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Agronomic efficiency of inoculant based on *Azospirillum brasilense* associated with nitrogen fertilization at maize

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The objective was to evaluate the agronomic efficiency in the field of the inoculant Fluid and Turfoso containing the bacterium *Azospirillum brasiliense*, strains AbV5 and AbV6 applied by treatment of seeds in the corn crop associated with nitrogen fertilization in different localities. Four experiments were carried out in different sites involving two in Paraná and one each from Mato Grosso do sul and Santa Catarina. The design was randomized blocks with 6 treatments: T1- 0 kg ha^{-1} of nitrogen (N), without inoculation (control); T2 - 80 kg ha^{-1} of N, without inoculation; T3 - 160 kg ha^{-1} of N, without inoculation; T4 - 80 kg ha^{-1} of N + seed inoculation AzoTotal® 'Liquid'; T5 - 80 kg ha^{-1} of N + seed inoculation Nitro 1000 Gramines 'Liquid'; T6 - 80 kg ha^{-1} of N + seed inoculation Nitro 1000 'Peat' grasses. The evaluations were composed of nutrient content in leaves and grains, and at the end of the cycle length and ear diameter, number of rows of grains and grains per row, mass of one thousand grains and productivity. The results showed that the inoculation of the seeds, regardless of the physical nature of the inoculant, was efficient for the maize crop, reducing the use of mineral nitrogen in all evaluated sites. It is concluded that the use of inoculation with *A. brasilense* regardless of the physical nature of the inoculant reduces the need for nitrogen fertilization of the corn crop by 50%, without reducing the final yield of the crop.

Key words: *Zea mays,* biological nitrogen fixation, productivity, *Azospirillum brasilense,* sustainable agriculture.

INTRODUCTION

The corn crop (*Zea mays* L.) is the most cultivated cereal in Brazil and in the world. National production in the 2015/2016 harvest was 83.33 million tons of grain (Conab, 2016; USDA, 2016). In the world scenario it has great social, economic and cultural relevance, being relevant for human consumption and mainly for animal

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consumption.

In relation to mineral nutrition corn is one of the most demanding into fertilizer, mainly the nitrogenous one (Carvalho et al., 2013), since this material needs high amout of nitrogen (Moda et al., 2014). In this study, the use of nitrogen fertilizers was considered to be an important determinant of the nutrient availability of the nutrient in the crop (Ferreira et al., 2007). Therefore, nitrogen fertilization is necessary because of the insufficient amount that the soil provides for adequate plant growth. This situation is particularly important for the maize crop, since, among the nutrients that influence its productivity, the N is one of the most absorbed during the development cycle of the plants.

The use of nitrogen fertilizers in maize crop presents high costs and high environmental impact (Garcia et al., 2013). In addition, there is a low efficiency of the current available sources, which are based on the 50% mark (Costa et al., 2003). The quest for maximizing the use of N is a challenge. One of the ways to increase yield and minimize the production costs is linked to plants that have a more developed root system (Costa et al., 2015), able to better exploit the soil and increase nutrient and water absorption. In this way, better development conditions are provided to the plant, resulting in increased productivity (Bassoi et al., 1994).

An alternative to reduce the use of mineral fertilizers is the use of seed inoculation by diazotrophic bacteria (Novakowiski et al., 2011; Quadros et al., 2014; Inagaki et al., 2015), widely used in leguminous crops.

Among the diazotic bacteria, the *Azospirillum* genus can colonize plant roots and shoots (Pedrinho et al., 2010). This is a promising alternative, since these microorganisms increase the availability of N to the plant, by breaking the triple bond of the atmospheric N_2 and making it available to the plants. This practice can provide up to 50% of the total N to the plant (Piccinin et al., 2013). In addition to the above, this specie is capable of producing plant growth promoting substances (Santi et al., 2013), with the most important being indole acetic acid, an auxin (Radwan et al., 2005; Perrig et al. 2007), resulting in a higher root development (Rodrigues et al., 2014) and thus increasing the area of root system exploration and nutrient absorption (Ferreira et al., 2013).

The use of diazotrophic bacteria as an alternative to increase the availability of nitrogen to crops may be a less costly and ecologically viable option, since it contributes to the reduction of atmospheric CO_2 up to 0.309 Mg CO_2-eq ha^{-1} demonstrated in Brachiaria (Hungria et al., 2016). In light of this finding, several studies have been conducted to verify the potential of *Azospirillum* spp. (Dartora et al., 2013; Repke et al., 2013; Guimarães et al., 2014; Quadros et al., 2014).

In this line, inoculation of the seeds with *A. brasilense* increases foliar area and shoot dry matter by 12% on the foliar area and dry issue of aerial part (Marini et al., 2015). Costa et al. (2015), showed an increase in plant height, dry stem and root mass, chlorophyll content, a thousand-grain mass, and final crop yield of the second crop. This increase was 29%.

The objective of the present work was to evaluate the agronomic efficiency in the field of Fluid and Turfoso inoculant containing the bacterium *Azospirillum brasiliense*, strains AbV5 and AbV6 applied by treatment of seeds in maize crop associated with nitrogen fertilization in different localities.

MATERIALS AND METHODS

The study was carried out by conducting experiments in four sites with different edaphoclimatic conditions in the 2013/2014 harvest. Four experiments were carried out in different sites involving two in Paraná and one each from Mato Grosso do sul and Santa Catarina., all in Brazil (Table 1).

The areas where the experiments were developed were being cultivated with annual and perennial crops under no-tillage system for at least five years. Therefore, in order to characterize it initially, that is to say, before sowing of the maize, samples were taken in twenty profiles of tradition for the collection of the soil with deformed structure, realized with a screw thread in the depth of 0 to 0.20 m, whose chemical and physical characteristics are presented in Table 2.

The count of diazotrophic microorganisms to determine the population of bacteria in cell numbers per mL was performed by estimating the Most Probable Number (MPN) using the MacCrady table in NFB (*Azospirillum* spp.) where the Semi-solid medium was used for bacterial growth according to methodology (Döbereiner et al., 1995). The results of the counts of diazotrophic microorganisms by the estimation of MPN in soils of the experimental areas were as follows: Site 1: 2x10^6 g^{-1} soil; site 2: 1.1x10^7 g^{-1} soil; site 3: 1.8x10^6 g^{-1} soil and site 4: 4.5 x10^6 g^{-1} soil.

The inoculant 'Nitro 1000 Gramines liquid' has the following characteristics: 2.0x10^8 CFU mL^{-1} (Colony Forming Units) of *A. brasilense* strains AbV5 and AbV6; Physical nature: Fluid; Density: 1.0 g / mL; Target Culture: Maize (*Z. mays* L.); Dosage tested 100 mL for 25 kg of seed: Lot: 001/2013; Manufacture: 10/31/13. The inoculant 'Nitro 1000 Gramines Peaty' has the following characteristics: Guarantee: 2.0x10^8 CFU g^{-1} of *A. brasilense* strains AbV5 and AbV6; Physical nature: Solid; Density: 1.0 g / mL; Target Culture: Maize (*Zea mays* L.); Dosage tested: 100 g for 25 kg of seed; Lot: 001/2013; Manufacture: 10/31/13.

The AzoTotal® inoculant was used as reference (standard inoculant) in the four experiments. The inoculant presents 2.0x10^8 CFU mL^{-1} of *A. brasilense*, strains AbV5 and AbV6; Physical nature: Liquid; Density: 1.0 g / mL. The dosage used in the test 100 mL for 25 kg of seed: Lot: 1101213; Manufactured: 09/13/13.

All the inoculants used were submitted to laboratory tests of concentration, purity and characterization. The analyzes followed official methods, according to Normative Instruction number 30, dated November 12, 2010 (MAPA). AzoTotal® Inoculant presented 2.15x10^8 CFU mL^{-1}; Nitro 1000 Grasses 'Liquid' presented 2.33x10^8 CFU mL^{-1} and Nitro 1000 Grasses 'Turfa' presented 2.12x108 CFU g^{-1}.

The four experiments used Piooner® 30F53 YH hydrid simple and were conducted in a randomized block design with six treatments and four replicates. The treatments were: T1- 0 kg ha^{-1} of nitrogen (N), without inoculation (control); T2 - 80 kg ha^{-1} of N, without inoculation; T3 - 160 kg ha^{-1} of N, without inoculation; T4 - 80 kg ha^{-1} of N + seed inoculation with standard AzoTotal® 'Liquid' inoculant at the dose of 100 mL 25 kg^{-1} of seeds; T5 - 80 kg ha^{-1} of N + seed inoculation with inoculant Nitro 1000 Gramines 'Liquid' at the dose of 100 mL 25 kg^{-1} of seeds; T6 - 80 kg ha^{-1} of N + seed

Table 1. Geographic site, type of soil and climate of the places where the experiments were carried out.

Site	Coordinates	Level	Soil Type	Weather
Site 1 – PR	24°40'S 54°16'W	248	RED LATOSOL Eutrophic	Humid Subtropical Mesothermic
Site 2 – PR	24°43'S 53°46'W	565	RED LATOSOL Dystrophic	Humid Subtropical Mesothermic
Site 3 – SC	27°16'S 50°30'W	1000	CAMBISOL Acric	Mild weather
Site 4 – MS	20°18'S 52°39'W	357	RED LATOSOL Dystrophic	Tropical Umid

Table 2. Chemical and physical characterization of the soils before the implantation of the experiments.

L	Chemical characteristics											Granulometry		
	pH	V	P	MO	Ca^{2+}	Mg^{2+}	K^{+}	Al^{3+}	H+Al	SB	CTC	Argila	Silte	Areia
	$CaCl_2$	- % -	mg dm^{-3}	g dm^{-3}	---cmol$_c$dm^{-3}							g kg^{-1}		
1	5.07	62.16	21.12	28.71	6.11	1.32	0.29	0.10	4.70	7.72	12.42	559	359	83
2	5.20	61.43	16.70	24.94	6.62	2.92	0.35	0.00	6.21	9.89	16.10	720	170	110
3	6.30	79.80	10.70	44.23	8.35	4.11	0.10	0.00	3.18	12.56	15.74	550	363	87
4	5.5	62	17	25.00	26.00	18.00	3.00	0.00	29.00	47.00	76.00	700	250	50

L: sites. (P,K, Micronutrients) Mehlich^{-1} ;(Al, Ca, Mg); KCl 1 mol L^{-1} ; (H+Al) pH SMP (7,5);(pH); $CaCl_2$ 0,01 mol L^{-1} .

Table 3. N, P and K content in the cultural remains, at the time of the implantation of the experiments in the four sites.

Site	Haystack	N	P	K
	t ha^{-1}	kg ha^{-1}		
Site 1 – PR	8.0	105	17	75
Site 2 – PR	7.5	98	16	69
Site 3 – SC	13.0	171	28	117
Site 4 - MS	12.0	160	28	110

inoculation with inoculant Nitro 1000 'Peat' grasses at the dose of 100 g 25 kg^{-1} seeds.

Nitrogen fertilization was performed in two applications. At the time of sowing, 30 kg ha^{-1} of N was applied in all treatments, with the exception of T1 (control). In the V6 stage of maize 50 kg ha^{-1} of N were applied in treatments with 80 kg ha^{-1} of N (T2, T4, T5 and T6). For T3 treatment, 80 kg ha^{-1} of N at the V4 stage and 50 kg ha^{-1} of N at the V8 stage were applied. Urea nitrogen (45% N) was used as the source of both the sowing and coverage.

Prior to corn sowing, the areas were desiccated with glyphosate herbicide at a dose of 4 L of p.c. ha^{-1}. At this moment the cultural remains were collected for analysis of N, P and K in the samples (Table 3).

The experiments were implanted with the following sowing dates: Site 1 (10/10/2013); Site 2 (11/05/2013); Site 3 (10/25/2013) and Site 4 (12/9/2013). The seed were treated with (trichloromethylthio) cyclohex-4-ene^{-1},2-dicarboxy fungicide at a dose of 0.2 kg 100 kg^{-1} of seeds, as well as with insecticide imidacloprid + Thiodicarb at the dose of 0.2 L 100 kg^{-1} of seeds.

Inoculation of the seeds was realized in high density plastic bags, where the inoculants were directly deposited according to the treatments. Then they were agitated for approximately two minutes to standardize the distribution of the inoculant in the seeds. Sixty minutes after inoculation, the sowing was done, being the standard procedure for the four experiments.

Phosphorus fertilization (80 kg ha^{-1} of P_2O_5) and potassium (60 kg ha^{-1} of K_2O) were applied to the sowing furrow using the mechanized fertilizer sowing machine (Embrapa, 2012).

Sowing of the experiments was carried out with the aid of a manual seeder (matracas), with five seeds per meter being distributed in the sowing furrow, reaching a final population of 70000 ha^{-1} plants. Each experimental plot consisted of 6 lines of 0.70 m spacing, 6 m long and 4.2 m wide, totaling 25.2 m^2 per plot, spaced apart by 1 m, and total area of 604.80 m^2. To obtain the useful area of the plots the outer lateral lines and 1.0 m of the ends of the lines of each plot were disregarded.

During the conduction of the experiments, the control of weeds, pests and diseases were carried out according to the needs of the crop (Embrapa, 2012).

When the plants were in the full flowering stage, they were collected the middle third of 10 leaves opposite and immediately below the main spike, per plot. After harvesting the cobs and threshing of the grains, samples of grains corresponding to each experimental plot were taken. These were dried at 65°C in a forced air circulation oven until mass reached constant. Afterwards, they were ground and analyzed for N, P and K content (Malavolta et al., 1997).

Foliar and grain samples were ground and subjected to sulfur

Table 4. Nitrogen, phosphorus and potassium contents in foliar tissue and grain of plants of hybrid corn Piooner® 30F53 YH in Place 1, from October 2013 to February 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	------------------------------ ($g\ kg^{-1}$) ------------------------------					
Control	21.00[b1]	3.05[b]	24.07[ns]	9.40[b]	1.57[ns]	3.08[ab]
80 kg ha^{-1} N	26.47[ab]	3.70[ab]	23.66	10.94[ab]	1.39	3.43[ab]
160 kg ha^{-1} N	27.13[a]	4.13[a]	22.17	11.81[ab]	1.24	3.65[a]
Inoculum Pattern²	26.03[ab]	3.54[ab]	23.26	11.16[ab]	1.26	2.32[b]
Inoculum Liquid³	26.25[ab]	3.97[a]	26.36	9.62[ab]	1.26	3.11[ab]
Inoculum Peaty[4]	27.03[a]	3.92[a]	24.62	12.03[a]	1.12	3.62[a]
Avarage	25.58	3.72	24.02	10.83	1.31	3.20
C.V. (%)	13.76	12.49	10.60	13.97	24.08	15.81

[1]Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. ns not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; **[3]** 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; **[4]** 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

digestion according to the methodology of Embrapa (2009).

The experiments harvest was performed on the following dates: Site 1 (05/02/2014); Site 2 (3/26/2014); Site 3 (03/24/2014) and Site 4 (04/09/2014). The components of the production were determined by sampling ten ears per useful plot. The evaluations were length of cobs (CE, expressed in cm), ear diameter (DE, in mm), number of rows per cobs (NFGE), number of grains per row in the cobs (NGF) and mass of thousand grains (MMG, in grams).

For the determination of grain yield, all the cobs of the useful plot were milled and the grains weighed. The results were expressed in kg ha^{-1}, correcting the values for 13% moisture in the wet basis.

The data, after tabulation, were submitted to analysis of variance by the Fisher-Snedecor test (test F) and the means of the treatments were compared by Duncan's test ($P \leq 0.05$). The analyses were carried out using the GENES computer program of the Federal University of Viçosa (UFV) (Cruz, 2013).

RESULTS

The results obtained Site 1 showed that nutrient contents in foliar tissue and maize grains were significantly influenced by treatments with the exception of foliar K content and P content in the grains. The N and P contents in the foliar and K tissues in the grains were superior in the treatment with 160 kg ha^{-1} N, without inoculation; however, they presented significant difference only of the control, without addition of N and inoculation. For the N content in the grains, the highest levels were obtained in plants whose seeds were treated with turfous inoculant; however, this treatment only differed statistically from the control (Table 4).

For the production variables, there was no significant effect on the length of spikes (CE), number of rows per spike (NFGE) and number of grains per cobs (NGF) (Table 5). Regarding the cob diameter (DE) and mass of a thousand grains (MMG), the treatment was 160 kg ha^{-1} of N, which was statistically higher to the control. When the productivity was observed, the agronomic efficiency of *A. brasilense* was verified through seed inoculation, since the treatments that received inoculation with *A. brasilense* + 80 kg ha^{-1} N, were equal to the treatment with 160 kg ha^{-1} N (Table 5).

For Site 2, there were no significant effects of treatments on P and K accumulation in foliar tissue and P in corn grains. For the N contents in the foliar tissue and N and K in the grains, a significant effect of the treatments was observed. The foliar N content was higher when 160 kg ha^{-1} N was used, however, it did not differ statistically from the treatments that received inoculation with *A. brasilense*. When the N and K contents were observed, the treatments 160 kg ha^{-1} N and turfous inoculant were superior to the control (Table 6).

Regarding the production components for Site 2, no significant differences were observed in the DE and NFGE. For the CE, NGF and MMG the lowest averages were provided by the control, treatments with inoculation of the seeds with A. brasilense showed the highest averages, except for the standard inoculant, which promoted intermediate results (Table 7).

Productivity was significantly influenced. Seed inoculation treatment with A. *brasilense* promoted higher means, and fertilization with 160 kg ha^{-1} N was used. Seed inoculations with standard, liquid and turfous inoculant exceeded the treatment with 80 kg ha^{-1} N promoting increments of 15.65; 23.16 and 26.22%, respectively (Table 7).

In the experiment conducted at Site 3 no effect of treatments on N accumulation in foliar tissue as well as N, P and K on the grains was observed. For the accumulation of foliar P the control obtained higher average values, while for the accumulation of K the highest average was observed with the fertilization of 160 kg ha^{-1} N, however differentiating only from the control (Table 8).

At Site 3 all the productive variables were significantly

Table 5. Length of cob (CE), cob diameter (DE), number of row of grain per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Plants of hybrid corn Piooner® 30F53 YH at Site 1 during the months of October 2013 to February 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha^{-1})
Control	19.84^{ns1}	51.24^{b}	16.55^{ns}	36.57^{ns}	298.31^{b}	11.595.31^{b}
80 kg ha^{-1} N	20.05	52.09^{ab}	16.65	37.48	327.31^{ab}	12.560.80^{ab}
160 kg ha^{-1} N	19.93	52.71^{a}	16.45	37.46	346.59^{a}	13.564.43^{a}
Inoculum Pattern²	20.20	51.79^{ab}	16.05	37.34	338.57^{ab}	12.585.91^{ab}
Inoculum Liquid³	20.62	51.75^{ab}	16.40	38.91	329.77^{ab}	13.245.25^{a}
Inoculum Peaty^{4}	20.84	51.88^{ab}	16.00	39.93	333.14^{ab}	13.446.86^{a}
Média	20.24	51.86	16.35	37.95	328.94	12.834.75
C.V. (%)	3.08	0.83	2.86	5.26	5.40	6.16

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. ^{ns} not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

Table 6. Nitrogen, phosphorus and potassium contents in foliar tissue and grain of Piooner® 30F53 YH hybrid corn in Site 2 during the months of November 2013 to March 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	(g kg^{-1})					
Control	22.05^{b}	3.11^{ns}	24.72^{ns}	8.59^{b}	1.38^{ns}	3.08^{b}
80 kg ha^{-1} N	23.13^{b}	3.55	22.72	10.83^{ab}	1.26	3.65^{ab}
160 kg ha^{-1} N	28.52^{a}	3.58	23.15	12.89^{a}	1.44	3.78^{a}
Inoculum Pattern²	26.31^{ab}	3.51	24.31	11.22^{ab}	1.28	3.62^{ab}
Inoculum Liquid³	25.32^{ab}	3.56	26.32	10.25^{ab}	1.30	3.58^{ab}
Inoculum Peaty^{4}	26.25^{ab}	3.59	25.52	12.25^{a}	1.29	3.70^{a}
Média	25.09	3.66	24.46	11.14	1.33	3.57
C.V. (%)	10.25	8.56	9.56	12.56	18.54	13.25

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. ^{ns} not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

Table 7. Spigot length (CE), ear diameter (DE), number of grain row per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Piooner® 30F53 YH hybrid corn plants at Site 2 during the months of November 2013 to March 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha^{-1})
Control	15.61^{c1}	48.92^{ns}	17.02^{ns}	33.20^{b}	340.68^{c}	6.255.56^{c}
80 kg ha^{-1} N	17.28^{ab}	51.00	17.21	35.07^{ab}	373.63^{b}	7.025.63^{bc}
160 kg ha^{-1} N	17.30^{ab}	53.19	17.42	35.15^{ab}	386.96^{a}	9.164.23^{a}
Inoculum Pattern²	16.45^{bc}	52.79	17.11	33.60^{b}	374.50^{b}	8.125.36^{a}
Inoculum Liquid³	17.66^{ab}	53.72	17.65	36.35^{a}	390.07^{a}	8.653.21^{a}
Inoculum Peaty^{4}	17.78^{a}	53.53	17.44	37.52^{a}	392.10^{a}	8.868.23^{a}
Média	17.01	52.19	17.28	35.15	376.32	8.015.37
C.V. (%)	5.55	5.21	3.31	5.47	1.97	10.52

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. ^{ns} not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

Table 8. Nitrogen, phosphorus and potassium contents in foliar tissue and grain of Piooner® 30F53 YH hybrid corn in Site 3 during the months of October 2013 to March 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	------------------------------------- (g kg^{-1}) -------------------------------------					
Control	12.91^{ns1}	3.44^{a}	14.10^{b}	8.10^{ns}	1.47^{ns}	5.46^{ns}
80 kg ha^{-1} N	13.78	1.93^{d}	17.00^{ab}	7.88	1.28	4.58
160 kg ha^{-1} N	13.35	2.81^{b}	22.46^{a}	8.32	1.47	4.82
Inoculum Pattern²	14.00	2.17^{cd}	17.90^{ab}	8.10	1.34	4.20
Inoculum Liquid³	13.91	2.78^{bc}	17.56^{ab}	8.31	1.44	5.48
Inoculum Peaty[4]	13.52	2.25^{bcd}	21.09^{a}	8.53	1.42	5.01
Média	13.31	2.56	18.35	7.89	1.40	4.91
C.V. (%)	9.08	15.40	20.74	17.99	15.39	17.64

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. [ns] not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

Table 9. Cob length (CE), cob diameter (DE), number of row per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Piooner® 30F53 YH hybrid corn plants at Site 3 during the months of November 2013 to March 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha^{-1})
Control	43.98^{c}	10.70^{c}	13.94^{b}	21.13^{c}	273.40^{c}	1954.38^{c}
80 kg ha^{-1} N	48.50^{b}	48.50^{b}	15.15^{a}	26.75^{b}	293.50^{bc}	6501.10^{b}
160 kg ha^{-1} N	52.18^{a}	52.18^{a}	15.85^{a}	35.43^{a}	316.65^{a}	9175.55^{a}
Inoculum Pattern²	48.58^{b}	48.58^{b}	15.38^{a}	26.65^{b}	297.85^{ab}	7935.18^{ab}
Inoculum Liquid³	48.58^{b}	48.90^{b}	15.20^{a}	26.35^{b}	299.30^{ab}	7813.90^{ab}
Inoculum Peaty[4]	49.28^{b}	49.28^{b}	15.58^{a}	28.25^{b}	301.83^{ab}	8308.83^{a}
Média	14.75	48.40	15.17	27.43	291.42	6114.82
CV (%)	7.96	2.82	4.50	10.50	4.72	15.93

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. [ns] not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

influenced by the treatments with nitrogen fertilization and inoculation of seeds with *A. brasilense*. The variables CE, DE and NGF were superior to the other treatments when the fertilization with 160 kg ha^{-1} N.

When evaluating MMG and productivity seed inoculations promoted averages similar to fertilization with 160 kg ha^{-1} N, which was the highest average. Seed inoculations with standard, liquid and turfous inoculant exceeded the treatment with 80 kg ha^{-1} N promoting increases of 22.05, 20.19 and 27.80%, respectively (Table 9).

For the experiment conducted at Site 4, N, P and K foliar contents as well as N content in maize grains were not influenced by the treatments used (Table 10).

Unlike the previous site, at Site 4, the variables CE, DE, NFGE, NGF and MMG were not influenced by the treatments used. Productivity was statistically influenced by the treatments employed, and the highest average was provided by fertilization with 80 kg ha^{-1} N, which differed from the control and fertilization treatments with 160 kg ha^{-1} N (Table 11).

DISCUSSION

Results of the interaction between diazotrophic bacteria and maize in terms of agronomic potential, nitrogen fixation or growth promotion, depends on many biotic and environmental factors such as plant genotype, soil microbiological community and nitrogen availability (Roesch et al*.,* 2006).

It is important to note that the effect of inoculation with *Azospirillum* in the experiments conducted can not be correlated only with the increase of N, but also with other

Table 10. Nitrogen, phosphorus and potassium contents in foliar tissue and grain of hybrid corn plants Piooner® 30F53 YH at Site 4 during the months of December 2013 to April 2014.

Treatment	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	------ ($g\ kg^{-1}$) ------					
Control	25.40^{ns}	3.00^{ns}	22.50^{ns}	14.00^{ns}	1.38^{ns}	4.44^{ns}
80 kg ha^{-1} N	26.00	3.00	22.50	13.30	1.40	4.58
160 kg ha^{-1} N	25.90	3.00	20.60	13.70	1.51	5.56
Inoculum Pattern²	24.70	3.10	20.60	13.50	1.41	4.57
Inoculum Liquid³	26.00	3.10	25.60	13.60	1.44	5.10
Inoculum Peaty[4]	26.60	3.40	25.60	13.90	1.46	5.25
Média	25.76	3.10	22.90	13.66	1.43	4.92
CV (%)	5.61	7.22	11.50	7.98	13.45	15.52

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. [ns] not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

Table 11. Cob length (CE), cob diameter (DE), number of row of grain per cob (NFGE), number of grains per row (NGF), mass thousand grains (MMG) and productivity (PROD), of plants of hybrid corn Piooner® 30F53 YH at Site 4 during the months of December 2013 to April 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha^{-1})
Control	13.8^{ns}	4.0^{ns}	15.4^{ns}	30.12^{ns}	218.4^{ns}	4967^{b}
80 kg ha^{-1} N	14.8	4.1	16.1	31.85	214.2	5993^{a}
160 kg ha^{-1} N	14.5	4.0	15.4	31.36	211.5	4953^{b}
Inoculum Pattern²	15.0	4.2	16.0	31.71	231.3	5590^{ab}
Inoculum Liquid³	14.4	4.1	15.5	30.92	230.2	5746^{ab}
Inoculum Peaty[4]	14.6	4.1	15.5	30.25	234.0	5586^{ab}
Média	6.26	2.76	4.61	31.04	10.06	19.47
C.V. (%)	2.09	0.26	1.65	1.25	26.3	963

[1] Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. [ns] not significant at 5% error; [2] 80 kg ha^{-1} de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; [3] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; [4] 80 kg ha^{-1} de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

nutrients (Holguin and Bashan, 1996; Ferreira et al., 2013). The results obtained in the present research for potassium in Site 3 and phosphorus at Sites 1 and 3.

Thus, based on research data with field inoculation experiments (Okon and Vanderleyden, 1997), the genus *Azospirillum* spp. promotes gains in productivity of important crops in the most varied conditions of climate and soil. However, they point out that the gain with inoculation goes beyond simply aiding in the biological fixation of N_2, also helping in the increase of the absorption surface of the roots of the plant and, consequently, in the increase of the volume of the explored soil, being able to increase the absorption of other nutrients.

According to the authors Okon and Vanderleyden (1997), this finding is justified by the fact that the inoculation modifies the morphology of the root system, increasing not only the number of radicels but also the diameter of the lateral and adventitious roots.

The modifications of the root system is related that *Azospirillum* spp. In plants, produce and stimulate the production of growth promoting substances, among them auxins, gibberilins and cytokinins, and not only the biological fixation of nitrogen.

According to Cantarella (2007), the N foliar sufficient level is 27.5 to 32.5 g kg^{-1} N, our result showed below the critical level for the maize crop. For the contents of the other nutrients, the values are within the appropriate, regardless of the treatments. When considering the critical levels established P and K sufficiency range in foliar tissue is, 2.5 to 4.0, and 17.0 to 22.5 g kg^{-1}, respectively (Cruz et al., 2008). In this way, the results of P and K in all studied sites are presented in the range of sufficiency, except for the K content obtained in the control treatment in Site 3, which was below the critical level.

The results demonstrated for the foliar N content evidenced that the effect of plant growth promotion by the action of *A. brasilense* is not restricted to biological fixation of nitrogen, although it contributes in part to the supply of N to the corn plant. About 50% as demonstrated in the present study. However, part of this 50% may have been supplied via the promotion of root growth, due to the induction of plant hormone synthesis, such as auxins (Radwan et al., 2004; Kuss et al., 2007), increasing the nutrient absorption capacity from the decomposition of the pre-existing straw.

Results similar to the present study are demonstrated (Salomone and Dobereiner, 1996) which verified increases in productivity with the inoculation of *Azospirillum* spp. In different crop conditions. In this same sense Cavallet et al. (2000), verified higher yield indexes in the corn crop, as a consequence of inoculation *A. brasilense.*

Okon and Labandera-Gonzalez (1994), when evaluating twenty years of studies with the inoculation of *Azopsirillum sp*. it was found that 60 to 70% of the experiments had positive results. In this sense, the beneficial effects of inoculation with *A. brasilense* have already been reported in several studies in the literature (Dartora et al., 2013; Repke et al., 2013; Quadros et al., 2014; Costa et al., 2015; Marini et al., 2015; Morais et al., 2015), in addition to the increase in productivity, the characteristics that these bacteria have in synthesizing growth-related phyton- mones are shown in Figure, such as auxins, gibber- linins and cytokinins (Kuss et al., 2007; Perrig et al., 2007) and the availability of N_2 present in the soil in absorbable forms for plants.

For the experiment conducted at Site 4, it is worth highlighting that after flowering, the high incidence of foliar diseases in maize was observed, which may have impaired the photosynthetic process and the transsite of assimilates to the grains, explaining in part the average productivity of grains.

In general, the results observed in the present work, involving four sites, developed in three distinct regions, show the agronomic efficiency of *A. brasilense* (strains AbV5 and AbV6) in promoting plant growth, contributing to good maize of the hybrid Piooner® 30F53 YH, which received inoculation via seed, with half of the nitrogen dose recommended.

Conclusions

The inoculation of the seeds of corn with 100 mL to 25 kg of seed with the liquid and turfous inoculants, based on the bacterium *Azospirillum brasilense*, presents agronomic efficiency.

Inoculation with *Azospirillum brasilense*, regardless of the physical nature of the inoculant (liquid or turfous), allowed to reduce nitrogen fertilization in the corn crop by 50% without compromising final crop yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Bassoi LH, Júnior LF, Jorge LA, Crestana S, Reichardt K (1994). Distribution of maize root system in a kanduidalfic eutrudox soil: II. Comparison between irrigated and fertirrigated crops . Sci. Agric. 51(3):541-548.

Cantarella L (2007). Nitrogênio. In: Novais RF, Alvarez VVH, Barros RL, Cantarutti RB, Neves JCL (Ed.). Fertilidade do Solo. Viçosa: Soc. Bras. Ciênc. Solo pp. 376-470.

Carvalho EV, Cancellier LL, Afférri FS, Dotto MA, Peluzio JM, Cruz OS (2013). Growth of corn at contrasting nitrogen levels and the correlation with grain yield. Rev. Bras. Ciênc. Agrár. 8(3):351-357.

Cavallet LE, Pessoa ACS, Helmich JJ, Helmich PR, Ost CF (2000). Corn produntivity in response to nitrogen application and seed inoculation with *Azospirillum* spp. Rev. Bras. Eng. Agríc. Ambien. 4(1):129-132.

CONAB (2016). Acompanhamento da safra brasileira de grãos. Brasilia: Conab.

Costa MCG, Vitti GC, Cantarella H (2003). N-NH3 losses from nitrogen sources applied over unburned sugarcane straw. Rev. Bras. Cienc. Solo 27(1):631-637.

Costa RRGF, Quirino GSF, Naves DCF, Santos CB, Rocha AFS (2015). Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize. Pesqui. Agropecu. Trop. 45(3):304-311.

Costa EM, Nóbrega RSA, Carvalho F, Trochmann A, Ferreira LDVM, Moreira FMDS (2013). Plant growth promotion and genetic diversity of bacteria isolated from cowpea nodules. Pesqui. Agropecu. Bras. 48(9):1275-1284.

Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35(3):271-276.

Cruz J, Karam DC, Monteiro MAR, Magalhães PC (2008). A cultura do milho. Sete Lagoas: Embrapa Milho e Sorgo.

Dartora J, Guimarães VF, Marini D, Sander G (2013). Nitrogen fertilization associated to inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* in the maize Rev. Bras. Eng. Agríc. Ambien. 17(10):1023-1029.

Döbereiner J, Baldani VLD, Baldani JI (1995). Como isolar e identificar bactérias diazotróficas de plantas não leguminosas. Brasilia: Embrapa.

Ferreira AS, Pires RR, Rabelo PG, Oliveira RC, Luz JMQ, Brito CH (2013). Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. Appl. Soil Ecol. 72:103-108.

Garcia G, Cardoso AA, Santos OAM (2013). From shortage to stress on earth: a century of changes in the nitrogen cycle. Quim. Nova 36(9):1468-1476.

Holguin G, Bashan Y (1996). Nirogen-fixation by *Azospirillum brasilense* cd is promoter when co-cultured with a mangrave rhizosphere bacterium (*Staphylococcus* sp). Soil Biol. Biochem. 28(12):16511660.

Hungria M, Nogueira MA, Araujo RS (2016). Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. Agric. Ecosyst. Environ. 221:125-131.

Inagaki AM, Guimarães VF, Lana MC, Klein J, Rodrigues ACPC, Rodrigues LOS, Rampim L (2015). Maize initial growth with the inoculation of plant growth-promoting bacteria (PGPB) under different soil acidity levels. Austr. J. Crop Sci. 9(4):271-280.

Kuss AV, Kuss VV, Lovato T, Flôres ML (2007). Nitrogen fixation and in vitro production of indolacetic acid by endophytic diazotrophic bacteria. Pesqui. Agropecu. Bras. 42(10):14591465.

Marini D, Guimarães VF, Dartora J, Lana MC, Pinto Jr AS (2015). Growth and yield of corn hybrids in response to association with *Azospirillum brasilense* and nitrogen fertilization. Rev. Ceres 62(1):117-123.

Moda LR, Santos CLR, Flores RA, Borges BMMN, Andrioli I, Prado RM (2014). Response of corn grown in tillage system for the application of nitrogen doses in a sequence of plant cover. Biosci. J. 30:178-187.

Morais TP, Brito CH, Ferreira ADS, Luz JMQ (2015). Morphophysiological aspects of maize plants and soil biochemistry due to nitrogen fertilization and maize seed inoculation with *Azospirillum brasilense*. Rev. Ceres 62(6):589-596.

Novakowiski JH, Sandini IE, Falbo MK, Moraes A, Novakowiski JH, Cheng NC (2011). Residual effect of nitrogen fertilization and Azospirillum brasilense inoculation in the maize culture. Semina: Ciênc. Agrár. 32:1687-1698.

Okon Y, Labandera-Gonzalez CA (1994). Agronomic applications of azospirillum: An evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem. 26(12):1591-1601.

Okon Y, Vanderleyden J (1997). Root-associated *Azospirillum* species can stimulate plants. Appl. Environ. Micro. 63:366-370.

Pedrinho EAN, Galdiano Jr RF, Campanharo JC, Alves LMC, Lemos EGDM (2010). Identification and evaluation of bacteria isolated from roots of maize. Bragantia 69(4):905-911.

Perrig D, Boiero ML, Masciarelli AO, Penna C, Ruiz AO, Cassán FD, Luna MV (2007). Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. Appl. Microbiol. Biol. 75:1143-1150.

Piccinin GG, Braccini AL, Gomes L, Dan DM, Loli G, Hossa KR, Ponce RM (2013). Yield and agronomic performance of wheat in management with *Azospirillum brasilense*. Rev. Agrar. 6(22):393-401.

Quadros PD, Roesch LFW, Silva PRF, Vieira VM, Roehrs DD, Oliveira CFA (2014). Field agronomic performance of maize hybrids inoculated with *Azospirillum*. Rev. Ceres 61(2):209-218.

Radwan TESED, Mohamed ZK, Reis VM (2005). Aeration and salt effects on indol acetic production by diazotrophic bacteria. Pesqui. Agropecu. Bras. 40(10):997-1004.

Radwan TESED, Mohamed ZK, Reis VM (2004). Effect of inoculation with *Azospirillum* and *Herbaspirillum* on production of indolic compounds and growth of wheat and rice seedlings. Pesqui. Agropecu. Bras. 39(10):987-994.

Repke RA, Júlio S, Cruz S, Jorge C, Silva DA, Gonzales PJS (2013). *Azospirillum brasilense* efficiency in combination with doses of nitrogen in the development of maize. Rev. Braz. Milho Sorgo 12(3):214-226.

Rodrigues LFOS, Guimarães VF, Silva MB, Pinto Jr AS, Klein J, Costa ACPR (2014). Agronomic characteristics of wheat due to *Azospirillum brasilense*, humic acids and nitrogen in greenhouse. Rev. Bras. Eng. Agríc. Ambien. 18(1):31-37.

Roesch LFW, Olivares FL, Pereira PLM, Selbach PA, Sá ELS, Camargo FAO (2006). Characterization of diazotrophic bacteria associated with maize: effect of plant genotype, ontogeny and nitrogen-supply. World J. Microbiol. Biotechnol. 22:967-974.

Salomone IG, Dobereiner J (1996). Maize genotype effects on the response to *Azospirillum inoculation*. Biol. Fertil. Soils 21:193-196.

Santi C, Bogusz D, Franche C (2013). Biological nitrogen fixation in non-legume plants. Ann. Bot. 111:743-767.

USDA (2016). World agricultural supply and demand estimates. Washington D. C.: United States Departmente of Agriculture.

Zuffo AM, Rezende PM, Bruzi AT, Oliveira NT, Soares IO, Neto FG, Cardillo BES, Silva LO (2015). Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasilense* in the soybean crop. Rev. Cienc. Agrar. 38(1):87-93.