Table 1) of the top layer (0-0.20m) was determined before the start of the study in 2015, following the methodologies proposed by Raij et al. (2001), Bremner (1996) for total N (determined by the regular Kjeldahl method using a block digester, and Korndörfer et al. (2004) to determinate Si (Ca chloride 0.01 mol L⁻¹).

Weed management followed best management practices for the region. The remaining straw (predecessor crop) was collected at corn planting to characterize and

Figure 1: Study area at the Selvíria, Mato Grosso do Sul state, Brazil (20°22'S, 51°22'W, altitude of 335 m)

Table 1: Soil chemical and granulometric attributes in the arable layer. Selvíria – MS, Brazil, 2015

P-resin	S-SO ₄	OM	pH	K	Ca	Mg	H+Al	Al	B
	mg dm ⁻³	g dm ⁻³	CaCl ₂			mmol _c dm ⁻³			Hot water
19	10	21	5.0	2.1	19.0	13.0	28.0	1.0	0.17
Cu	Fe	Mn	Zn	V	Total N	Si	Clay	Sand	Silt
DTPA				%	g kg ⁻¹	mg dm ⁻³	g kg ⁻¹		
3.1	20.0	27.2	0.8	55	1.04	9.4	433	471	90

OM = organic matter; V = base saturation

estimate the accumulation of nutrients: 78.2; 7.2; 68.8; 23.3; 21.1; 16.3 and 13.1 kg ha⁻¹ of N, P, K, Ca, Mg, S and Si, respectively, and 260.4; 74.0; 1018.1; 709.6 and 185.1 g ha⁻¹ of B, Cu, Fe, Mn and Zn, respectively and a C/N ratio of 38.3 (Galindo *et al.* 2018).

Based on the soil analysis and with the aim of increasing the saturation by bases to 80%, the dose of 1.94 t ha⁻¹ of dolomitic limestone and 1.76 t ha⁻¹ of calcium and magnesium silicate was applied 30 days before sowing of corn, as topdress and without incorporation. During blanket fertilization, for both years, 375 kg ha⁻¹ of the 08-28-16 for-

mulation were used, corresponding to 30 kg ha⁻¹ N, 105 kg ha⁻¹ P₂O₅, and 60 kg ha⁻¹ K₂O, based on the soil analysis and the requirements of the corn crop.

For seed treatment, the fungicides pyraclostrobin + thiophanate-methyl (6 g + 56 g of a.i. per 100 kg of seed) and the insecticide fipronil (62 g of a.i. per 100 kg of seed) were used. Seeds corn inoculation with the *Azospirillum brasilense* bacterial strains Ab-V5 and Ab-V6 (guarantee of 2x10⁸ CFU mL⁻¹ - Inoculants consisted of a mixture of strains CNPSO 2083 (=Ab-V5) and CNPSO 2084 (=Ab-V6) was carried out at doses of 300 mL of inoculant (liquid)

Figure 2: Rainfall, air relative humidity and maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the corn cultivation in the period November 2015 to April 2016 (A) and November 2016 to April 2017 (B)

per hectare of planted seeds, with the aid of a clean mixer for incorporation of the seeds and was performed one hour before sowing the seeds and after treatment of the seeds with insecticide and fungicide (Galindo et al., 2018).

The mechanical sowing of the simple hybrid DOW 2B710 PW was carried out on 13 November 2015 for the 2015/16 crop and 11 November 2016 for the 2016/17 crop, being sown at 3.3 seeds per meter and emergence of seedlings was five days after sowing, on 18 November 2015 and 16 November 2016, respectively. The corn crop was irrigated using a center pivot sprinkling system, with a mean water depth of 14 mm and an irrigation interval of approximately 72 h. The herbicide tembotrione (84 g ha⁻¹ of a.i.) and atrazine (1000 g ha⁻¹ of a.i.) were applied for the control of post-emergence weeds, plus the addition of an adjuvant in the herbicide syrup, oil (720 g ha⁻¹ of a.i.), on 4 December 2015 and 2 December 2016, respectively. Insect control was performed with methomyl (215 g ha⁻¹ a.i.) and triflumurom (24 g ha⁻¹ a.i.), on 20 December 2015 and 17 December 2016, respectively (Galindo et al. 2018).

The N topdressing was hand applied without soil incorporation, between the plant lines, on soil surface approximately 0.10 m from the rows, on 1 December 2015

and 10 December 2016 in the V6 corn stage. After N fertilization, the area was irrigated by sprinkling (depth of 14 mm) at night to minimize losses by volatilization of ammonia. The harvest was carried out on 1 March 2016 and 21 March 2017, which corresponds to 117 and 125 days after plant emergence, respectively.

2.4 Evaluations

Evaluations were performed as follows: a) N, P, K, Ca, Mg and S contents in corn shoot and root, collecting the aerial part and roots of five corn plants per plot, in the female flowering (R1 corn) stage. The macronutrients determination followed the methodology described in Malavolta et al. (1997).

2.5 Statistical analysis

Data were compared by the Shapiro and Wilk (1965) test and analysis of variance (F test) using a triple factorial scheme. When a significant result was verified by the F test ($p \leq 0.01$ and $p \leq 0.05$), the Tukey test ($p \leq 0.05$) was used for comparison of means of inoculation or not with *Azospirillum brasilense* and soil acidity correctives sources, and adjusted to polynomial regression for the nitrogen rates using SAS program (SAS Inst. Inc., Cary, NC, 2015).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

The N rates positively influenced the N and K concentrations in the 2016/17 crop and Ca in the 2015/16 in the shoots. There was a linearly response for N and K and a quadratic response for Ca up to 130 kg ha⁻¹ of N (Table 2, Figures 3A, B and C). There was also a positive influence on N and P concentrations in both crops, K in the 2016/17 crop and S in 2015/16 in the roots. There was a linearly response for the above-mentioned macronutrients, except N and K in 2016/17 crop, which set the quadratic response until doses of 180 and 166 kg ha⁻¹ of N, respectively (Tables 2 and 3 and Figures 3D, E, F and 4A, B and C). In relation to the sources of soil acidity correctives, the use of dolomitic limestone resulted in higher concentrations of N, Ca and Mg in shoot and Mg in roots in the 2015/16 crop (Tables 2 and 3). Regarding inoculation with *A. brasilense*, seed application increased the N concentration in shoot,

Figure 3: Effect of N rates in N (A), K (B) and Ca (C) concentration in shoot, and N (D) and (E) and P (F) concentration in root. Selvíria, MS – Brazil, agricultural crop 2015/2016¹ and 2016/2017²

however, it reduced the concentration of Ca in roots in the 2016/17 crop (Tables 2 and 3).

The interaction between N doses x inoculation with *A. brasilense* was significant for N and S concentrations in shoots in 2015/16 crop. At the dose of 100 kg ha⁻¹ of N, the inoculated treatments provided a higher N concentration,

whereas at the doses of 100, 150 and 200 kg ha⁻¹ of N, the inoculated treatments increased S concentration (Figures 4D and 4E). A linearly response was observed for the treatments with *A. brasilense* inoculated for both macronutrients mentioned above (Figures 4D and 4E).

Table 2: N, P, K, Ca, Mg and S concentrations in corn shoot as functions of N rates, sources of acidity correctives and seed inoculation with *Azospirillum brasilense*. Selvíria, MS – Brazil, 2015/2016¹ and 2016/2017²

Shoot	N	(g kg ⁻¹ D.M.)									
		P		K		Ca		Mg		S	
N rates		2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	19.21	14.72	3.48	3.27	21.56	20.41	2.84	3.39	2.86	1.83	2.48
50	19.30	16.40	3.89	3.53	21.56	20.77	3.11	3.58	2.81	1.74	2.71
100	20.26	16.31	3.74	3.35	22.19	21.36	3.61	3.50	2.86	1.80	2.65
150	19.78	16.38	3.89	3.49	22.25	22.15	3.61	3.34	2.91	1.92	2.86
200	21.00	16.87	3.92	3.34	20.38	22.03	3.18	3.34	2.89	1.87	2.81
Sources of acidity correctives											
Limestone	20.51 a	16.33 a	3.85 a	3.25	23.15	20.86	3.51 a	3.28	3.15 a	1.80	3.00
Ca and Mg silicate	19.30 b	15.94 a	3.72 a	3.54	20.03	21.83	3.03 b	3.58	2.58 b	1.86	2.40
L.S.D. (5%)	1.08	0.92	0.29	0.19	1.51	0.93	0.32	0.21	0.20	0.14	0.18
Inoculation											
Without <i>A. brasilense</i>	19.79	15.53 b	3.77 a	3.37	22.03	21.78	3.12 a	3.39	2.92 a	1.77	2.51
With <i>A. brasilense</i>	20.03	16.74 a	3.80 a	3.42	21.15	20.91	3.43 a	3.46	2.81 a	1.89	2.90
L.S.D. (5%)	1.08	0.92	0.29	0.19	1.51	0.93	0.32	0.21	0.20	0.14	0.18
Overall Mean	19.91	16.13	3.78	3.40	21.59	21.34	3.27	3.43	2.86	1.83	2.70
C.V. (5%)	8.19	8.64	11.72	8.63	10.54	6.61	14.93	9.27	10.72	11.22	10.33
P value											
RATES (R)	0.0376*	0.013*	0.2823ns	0.3916ns	0.4958ns	0.0090**	0.0080**	0.4956ns	0.9725ns	0.5125ns	0.020*
SOURCES (S)	0.0300*	0.3851ns	0.3783ns	0.0068**	0.0003**	0.0431*	0.0056**	0.0080**	0.0000**	0.3371ns	0.0000**
INOCULATION (I)	0.6519ns	0.0127*	0.8137ns	0.5386ns	0.2387ns	0.0681ns	0.0585ns	0.4826ns	0.2887ns	0.0737ns	0.0003**
R X S	0.1280ns	0.7467ns	0.9312ns	0.4914ns	0.6225ns	0.0954ns	0.8386ns	0.4787ns	0.0866ns	0.7099ns	0.3036ns
R X I	0.0351*	0.3768ns	0.2684ns	0.2738ns	0.3763ns	0.1660ns	0.5986ns	0.6491ns	0.4408ns	0.9836ns	0.0070**
S X I	0.0829ns	0.4056ns	0.2997ns	0.0062**	0.0311*	0.0002**	0.9745ns	0.0331*	0.5178ns	0.0182*	0.0040**
R X S X I	0.6875ns	0.7421ns	0.1913ns	0.2883ns	0.1157ns	0.0998ns	0.5348ns	0.1856ns	0.3409ns	0.7429ns	0.8183ns

Means followed by the same letters in the column do not differ by Tukey at 0.05 probability level.

**, * and ns: significant at p<0.01, 0.01<p<0.05, and not significant, respectively

Table 3: N, P, K, Ca, Mg and S concentrations in corn root as functions of N rates, sources of acidity correctives and seed inoculation with *Azospirillum brasilense*. Selvíria, MS – Brazil, 2015/2016¹ and 2016/2017²

Root	(g kg ⁻¹ D.M.)											
	N		P		K		Ca		Mg		S	
N rates	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	5.69	5.24	1.24	1.26	9.44	11.00	1.68	2.39	0.94	0.93	5.89	3.05
50	5.80	5.85	1.31	1.28	9.25	12.93	1.58	2.53	0.92	0.95	6.98	3.19
100	6.00	6.52	1.39	1.32	9.81	13.07	1.56	2.44	0.95	0.95	6.64	3.16
150	6.20	6.89	1.39	1.38	8.50	12.49	1.73	2.57	0.95	0.90	7.06	3.14
200	6.69	6.82	1.45	1.41	9.88	12.24	1.63	2.41	0.96	0.87	7.14	3.36
Sources of acidity correctives												
Limestone	6.04 a	6.34 a	1.29 a	1.34 a	9.20 a	12.54	1.59 a	2.43 a	1.00 a	0.92 a	6.85 a	3.14 a
Ca and Mg silicate	6.11 a	6.19 a	1.42 a	1.32 a	9.55 a	12.15	1.69 a	2.51 a	0.89 b	0.92 a	6.63 a	3.22 a
L.S.D. (5%)	0.55	0.24	0.09	0.06	1.42	0.62	0.12	0.14	0.09	0.06	0.70	0.19
Inoculation												
Without <i>A. brasilense</i>	5.81 a	6.34 a	1.37 a	1.32 a	9.70 a	12.38	1.68 a	2.55 a	0.97 a	0.92 a	6.78 a	3.27 a
With <i>A. brasilense</i>	6.34 a	6.19 a	1.34 a	1.35 a	9.05 a	12.31	1.60 a	2.38 b	0.92 a	0.92 a	6.70 a	3.09 a
L.S.D. (5%)	0.55	0.24	0.09	0.06	1.42	0.62	0.12	0.14	0.09	0.06	0.70	0.19
Overall Mean	6.07	6.26	1.35	1.33	9.38	12.35	1.64	2.47	0.94	0.92	6.74	3.18
C.V. (5%)	13.64	5.69	10.27	7.11	22.81	7.55	10.64	8.46	15.02	9.10	15.71	9.12
P value												
RATES (R)	0.0176*	0.0000**	0.0051**	0.0015**	0.7105ns	0.0022**	0.2839ns	0.3530ns	0.9857ns	0.2733ns	0.0409*	0.3384ns
SOURCES (S)	0.7707ns	0.2211ns	0.0115*	0.5657ns	0.6107ns	0.1951ns	0.0732ns	0.2667ns	0.0223*	0.8086ns	0.5230ns	0.4115ns
INOCULATION (I)	0.0581ns	0.1889ns	0.5465ns	0.3210ns	0.3484ns	0.7968ns	0.1372ns	0.0171*	0.3027ns	0.8668ns	0.8115ns	0.0645ns
R X S	0.2882ns	0.1159ns	0.1281ns	0.7010ns	0.7669ns	0.2908ns	0.4412ns	0.1629ns	0.5306ns	0.4214ns	0.2894ns	0.1148ns
R X I	0.7875ns	0.0517ns	0.3323ns	0.9099ns	0.6675ns	0.2235ns	0.9730ns	0.3220ns	0.8212ns	0.6474ns	0.1682ns	0.0732ns
S X I	0.7337ns	0.5219ns	0.4106ns	0.5439ns	0.4260ns	0.0342*	0.3616ns	0.1862ns	0.6215ns	0.3856ns	0.7154ns	0.1207ns
R X S X I	0.7936ns	0.1028ns	0.7852ns	0.5948ns	0.5594ns	0.8315ns	0.7742ns	0.0930ns	0.9498ns	0.1517ns	0.3683ns	0.1068ns

Means followed by the same letters in the column do not differ by Tukey at 0.05 probability level.

**, * and ns: significant at p<0.01, 0.01<p<0.05, and not significant, respectively

The interaction between sources of acidity correctives x inoculation with *A. brasilense* was significant for P, Ca and Mg concentrations in the 2016/17 crop, K in both crops and S in the 2015/16 crop in corn shoot, and K concentration in the 2016/17 crop in the roots (Figures 4F, 5A, B, C, D, E and F). In the absence of inoculation, the application of Ca and Mg silicate increased the P concentration compared to the use of dolomitic limestone. The use of limestone increased the P concentration for the seeds with *A. brasilense* inoculated compared to the treatments which were not inoculated (Figure 4F).

In the 2015/16 crop the use of limestone increased the K concentration compared to the use of Ca and Mg silicate for inoculation with *A. brasilense* (Figure 5A). The use of dolomitic limestone increased the K concentration in the presence of *A. brasilense*, while the use of Ca and Mg silicate increased the K concentration in the absence of inoculation. However, in the 2016/17 crop, when inoculation was performed, the use of Ca and Mg silicate increased the K concentration when compared to the use of limestone (Figure 5B). However, dolomitic limestone increased the K concentration in the absence of inoculation, while the use of Ca and Mg silicate increased the K concentration when *A. brasilense* was applied (Figure 5B).

For Ca and Mg concentrations, when *A. brasilense* was inoculated, associated with the use of Ca and Mg silicate, an increase of the concentrations of these macronutrients compared to the use of limestone was observed. The use of Ca and Mg silicate increased Ca and Mg concentration with *A. brasilense* inoculation (Figures 5C and D).

Regardless of inoculation or not with *A. brasilense*, the S concentration was higher with the use of dolomitic limestone. In the treatments in which limestone was applied, the inoculation increased the S concentration compared to the treatments without inoculation (Figure 5E).

For K concentration in roots, when the inoculation was performed, the use of dolomitic limestone increased the values of this nutrient compared to the use of Ca and Mg silicate (Figure 5F).

4 Discussion

Applied nitrogen was absorbed as evidenced by increased N concentration in shoot and root, and favored macronutrients uptake, with increase in K (2016/17 crop), Ca and S (2015/16) concentration in shoots and P (both crops), K (2016/17) and S (2015/16) in roots. N is the nutrient that most interferes in the development and productivity of crops and is the nutrient most demanded by the corn

plant, being found in higher concentrations in plant tissues and grains. The increased N availability favored root development, which, by exploiting a larger volume of soil, may give a greater amount of macronutrient uptake and water, reflecting on shoot removal.

The average nutrients concentration in the corn shoot and root in descending order was $K > N > P > Ca > Mg > S$ in the shoot and $K > N > S > Ca > P > Mg$ in the root, over the two years of cultivation. Phosphorus levels in the soil solution in Brazilian Cerrado (Savannah) are generally very low, making it necessary to apply high amounts of phosphate fertilizer to meet the demands of the crops, which has a low fertilization efficiency on acidic soils. In addition, P has an important role in the composition of ATP, responsible for the storage and transport of energy for endergonic processes, such as the synthesis of organic compounds and the active uptake of nutrients (Marschner 2012). The K acts in osmoregulation (control of salt concentrations in tissues or cells) and resistance of the wheat plant to the dry matter, K also acts in important functions such as grain filling and final product quality (Barker and Pilbeam 2015). The Ca can contribute to the formation and growth of the plant root system, since this nutrient is essential in the synthesis of new cells of the root apical region (meristems) when acting on the composition of the cell wall structure (Marschner 2012), and adequate S contents in the soil are very important for the success of the corn crop because adequate availability of this nutrient increases the efficiency of use of N (protein and amino acids synthesis such as cystine, cysteine and methionine) (Wieser 2007).

The silicate did not positively affect the macronutrients absorption as verified by the decreased N, Ca and Mg concentration in shoot and Mg concentration in roots in 2015/16 compared to the use of limestone. The increased pH after Si application associated with corn straw decomposition in the first crop second predecessor crop provided a higher Si amount in the second year (Galindo et al. 2018). This greater availability probably caused greater uptake and accumulation of this beneficial element in the 2016/17 crop. Therefore, the difference in Ca and Mg silicate efficiency in promoting macronutrient uptake between the first and second crops (e.g. in the second crop the Si use numerically provided an increase in 8.92; 4.65; 9.15; 3.33 and 2.55% for P, K, Ca and Mg concentration in shoot and S in root, respectively) can be explained.

The *A. brasilense* inoculation alone favored the N concentration in corn shoots with an increase in the 2016/17 crop, and when associated to the applied N rates, increased the N concentration in the 2015/16, with the application of 100 kg ha⁻¹ of N and S concentrations with

Figure 4: Effect of N rates in P (A), K (B) and S (C) concentration in root, interaction between rates and inoculation with *Azospirillum brasilense* in N (D) and S (E) concentration in shoot and interaction between sources of acidity correctives and inoculation with *Azospirillum brasilense* in P (F) concentration in shoot. Selvíria, MS – Brazil, agricultural crop 2015/2016¹ and 2016/2017².

Means followed by the same letters in the rates column do not differ by Tukey at 0.05 probability level and means followed by the same small letters for sources of acidity correctives and uppercase for inoculation with *Azospirillum brasilense* do not differ by Tukey at 0.05 probability level.

the application of 100, 150 and 200 kg ha⁻¹ also in the 2015/16 crop and in the corn shoot. It is important to point out that in isolation, the inoculation reduced the Ca con-

centration in roots in the 2016/17 crop, however, caused a numerical increase in the concentration of this nutrient in the aerial part by 2.06%. Besides that, when associated

Figure 5: Interaction between sources of acidity correctives and inoculation with *Azospirillum brasilense* in K (A and B), Ca (C), Mg (D) and S (E) concentration in the shoot and K (F) in the root. Selvíria, MS – Brazil, agricultural crop 2015/2016¹ and 2016/2017².

Means followed by the same small letters for sources of acidity correctives and uppercase for inoculation with *Azospirillum brasilense* do not differ by Tukey at 0.05 probability level

with Si applied as Ca and Mg silicate favored K, Ca and Mg concentrations in shoot in the 2016/17 crop, and when associated with limestone favored P and K concentrations in the shoot and root of the 2016/17 crop and K concentration in the shoot in 2015/16.

The broadly favorable results obtained by *Azospirillum* inoculation in plants is well characterized (Hungria et al. 2010; Santos et al. 2017) and occurs, among other factors, because BNF increase in nitrate reductase activity when they grow endophytically in plants, increase mineral and water uptake, improve tolerance to salinity and drought, as well as the ability to produce phytohormones such as indole-3-acetic acid (IAA) (Dardanelli et al. 2008; Hungria et al. 2010; Zawonski et al. 2011; Meza et al., 2015; Pankiewicz et al. 2015). *A. brasilense* synthesizes IAA by the tryptophan-dependent pathway (Duca et al. 2014). Tryptophan synthesis requires a high quantity of ammonium (Günes, et al. 2014). Therefore, the N uptake must be increased to synthesize tryptophan properly and to produce plant hormones that will increase macronutrients and water absorption, positively reflecting on corn development. This study demonstrates benefits in corn nutrition, even high N doses considered for BNF, elucidating that *A. brasilense* associated with N fertilization provide beneficial effects for plants. In addition, due to the low cost, ease of acquisition and application, non-toxicity, this technology will possibly be increasingly adopted by farmers.

The Si use provides several benefits on grasses, mainly when it is associated with abiotic and biotic conditions (Reis et al. 2008). This element will mainly benefit the Si hyperaccumulators plants, which include some grasses such as rice (*Oryza sativa*) and sugarcane (*Saccharum officinarum*), that present SiO₂ concentrations above 4% (Lima et al. 2011). However, some factors will influence the Si uptake by plants, such as genotype, plant species, and type of soil in which the application was performed (Camargo et al. 2014a, 2014b; Galindo et al. 2018). Therefore, the slight response to silicate use, even with a high Si absorption would be expected. In this sense, new studies with Si use in accumulator crops should be performed.

Finally, we can conclude that the inoculation with *A. brasilense* alone favored the concentration of N in the shoot, and when associated to the N rates, increased the concentration of N and S even when associated with high N rates in topdressing, being a complement and optimizing nitrogen fertilization. Besides that, when associated with Si in the form of Ca and Mg silicate favored K, the Ca and Mg concentration in the shoot in the 2016/17 crop, and when associated with limestone increased P and K concentrations in the shoot and root in the 2016/17 crop, and the K concentration in the shoot in 2015/16. We veri-

fied that the Si when applied as Ca and Mg silicate does not promote an increase in nitrogen fertilization efficiency enough to increase uptake of macronutrients. Although it did not favor the N use, the Si also did not harm the inoculation with *A. brasilense* and nitrogen fertilization.

Acknowledgments: The authors are grateful to CAPES and FAPESP (process number 2017/06002-6).

Conflict of interest: Authors declare no conflict of interest.

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