

Full Length Research Paper

***Azospirillum brasilense* promotes increment in corn production**

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Received 11 December, 2015; Accepted 19 April, 2016

The corn crop is one of the most cultivated in the world and one of the most need studies that seek alternatives on the use of *Azospirillum brasilense*. This bacterium produces growth hormones that may benefit the corn crop. The work objective was to verify the agronomical performance of corn crop, in function of foliar *Azospirillum brasilense* inoculation, associated with nitrogen doses. The research was performed in Selvíria, Mato Grosso do Sul State, Brazil, which is in the Brazilian Cerrado. The corn crop was cultivated during season (Spring/Summer) and late season (Summer/Autumn), under conventional tillage system. Experimental design of randomized blocks was used, with factorial scheme 4 × 2. The treatments were made in four nitrogen doses (0, 30, 60 and 90 Kg ha⁻¹) with and without the foliar applying (in stage V6) of the inoculant containing *A. brasilense*. The inoculant used had the strains AbV₅ and AbV₆ of *A. brasilense* (2×10⁸ viable cells mL⁻¹) and, the dose 200 mL ha⁻¹ was used. The nitrogen fertilization was made with ammonium sulfate, also on phenological stage V6. The following evaluations were made: Final plant population, foliar nitrogen content, foliar chlorophyll index, ear insertion height, plants height, stalk diameter, ears length, ears diameter, thousand grain weight and grain productivity. The foliar inoculation with the *A. brasilense* bacterium proved to be advantageous for the corn crop, and therefore, an option for the farmer. Because of the large volume of products used in seed (fungicide, insecticide, plant growth regulators) and the lack of information on the degree of interference of these products on the bacteria, it becomes pertinent the study of other inoculation forms on crops. Other studies that verify the influence of *A. brasilense* bacteria in foliar application on the physiology of the shoot and root of crops should be encouraged.

Key words: *Zea mays* L., inoculation, nitrogen, diazotrophic bacteria.

INTRODUCTION

The corn crop (*Zea mays* L.) has indisputable role in the world and Brazilian economy, due to its outstanding position among the agricultural species explored (Môro and Fritsche, 2015). Besides human feeding, corn is the main component in animal food, highlighting birds, cattle

and pigs. Therefore, much research should be conducted in order to increase productivity (Purwanto et al., 2015).

Plant growth-promoting bacteria, called associative, as they do not make symbiosis with the host plant and belong to the genus *Azospirillum*, can help through

Table 1. Soil analysis result on depth of 0.0 - 0.2 m, before the experiment in the area, Selvíria, MS, Brazil, 2011/2012 and 2012/2013.

P (resin)	O.M.	pH	K	Ca	Mg	H+Al	CEC	BS
mg dm ⁻³	g dm ⁻³	CaCl ₂mmol _c dm ⁻³					%
11	21	5.2	3.0	15.0	10.0	19.0	47.0	59

several mechanisms in the crops nitrogen nutrition (Bashan and Bashan, 2005). Among the mechanisms, the process of nitrogen biological fixation and the plant growth promotion stand out for the production of several vegetable hormones, which result in higher growth, water and nutrients absorption (Moreira et al., 2010) which may increase the productive potential of crops. According Bashan et al. (2004), the most common explanation for the effect of the bacteria in plants is the production of hormones that alter the metabolism and the morphology of the plant, improving the nutrient and water uptake.

Costa et al. (2015) studied inoculation with *Azospirillum brasilense* in seed and nitrogen doses in corn crop in the Cerrado region and reported that the use of bacteria in corn promoted greater plant height, stalk diameter, chlorophyll index, dry mass of stalk and root, ear length, thousand grain weight and yield. Similarly, Souza (2014) also in Cerrado region, noted that the use of bacteria in seed promoted greater plant height and ear insertion height, hundred grain weight and yield. Morais et al. (2016) researched several doses of inoculant containing *A. brasilense* applied in sowing furrow of corn and verified that the dose of 200 mL ha⁻¹ promoted increased in grain yield. One of the most pronounced effects of inoculation with *Azospirillum* on root morphology is represented by the proliferation of root hairs (Saikia et al., 2012) and, according Fulchieri et al. (1993) the promotion of root growth can lead to better exploration of the soil and enhance the growth and development of plants by the greater water and nutrients uptake.

Studies that promote knowledge about bacteria of genera *Azospirillum* must be stimulated (Moreira et al., 2010; Lana et al., 2012) aiming not only low cost and low environmental impact agriculture, but also the biotechnological potential that these bacteria present (Moreira et al., 2010). Identifying the managing conditions which may contribute to the maximization of processes made by them is a challenge for the present research (Moreira et al., 2010), as an example, the study about foliar applying of the inoculant containing the bacterium *A. brasilense* can be mentioned. According to Fukami et al. (2016) little is known about the effects of pesticides

used to treat seeds on *Azospirillum*. The authors report that alternative methods of inoculation in crops are needed and highlight the foliar application in the vegetative stage.

This way, the objective of the work was to evaluate the corn crop performance (season and late season) when submitted to foliar inoculation of *A. brasilense* and nitrogen doses applied in topdressing, in low altitude Cerrado region.

MATERIALS AND METHODS

The experiment was developed during the season in 2011/12 (Spring/Summer) and the late season in 2012/13 (Summer/Autumn) at the Teaching, Research and Extension Farm of Engineering College of Ilha Solteira - UNESP, located in Selvíria city, Mato Grosso do Sul State, Brazil, within the geographical coordinates of 22° 23' south latitude and 51° 27' west longitude and 335 m of altitude. Soil is classified according to Santos et al. (2013), in RED OXISOL typical dystrophic clayed. Before of experiment installation was collected soil in the layer from 0.0 to 0.2 m for the chemical characterization of all experimental area, with the same values for all experimental plots (Table 1).

The soil preparation was made with scarifier and leveling harrow. The conventional seeding system was used. The corn seeding on season period was made under soybean residues and on late season period under corn residues. The experimental area was cropped with soybeans on summer of 2010/2011 and remained fallow until the sowing of corn on season period (2011/12). After the cropped of corn on season period (2011/2012), the area remained fallow until the sowing of corn on late season period (2013). It was opted to sow the corn in the beginning of period that characterizes the late season (January) to match the period of much of the crop development with a part of the rainy period, providing better water condition. Agricultural activity in the Cerrado is concentrated in the rainy period, usually between the months of October to March, when it occurs between 80 and 90% of the annual precipitation (Assad et al., 1993), so there is a preference for sow crops in the period that provides better conditions for development.

The annual average rainfall in the region is 1,313 mm, maximum annual temperature is 31°C, minimum annual temperature is 19°C and average annual temperature is 25°C (Portugal et al., 2015) with climate type A_w, according to Köppen's classification. The daily rainfall values, maximum temperature and minimum temperature on the experiment leading period are on Figure 1.

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Abbreviation: CEC, Cation Exchange Capacity; BS, Base Saturation

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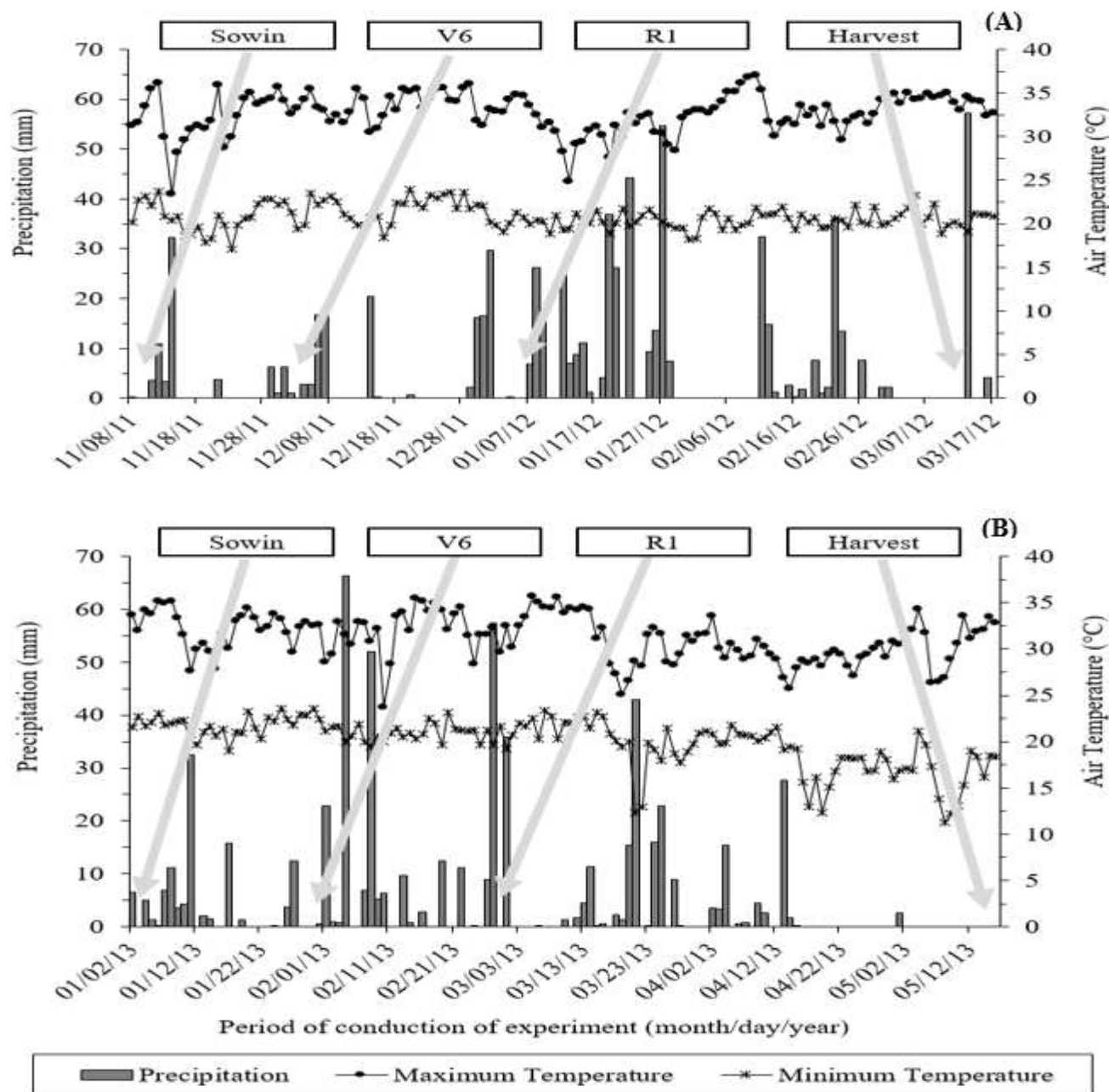


Figure 1. Daily values of rainfall, minimum and maximum temperature during the experiment leading period with season corn - 2011/12 (A) and late season corn - 2012/13 (B), in Selvíria - MS, Brazil.

The experimental design was of randomized blocks with 8 treatments, disposed in factorial scheme 4x2 with four replications. The treatments were composed by 4 nitrogen doses in ammonium sulfate form (0, 30, 60 and 90 Kg ha⁻¹) with and without the foliar application of the inoculant containing *A. brasilense*. The plots were composed by 5 lines with 5 m long, being considered as useful areas, the 2 central ones.

For the season corn the simple hybrid AG 8088 YG was used, of early cycle and hard orange seed; while for the late season corn, the simple hybrid DKB 390 VT PRO was used, of early cycle and semi hard yellow-orange seed (Cruz et al., 2013). The foliar inoculation was performed with Masterfix Gramineas® inoculant,

with the strains AbV₅ and AbV₆ of *A. brasilense* (2x10⁸ viable cells mL⁻¹) with dose of 200 mL ha⁻¹ diluted with water only. The sowing was made on 11/9th/2011 (season corn) and 01/2nd/2013 (late season corn) with row spacing of 0.90 m and the initial population was established in 50,000 plants ha⁻¹ for the corn in both cultivation seasons. According to Fornasieri-Filho (2007) the ideal population for the corn crop varies from 30,000 to 90,000 plants ha⁻¹. The mineral fertilization in the sowing furrow was defined based on the chemical soil analysis and expected productivity of 4,000 to 6,000 kg ha⁻¹, according to the recommendations of Raji and Cantarella (1997) using 250 Kg ha⁻¹ of 08-28-16 (season) and 400 kg ha⁻¹ of formulated 04-30-10 (late season).

The foliar inoculation with the bacterium, as well as the topdressing fertilization, were made when the plants were on developing stage V6 (six expanded leaves), according to the phenological scale proposed by Ritchie et al. (2003), at that phase the crop was with 17 DAE (days after the emergency) and 22 DAE on season and late season, respectively. The topdressing fertilization and the inoculant applying were made in the late afternoon, aiming mild temperature conditions, especially to favor the inoculation with the bacteria. For the inoculant applying, costal spray with cone jet tip and 180L ha⁻¹ output was used.

Aiming to keep the crop free from competition with weeds, atrazine and tembotrione were used in doses of 1,000 and 100 g ha⁻¹ of a.i., respectively, in tank mixture form. The adjuvant soy methylated ester was added to the application mixture (500 g ha⁻¹ of a.i.). The application was made through the use of tractor powered spray with flat jet tips and adjusted to apply 200 L ha⁻¹ of the mixture.

During the research, the following evaluations were performed: (a) final plant population, made through counting the plants in the useful area at the harvest moment and later extrapolated for hectare. This evaluation has an important role on the yield of corn crop, since small variations have great influence on the final yield (Cruz et al., 2015); (b) foliar nitrogen content, gotten through collect of the mid third of five opposite leaves and under the main corn ear on stage R1 (flowering – at 52 DAE and 48 DAE of season and late season corn, respectively), according to the phenological scale proposed by Ritchie et al. (2003), after that the drying of leaves was made and the nitrogen content was determined in laboratory. The analysis of plant tissue as a diagnostic criterion is based on the premise there is a relationship between growth and crops production and the nutrients content in their tissues (Coelho, 2008) among them, the nitrogen; (c) foliar chlorophyll index, made at the same moment and with the same leaf collect to obtain the foliar nitrogen content, using portable chlorophyll meter (model CFL 1030), which through sensors, analyzes three light frequency lines and through absorption relation of different frequencies, provides measurement of chlorophyll a, b, and total (a+b) levels, expressed in dimensioned units called FCI (Foliar Chlorophyll Index). The foliar chlorophyll index is a good parameter to indicate the nitrogen level in cereals (Argenta et al., 2001), because 50 to 70% of total nitrogen of the leaves is integral of enzymes that are associated to the chloroplasts (Lima et al., 2009); (d) plants height, gotten by measurement of five random plants per plot, using a graduated ruler, from the ground to the flag leaf, on R6 stage (physiological maturity), according to the phenological scale proposed by Ritchie et al. (2003); (e) ear insertion height, measured in the plants and at the same moment of plant height, however, the measurement was made from the ground to the ear insertion on the stalk. Higher plant height and ear insertion height on the stalk can contribute to the increase of crop lodging (Brachtvogel et al., 2012); (f) stalk diameter, measured at the second internode from the plants base, with digital caliper rule CD-6 CSX-B (Mitutoyo Sul Americana®), five random plants were considered. The stalk diameter is one of the characteristics that has been more related to the percentage of lodging and breakage of plants in corn crop (Kappes et al., 2013); (g) ear length, made after harvest, considering ten random ears without straw in each plot, which were measured from the base to the apex with graduated ruler; (h) ear diameter, was obtained by measuring the central third of the same ears used to measure the length, with the digital caliper ruler. The length and diameter of corn ears directly influence the yield of crop grain (Kappes et al., 2009); (i) thousand grain weight, obtained by counting a subsample of 250 grains per plot, which was submitted to weighting and humidity determination, making it possible to estimate the grains weight corrected to 13% of humidity (wet basis - w.b.). The grain mass depends entirely of the factors that control the supply of assimilates for grain filling (Fageria, 1989) and correlates positively with crop yield (Duarte et al., 2007); (j) grain productivity, it was obtained from

the threshing and weighting of grains from the ears collected from the two central lines in each plot, the values were extrapolated to kg ha⁻¹ and corrected to 13% humidity (w.b.). This is the most important variable to check if the crop was responsive to the different treatments in which it was subjected.

The corn ears harvest on the area was performed on 03/15/2012, at 120 DAE of corn on season and on 16/05/2013, at 127 DAE on late season.

The results were submitted to the F test of analysis of variance, comparing the treatments average with *A. brasilense* by the Tukey test at 5% de probability. The N doses average in topdressing were submitted to the regression analysis, adjusting meaningful equation models through the F test.

RESULTS AND DISCUSSION

During the corn crop cycle cropped in the season period (Figure 1A), the accumulated rainfall registered was 682 mm and on corn cycle cropped in late season (Figure 1B), the accumulated rainfall was 603 mm. In both cultivation seasons the precipitation was adequate to the corn crop because, according to Fancelli (2015) this crop requires 400 to 600 mm of rainfall during the cycle. It is noticed that in the period of late season occurred less rainfall and irregular distribution at the end of the reproductive phase.

The F test was significant at 5% to the inoculation in the final population variable only in the season period (Table 2). The foliar inoculation with *A. brasilense* provided higher final plant population. After 11 days of the inoculant applying, there was a 14-day dry period, what may have not favored the non-inoculated plants (Figure 1). At the initial development phase, the corn plants have fast absorption of nutrients and water; therefore, long periods without water may impair the development. It can be explained by the hormones production by the bacteria, which promoted the growth of the root system and, consequently the soil volume to be explored in search of water and/or nutrients (Okon and Labandera-Gonzalez, 1994), providing higher survival rate, while the non-inoculated plants did not have this competitive advantage. According Fornasieri-Filho (2007) in corn crops with excess of plants regarding the water supply capacity occur death of plants. Therefore, the dry periods are the main cause of mortality of plants in Cerrado region. What did not occur with the late season corn, because there was proper rainfall distribution in the vegetative and reproductive phases. For both corn crop periods, the F test showed that there was no influence of nitrogen doses (Table 2).

As to the foliar nitrogen content, it is noticed that on season corn, the F test was significant at 1% for inoculation and nitrogen doses, and the late season corn was significant at 5% only for nitrogen doses (Table 2). The inoculation had positive influence, resulting in higher foliar N content. However, both treatments obtained values within the suitable range, which, according to Cantarella and Furlani (1997) is between 27 and 35 g Kg⁻¹. In relation to the N doses, it's observed that in both crops,

Table 2. Average values of final plant population, nitrogen content and foliar chlorophyll index of season and late season corn according to the *A. brasilense* foliar inoculation and nitrogen applying in topdressing (Selvíria, MS, Brazil, 2011/12 and 2012/13).

Treatments	Final Population		Foliar Nitrogen Content		Foliar Chlorophyll Index		
	n° plants ha ⁻¹		g kg ⁻¹				
	Season	Late season	Season	Late season	Season	Late season	
Inoculation							
Presence	43,595 ^a	48,750	34.35 ^a	28.06	70.93	55.94	
Absence	40,972 ^b	46,736	33.46 ^b	27.49	71.75	58.43	
M.S.D	2,450	2,370	0.67	1.09	3.29	2.54	
N Dose (kg ha⁻¹)							
0	43,518	49,027	32.20 ⁽¹⁾	26.45 ⁽²⁾	67.59 ⁽³⁾	54.09 ⁽⁴⁾	
30	41,666	47,083	34.65	27.64	71.90	56.86	
60	41,512	47,777	33.65	28.30	74.20	58.61	
90	42,438	47,083	35.13	28.70	71.66	59.19	
	I	4.90 [*]	3.11	7.50 ^{**}	1.21	0.26	4,15
F Test	D	0.60	0.64	15.95 ^{**}	3.54 [*]	2.91 [*]	3,49 [*]
	I x D	2.60	0.56	1.55	2.89	0.86	0,29
General average		42,283	47,743	33.91	27.77	71.34	57.19
V.C. (%)		7.93	6.76	2.70	5.34	6.39	6.05

** and * - meaningful to 1 and 5% of probability, respectively; averages followed by distinct letter in the columns, differ by Tukey test at 5% of probability; V.C. - variation coefficient; M.S.D. - Minimum significant difference. ⁽¹⁾y = 32.74+0.026x (R²= 0.60**); ⁽²⁾ y = 26.66+0.025x (R²= 0.94**); ⁽³⁾y = 67.44+0.22x-0.0019x² (R²= 0.98*); ⁽⁴⁾y = 54.63+0.0567x (R²= 0.92**).

the foliar N contents had a linear positive response, the same was reported by Farinelli and Lemos (2012), Mota et al. (2015), Lange et al. (2006), Moda et al. (2014) and Valderrama et al. (2014).

For the foliar chlorophyll index the F test was significant at 5% for nitrogen doses in both crops (Table 2). On season, it is noticed that the foliar chlorophyll index adjusted to the quadratic equation, which had higher values with the approximate dose 58 kg ha⁻¹ of N. Maestro et al. (2014), working with N doses in corn at the same region of the present work, it was observed a higher foliar chlorophyll index with doses of 85 and 96 kg ha⁻¹, in the years 2009 and 2010, respectively. Torres et al. (2015) observed higher values for the chlorophyll index with the dose of 142 kg ha⁻¹, the authors report that above these doses, there was chlorophyll content reduction due to the fact that they stopped responding to the increase of N offer.

The foliar chlorophyll index is used to predict the nitrogen content in the leaves, however, making comparison it's observed that the chlorophyll index did not reflect on the foliar N content on season. According to Blackmer and Schepers (1995) the luxury consumption of nitrogen by the plant, in nitrate form is not detected by the chlorophyll meter. It happens because the N does not associate to the chlorophyll molecule (Sangoi et al., 2015). On late season, the foliar chlorophyll index adjusted to the positive linear equation with the nitrogen dose increase, reliably reflecting the foliar nitrogen level, the same was reported by Costa et al. (2012) and Mota

et al. (2015).

In relation to plant height, the F test was significant at 1% for inoculation only on late season corn (Table 3). The plants height was benefited by the foliar inoculation with inoculant containing *A. brasilense*, representing increase a little above of 7 cm under inoculation treatment. The growth hormones production characteristic, given by the bacteria, may be the reason for such superiority in plants height. According to Bashan and Bashan (2010) and Vacheron et al. (2013) these hormones change the plants metabolism and morphology, leading to better mineral and water absorption, consequently higher plants. Kappes et al. (2013), researching inoculation, applying nitrogen foliar and in topdressing in Cerrado region, obtained increase of 12.6 cm in corn plant height. Puentes et al. (2009), working with seeds inoculation with *A. brasilense* (without inoculation, 12 mL kg⁻¹ of seeds and 0.41 mL kg⁻¹ of seeds in three study locations) in Argentina, observed that at the second location, the plant height with treatment 0.41 mL kg⁻¹ of seeds was superior to the ones without inoculation.

It is observed that for the ear insertion height the F test was significant at 5% for the interaction between inoculation and N doses on season corn (Table 3). According to the Table 4 values, it is noticed that when the nitrogen in topdressing was not applied (0 kg ha⁻¹), the presence of inoculation with *A. brasilense* provided superior ear insertion height. The same thing did not happen when N in topdressing was made. This way, it is possible to infer that the inoculation with the bacteria *A.*

Table 3. Average values of plants height, ear insertion height and stalk diameter of season and late season corn according to the *A. brasilense* foliar inoculation and nitrogen applying in topdressing (Selvíria, MS, Brazil, 2011/2012 and 2012/2013).

Treatments	Plants height		Ear Insertion height		Stalk diameter		
	cm		cm		mm		
	Season	Late season	Season	Late season	Season	Late season	
Inoculation							
Presence	187.26	199.18 ^a	104.34	115.40 ^a	25.25	19.52	
Absence	189.41	192.11 ^b	105.22	112.40 ^b	24.52	19.29	
M.S.D	4.04	3.81	3.13	2.95	0.98	0.48	
N Dose (kg ha⁻¹)							
0	188.42	196.25	102.47	112.48	24.26	19.29	
30	189.72	198.25	106.10	115.93	24.48	19.49	
60	189.80	192.30	105.97	110.43	24.98	19.28	
90	185.40	195.78	104.57	116.78	25.82	19.57	
F Test	I	1.21	14.89 ^{**}	0.34	4.46 [*]	2.33	1.00
	D	1.10	1.827	1.23	4.37	2.11	0.39
	I x D	0.57	0.049	3.69 [*]	0.67	1.44	0.74
General Average	188.34	195.64	104.78	113.90	24.89	19.41	
V.C. (%)	2.94	2.65	4.10	3.53	5.43	3.36	

^{**} and ^{*} - meaningful at 1 and 5% of probability, respectively; Averages followed by distinct letter in the columns differ by Turkey test at 5% of probability; DMS - Minimum significant difference; V.C. - variation coefficient.

Table 4. Ear insertion height (cm) on season corn plants, according to *A. brasilense* foliar applying and nitrogen doses in topdressing (Selvíria, MS, Brazil, 2011/2012).

Inoculation	N doses (kg ha ⁻¹)				Regression equation	R ²
	0	30	60	90		
Presence	106.1 ^a	105.5 ^a	104.0 ^a	101.6 ^a	Not meaningful	-
Absence	98.8 ^b	106.6 ^a	107.9 ^a	107.5 ^a	$y = 99.04 + 0.298x - 0.002x^2$	0.98 [*]

^{*}, significant at a 5% of probability by F test. Averages followed by distinct letter in the columns differ by Turkey test at 5% of probability.

brasilense supplied, sufficiently, the N demanded by the corn plants. According to Vacheron et al. (2013) plant growth promoting bacteria can fix and provide nitrogen to the plant, promoting growth. From 30 kg ha⁻¹ of N may be available in the soil amount of nitrogen in the ammonium form sufficient to promote reduction of the bacteria activity, mainly, may have coincided with the V9 stage of development (elongation stage of stalk). According to Rudnick et al. (1997) and Hartmann (1988) the addition of nitrogen to the soil, especially in ammoniacal form decreases the activity of *A. brasilense* bacterium. Therefore, the presence of ammoniacal nitrogen in the soil at this stage of the plant, could have influenced the bacteria and their activity, interfering in the ear insertion height. According to Ritchie et al. (2003) in the V9 stage the plant stalk is in rapid elongation and one female inflorescence will develop from each of the nodes above the soil surface, except for the last six to eight nodes below tassel. The authors explain that the growth of female inflorescences of lower insertions in the stalk,

occasionally stays slower and only one or two female inflorescences in upper position in the plant will develop into productive ears.

Still on Table 4, it is noticed that in inoculation absence, the insertion height adjusted to a quadratic equation with N doses increase in topdressing, which presented maximum point with estimated dose of 76 kg ha⁻¹ of N.

On late season, the F test was significant at 5% for the ear insertion height (Table 3). This variable was positively influenced by the inoculation with *A. brasilense*, in a similar way to what happened to plant height, also on late season period. Therefore, we have the same explanation. It is noteworthy that higher plant height and ear insertion height can bring some implications. According to Sousa and Ferreira (2015), Carvalho et al. (2015) and Cabral et al. (2016) corn plants and ear insertion height higher have a greater tendency to tip over and stalk breakage, especially in regions of strong winds. However, in this study it was not observed such implication.

The corn stalk diameter did not respond to any of the

Table 5. Average values of ear length and ear diameter of season and late season corn according to the *A. brasilense* foliar inoculation and nitrogen applying in topdressing (Selvíria, MS, Brazil, 2011/12 and 2012/13).

Treatments	Ear length		Ear diameter	
	mm		mm	
	Season	Late season	Season	Late season
Inoculation				
Presence	178.77	151.57 b	49.50	47.70
Absence	177.49	162.06 a	49.97	47.48
M.S.D	1.70	1.67	0.60	1.09
N Dose (kg ha⁻¹)				
0	178.00	153.45	49.44	48.01
30	176.12	157.75	49.25	47.81
60	179.87	157.69	49.97	46.84
90	178.53	158.37	50.29	47.70
F Test	I	2.46	10.72**	2.72
	D	3.62*	0.50	2.71
	I x D	4.55**	0.11	1.48
General Average	178.13	156.80	49.74	47.59
V.C. (%)	1.30	5.78	1.65	3.12

** and *, significant at 1 and 5% of probability, respectively; averages followed by distinct letter in the columns differ by Turkey test at 5% of probability; M.S.D. - Minimum significant difference; V.C., variation coefficient. ⁽¹⁾ $y = 32.74 + 0.026x$ ($R^2 = 0.60^{**}$); ⁽²⁾ $y = 26.66 + 0.025x$ ($R^2 = 0.94^{**}$); ⁽³⁾ $y = 67.44 + 0.22x - 0.0019x^2$ ($R^2 = 0.98^*$); ⁽⁴⁾ $y = 54.63 + 0.0567x$ ($R^2 = 0.92^{**}$).

Table 6. Ear Length (mm) on season corn according to the foliar applying of *A. brasilense* and nitrogen doses in topdressing (Selvíria, MS, Brazil, 2011/12).

Inoculation	N doses (kg ha ⁻¹)				Regression Equation	R ²
	0	30	60	90		
Presence	177.62	178.12 ^a	182.10 ^a	177.25	$y = 177.01 + 0.14x - 0.001x^2$	0.50*
Absence	178.37	174.12 ^b	177.65 ^b	179.82	$y = 177.92 - 0.13x + 0.002x^2$	0.76**

** and *, significant at 1 and 5% of probability, respectively. Averages followed by distinct letter in the columns differ by Turkey test at 5% of probability.

treatments (Table 3). The same way, Fernandes et al. (2005), Meira et al. (2009), Goes et al. (2014) and Gazola et al. (2014) did not observe stalk diameter response with the increase in N doses. Dotto et al. (2010), Kappes et al. (2013), Souza (2014) and Marini et al. (2015) working with diazotrophic bacteria inoculation, did not observe influence on the corn stalk diameter.

According to the significance of 1% of the F test, it is verified that there was interaction between inoculation and N doses for ear length evaluation on season corn (Table 5).

According to the deployment shown on Table 6, it is noticed that only in doses of 30 and 60 kg ha⁻¹ of N the inoculation presence provided superior ear length. Probably, until the dose of 60 kg ha⁻¹ of N there was significant reduction of ammoniacal nitrogen in the soil in the course of time, allowing the bacteria resume their activity on the main moment in which the plant determines the ear length (V12). According to Rudnick et al. (1997) and Hartmann (1988) the addition of nitrogen

to the soil, especially in the ammoniacal form decreases the activity of *A. brasilense* bacteria. In the course of time, after nitrogen fertilization in topdressing (V6), the ammoniacal nitrogen passes to the nitrate form by the action of Nitrosomonas and Nitrobacter bacteria. Cantarella (2007) states that in soils with aerobic conditions and high temperatures, the ammoniacal nitrogen are oxidized to nitrate form in approximately 15 to 30 days. This process decreases the amount of ammoniacal nitrogen in the soil that would be a limiting factor for the bacterium. Possibly, at this stage there was a reduction of the amount of ammoniacal nitrogen and allowed the *Azospirillum* bacteria resume their activity, resulting in increased ear length.

At the inoculation presence, the ear length adjusted to a quadratic model with N doses increase, showing longer length with the dose of 70 kg ha⁻¹, but at the inoculation absence, there was the opposite, in other words, the ear length adjusted to a quadratic model, however, showing minimum point with the dose of 33 kg ha⁻¹. Dotto et al.

Table 7. Average values of thousand grain weight and grain productivity of season and late season corn according to the *A. brasilense* foliar inoculation and nitrogen applying in topdressing (Selvíria, MS, Brazil, 2011/12 and 2012/13).

Treatments	Thousand grain weight		Grain productivity			
	g		kg ha ⁻¹			
	Season	Late season	Season	Late season	Average	
Inoculation						
Presence	314.00	319.34	6,751 ^a	6,098 ^a	6,425 ^a	
Absence	314.62	311.54	5,883 ^b	5,405 ^b	5,644 ^b	
M.S.D	8.53	4.50	573.91	420.17	332.20	
N doses (kg ha⁻¹)						
0	306.19	316.65	6,273	5,847	6,060	
30	312.95	311.59	6,353	5,917	6,135	
60	319.44	312.02	6,172	5,558	5,865	
90	318.65	321.51	6,470	5,685	6,078	
F Test	I	0.02	13.00**	8.07**	11.77**	23.89**
	D	2.20	4.63**	0.17	0.64	0.54
	I x D	0.26	8.26**	0.70	0.31	1.25
General average	314.31	315.44	6.317	5,752	6,034	
V.C. (%)	3.72	1.94	13.69	9.94	7.49	

** and *, significant at 1 and 5% of probability, respectively. Averages followed by distinct letter in the columns differ by Turkey test at 5% of probability; M.S.D. – Minimum significant difference; VC, variation coefficient.

(2010) report that a higher contribution of inoculation associated to nitrogen fertilization is generally noticed. Nevertheless, there are many papers performed in Brazil in regions with similar climate to the present study (Kappes et al., 2013, Cunha et al., 2014) and regions with different climate (Cavallet et al., 2000; Repke et al., 2013; Marini et al., 2015) showing absence of significant results for the interaction between seed inoculation with *A. brasilense* and N doses for the ear length. According to Basi (2013) it is essential that field experiments be performed to evaluate the effects of inoculation with *A. brasilense* in order to obtain more results of this technology in corn crop in different conditions of year, climate and soil. This information reinforces the importance of the study of other forms of inoculation, since researches with foliar inoculation in different crops are scarce. Morais et al. (2016) reported that the use seed inoculation becomes impractical in the field, because the seeds generally marketed have already been treated with phytosanitary products, and the need to treat seeds again with bacteria is not attractive to farmers.

On late season, the F test showed significance of 1% for the inoculation on the ear length (Table 5). The inoculation presence provided decrease of 6.5% on ear length, in relation to the inoculation absent treatment. Differently, Cavallet et al. (2000) and Kappes et al. (2013), obtained increase of 6 and 3.7%, respectively, on the ear length.

The ear diameter was not influenced by the inoculation and the N doses, as seen by the F test that was not

significant (Table 5). Similarly, Cunha et al. (2014) and Marini et al. (2015), using inoculation and Kappes et al. (2013), using inoculation and N doses applying in topdressing, did not find differences from the treatments about the ear diameter of corn.

The thousand grain weight on season was not influenced by the treatments, as verified by non-significant F test. But on late season period, the F test was significant at 1% for the interaction between inoculation and N doses (Table 7). According to the deployment (Table 8), it is noticed that on the doses of 0 and 30 kg ha⁻¹ of N the inoculation presence provided increase of 7% and 4% on the thousand grain weight, respectively. Biari et al. (2008) verified increase of 30% on the hundred grain weight, from the inoculation treatment with *A. brasilense* associated to the applying of 50 kg ha⁻¹ N in topdressing in comparison to the control treatment.

When the inoculation was performed (Table 8), the thousand grain weight adjusted to a quadratic model, presenting minimum point with the dose of 57 kg ha⁻¹ of N. At inoculation absence, the thousand grain weight adjusted to a positive linear model with increase of N doses.

Costa et al. (2015) verified that the thousand grain weight responded in a positive linear way with N doses increase, at the inoculant presence on the seed, foliar and also on the inoculant absence. Rodrigues et al. (2014) observed that the hundred grain weight of corn responded in a negative linear way, with the N doses increase, when the seeds were inoculated with the strain

Table 8. Thousand grain weight (g) on late season corn, according to the foliar applying of *A. brasilense* and nitrogen doses in topdressing (Selvíria, MS, Brazil, 2012/2013).

Inoculation	N doses (kg ha ⁻¹)				Regression Equation	R ²
	0	30	60	90		
Presence	327.6 ^a	318.0 ^a	313.8 ^a	318.0 ^a	$y = 327.76 - 0.457x + 0.004x^2$	0.99*
Absence	305.7 ^b	305.2 ^b	310.3 ^a	325.0 ^a	$y = 302.08 + 0.2104x$	0.77**

** and *, significant at 1 and 5% of probability, respectively. Averages followed by distinct letter in the columns differ by Turkey test at 5% of probability.

AbV₅ of Azospirillum.

In relation to the corn productivity, the F test was significant at 1% for inoculation in both crops and in the average (Table 7). The presence of foliar inoculation with *A. brasilense* provided higher values, whereas the average of the two periods of cultivation, there was increase of 14% in productivity. Several authors reported increase in corn grain productivity with the use of inoculation in seeds with *A. brasilense*. Cavallet et al. (2000) reported increase of 17% in productivity, Hungria et al. (2010) 27%, Bartchechen et al. (2010) 15%, Lana et al. (2012) 11%, Braccini et al. (2012) 26%, Kappes et al. (2013) 9%, Souza (2014) 6% and Mazzuchelli et al. (2014) reported increase of 22% in productivity. Works that evaluate foliar inoculation with *A. brasilense* in corn crops are scarce. Costa et al. (2015), working with inoculation in the seed, foliar (stage V4) and control, associated to foliar applying of N doses (0, 25, 50, 75 and 100%, with the dose of 100% corresponding to 50 kg ha⁻¹ of N), verified that there was increase of 36% in corn grains productivity when the seed inoculation was made in relation to the control. The authors also verified increase of 22% in productivity with foliar applying without foliar N applying. According to Okon and Labandera-Gonzalez (1994) the productivity increase in response to the inoculation is usually about 5 to 30%.

In general, it is noticed that the crops were little responsive to the doses of N. This can be attributed to the fact that corn was sown under soybean residues, which showed average yield of 3,600 kg ha⁻¹. According to Duarte et al. (2013) the nitrogen present in soybean residues can be used by the late season corn. The authors estimate that for the corn grown in succession, are availed about 15 kg of N for each ton of soybean, or 54 kg ha⁻¹ of N when produces 3.6 ton ha⁻¹ of soybean.

When observing the previous assessments, it is noticed that for corn grown on season, what probably influenced the higher productivity in the presence of inoculation was the final plant population, expressing the higher potential to overcome the productivity of the treatment without inoculation. According to Von Pinho et al. (2008), there is linear relation between grain productivity and plants density. The authors found that for each increase of 1,000 plants ha⁻¹ in plant population, there was increase in grains productivity.

For the late season corn, the increase on plant height

and the thousand weight grains with the treatment in inoculation presence, influenced to culminate in higher productivity. Higher plants tend to be more productive because suffer less stress during the development and accumulate greater amounts of reservation in the stalk (Silva et al., 2006). According to Braccini et al. (2012) the corn inoculation with *A. brasilense* promotes increase in plant height and in corn grains productivity, when compared to the control group.

Conclusions

The foliar inoculation with the *A. brasilense* bacterium proved to be advantageous for the corn crop, and therefore, an option for the farmer. Because of the large volume of products used in seed (fungicide, insecticide, plant growth regulators) and the lack of information on the degree of interference of these products on the bacteria, it becomes pertinent the study of other inoculation forms on crops. Other studies that verify the influence of *A. brasilense* bacteria in foliar application on the physiology of the shoot and root of crops should be encouraged.

Conflict of Interests

The authors have not declared any conflict of interests.

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