

Methods of application and doses of inoculation with *Azospirillum* in agronomic performance of off-season corn crop

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Abstract

In order to achieve high yields for off-season corn crop sown after soybean harvest, high doses of nitrogen fertilizers are required. Biological nitrogen fixation helps reduce the use of nitrogen fertilizers through the inoculation of nitrogen-fixing associative bacteria, such as those of the genus *Azospirillum*. This study aimed to evaluate the agronomic performance of corn hybrid DKB390Y in response to different application methods, such as using inoculant and doses of inoculant with *Azospirillum brasilense*. The experiment was conducted in a property adjacent to Federal University of Mato Grosso (Sinop campus), between February and July 2018. The experimental design included randomized blocks in a 3×5 factorial arrangement, replicated four times (with three application methods: foliar spraying; spraying foliar with bovine gelatin; and with a paint roller (Black & Decker Rapid Roller BDPR400-wool®) and five doses of inoculant: 0, 100, 200, 400 and 800 mL ha⁻¹). No significant differences were obtained through the application methods in the rates of chlorophyll, plant height, stem diameter, number of rows of grains, number of grains per row, ear length, ear diameter and weight of one thousand grains, except for grain yield. Grain yield was found to be superior with foliar inoculation at 100 mL ha⁻¹ method foliar spray, and 200 mL ha⁻¹ at method foliar application with bovine gelatin.

Keywords: *Zea mays* L. Biostimulant. Chlorophyll. Foliar application. Roller application.

Introduction

Corn (*Zea mays* L.) is one of the the most cultivated cereals in Brazil, and has great economic importance due to its role as a raw material for industries, animal feed and human food (PEDRINHO et al., 2010). According to CONAB (2019), the overall production of the second corn crop of 2018/2019 in Mato Grosso, Brazil, reached 31,045,000 tons, with an average productivity of 6,376 kg ha⁻¹, which means an increase of 18.5% in production and 8.8% in productivity in relation to the previous

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harvest in the state, that was favored by the regular rainy season, which allowed a bigger window in the sowing and a full development of the plant.

There are several factors that can decrease the productivity of a crop, among which the following stand out: pests and diseases, inefficient soil management, low fertility and meteorological problems (water stress, high temperatures etc.). For corn, the limiting factor in obtaining high yields is nitrogen (N) deficiency (FIORINI et al., 2019). This nutrient can be made available to plants in different manners, for example, via chemical fertilizer, organic matter from the soil, or even an organic fertilizer of animal or vegetable origin. The nitrogen can also be made available through biological nitrogen fixation (BNF), an option that has been introduced only recently, but has always been present in nature (PACENTCHUK et al., 2012).

Brazil is a pioneer in inoculation of seeds with microorganisms associated with the BNF process. Proof of this is the massive use of *Rhizobium spp.* inoculants in soybean cultivation; these bacteria are widely recommended due to their efficiency in supplying the high nitrogen requirement of soybean. Estimates point to BNF contributions of more than 300 kg of N per hectare, in addition to the release of 20–30 kg N per hectare for the next crop (HUNGRIA et al., 2010).

According to Hungria (2011), the nitrogen fixed for bacterial varies from 30 to 50 kg per hectare year¹. As stated by Campos et al. (2001), the annual gain in soybean productivity due to inoculation varies from 80.0 to 291.0 kg ha⁻¹, corresponding to an increment from 4.0 to 12.5%. In addition, according to the authors, a problem that was being faced due to the use of mineral N was the low content of proteins translocated to grain, which was often insufficient to satisfy the requirements of industries. Thus, the use of BNF played a key role in solving this, as nitrogen from biological fixation is more easily translocated to grain than mineral N (HUNGRIA et al., 2010).

Therefore, interest arose in obtaining the benefits provided by diazotrophic bacteria for the cultivation of non-legumes, as well. The Brazilian agriculture sector has taken many strides in this direction and today it is possible to identify commercial *Azospirillum spp.* inoculants that are recommended for the most diverse cereal species belonging to the *Poaceae* family. In fact, such inoculants are being commonly used for the cultivation of corn (CADORE et al., 2016).

Azospirillum is a genus of free-living bacteria in soil that has a good capacity for BNF associated with plants but without the complexity of nodule formation. It is believed that *Azospirillum* population varies for each hybrid, depending on the different qualitative and quantitative characteristics of the root exudates (CADORE et al., 2016). These microorganisms are considered promoters of plant growth, because not only do they have the capacity for BNF (HUERGO et al., 2008), but they also produce hormones, such as auxins, gibberellins and cytokinins, as well as solubilize phosphates; further, they serve as agents in the biological control of pathogens (CORREA et al., 2008).

The most commonly used method of inoculation is through seed treatment; however, studies conducted in other cultures show that there is a positive effect when the inoculant is applied in the sowing furrow (MÜLLER, et al. 2013), or through foliar application (CICILIATO et al. 2015). An innovative alternative found was the use of a paint roller (Black & Decker Rapid Roller BDPR400-wool) to apply the inoculant between the corn plant lines, in order to achieve a better spread and a greater uniformity of application. Pereira et al. (2018) obtained positive results using this method, while experimenting with herbicides for the control of weeds.

Following the discussion above, it is necessary to deepen our understanding of the topic by evaluating which inoculation method is more efficient. Therefore, this study aimed to evaluate the

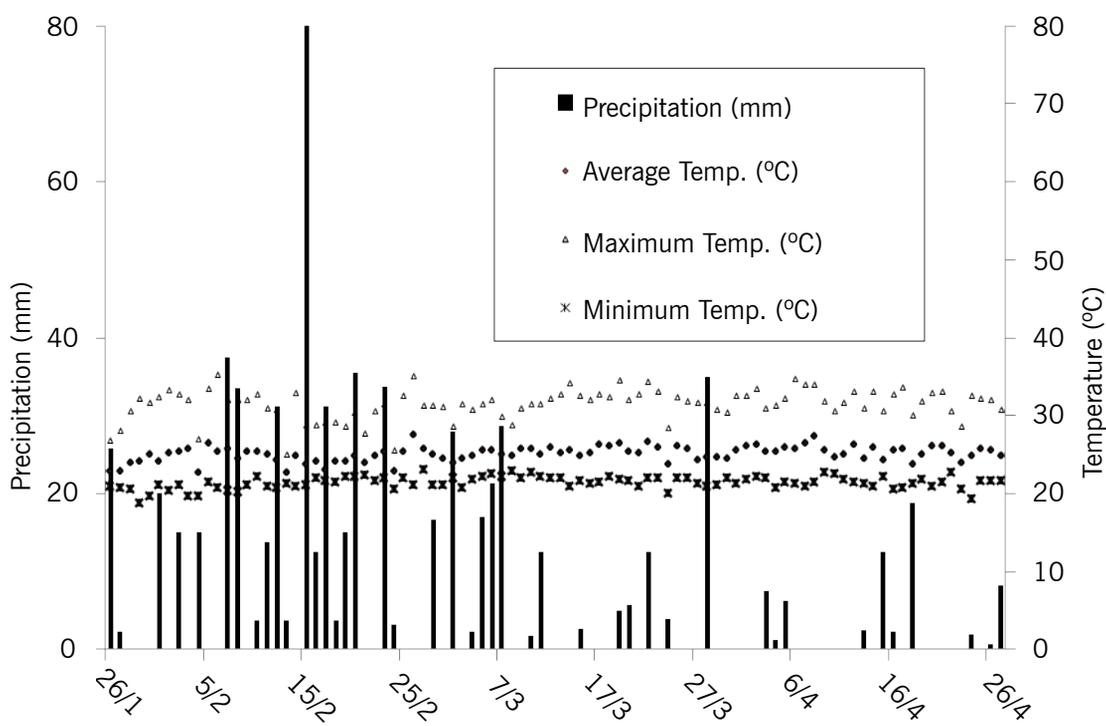
inoculation of *Azospirillum brasilense* through different application methods and at various inoculant dose levels.

Materials and methods

The experiment was conducted in a commercial area adjacent to Federal University of Mato Grosso (UFMT), Sinop campus, between February and July 2018. The chosen site is located at latitude 11°86'32"S, longitude 55°47'89"O and at an altitude of approximately 380.0 m, with a flat topography. The climate according to Köppen (1948) is classified as Aw (tropical with dry winter), having two well-defined seasons, one rainy (between October and April) and the other dry (from May to September), with low annual thermal amplitude varying between 24.0 and 27.0 °C. The average annual precipitation in the region is approximately 2100 mm (SOUZA et al., 2013).

The Figure 1 is presents the climatologically data of the experiment conducted in the period between January 26 and April 27, 2018.

Figure 1 – Precipitation (mm) and average, maximum and minimum temperatures (°C) per week during the experiment period. Sinop/MT.



Source: Elaborated by authors (2017).

Soil was collected six months before soybean sowing, with the aid of a Dutch auger in the 0.0 to 20.0 cm depth layer, and subsequently a chemical analysis of the soil was conducted at the Soil Laboratory of UFMT (Sinop campus).

From these steps, the following results were obtained: pH (CaCl₂): 5.1; Organic Matter: 18.55 g dm⁻³; P (Melich⁻¹): 6.07; K: 52.00 mg dm⁻³; Ca: 2.84; Mg: 0.93; S: 0.40; Al: 0.0; H: 2.92; cation exchange capacity (CTC pH 7.0): 6.82 cmol dm⁻³; V: 57.2%; Ca/Mg ratio: 3.05; Ca/K ratio: 21.85 and Mg/K ratio: 7.16.

The micronutrient values (in mg dm⁻³) were as follows: Zn: 5.51; Cu: 0.44; Fe: 199.16; Mn: 11.25; and B: 0.15. A physical analysis of the soil revealed the following compositions (in g dm⁻³): sand: 497.0; silt: 125.0 and clay: 378.0. Therefore, it was confirmed that base saturation is in accordance with the requirement for soybean and no liming is required. The soil in the region is classified as Red-Yellow Latosol (SANTOS et al., 2013).

The soybean cultivar (“Bonus”) with industrial treatment of seeds Basf® was sown at a density of 15 seeds per meter, for an average population of 260,000 plants per hectare. Fertilization was realized with 500.0 kg ha⁻¹ 00-18-18 (NPK), according to the producer’s management, with sufficient phosphorus and potassium required for a good soybean yield (SOUZA and LOBATO, 2004). The culture was inoculated with a liquid inoculant with *Azospirillum* and peat inoculant with *Bradyrhizobium*. Further, fungicide and insecticide were applied three times each one during the development stages, in accordance with necessity and the management of production.

Weed desiccation was carried out twice: once before sowing the crop corn, with the application of 1.5 kg ha⁻¹ of glyphosate (granulated), and then post-emergence at 30 days, with another 1.5 kg ha⁻¹ of glyphosate with a spray volume of 100 L ha⁻¹ through a tractor-mounted bar sprayer.

Next, on January 26, 2018, the corn hybrid sample DKB 390Y was sown, with 0.5 m of spacing between lines and a final stand of 60,000 plants per hectare. The hybrid’s seeds received industrial chemical treatment from Basf®. For sowing fertilization, the doses of 40.0 kg ha⁻¹ of N, 98.4 kg ha⁻¹ P₂O₅, and 52.5 kg ha⁻¹ K₂O was used as per requirements for a good corn crop yield. The application of nitrogen in cover was done manually using urea (45.0% N) at a dose of 30.0 kg ha⁻¹ (SOUZA and LOBATO, 2004).

The experimental design included randomized blocks in a 3×5 factorial arrangement, replicated four times, thereby totalizing 60 plots. A commercial inoculant — Ab-V5 and Ab-V6 of *Azospirillum brasilense* (10⁸ UFC mL⁻¹) — was applied in three different methods: foliar spray, foliar application with bovine gelatin, and with the aid of a size 23 paint roller (Black & Decker Rapid Roller BDPR400--wool®), size 23.0 cm, sheep wool material, with 9.0 mm application in the soil between the corn lines. The controls without inoculant *Azospirillum* was the doses 0.0 mL ha⁻¹ for each application method, using bomb of CO₂ for foliar spray and foliar application with bovine gelatin methods.

For each application method, five different inoculant doses were used: 0, 100, 200, 400.0 and 800.0 mL ha⁻¹. The treatments were applied in the V4 development stage, period with rapid accumulation of dry mass and nutrients in a corn plant (RITCHIE et al., 2003). The experimental plots consisted of four cultivation lines, with five meters in length and 0.5 meter of spacing between lines. The usable area of the plot for samples was considered to be between the 2.0 central lines and 4.0 meters in length, thereby disposing off 0.5 meter at each end of the plots and the two lateral lines.

The following characteristics were evaluated for chlorophyll content, plant height and diameter of the plant. The measurements were obtained at the full flowering stage (R2) from six plants selected at random from each useful plot area, approximately 60 days after corn germination. The chlorophyll content was determined using the superior leaf, below the main ear, with the aid of a chlorophyll meter ClorofiLoG® (model CFL-1030) of the industries Falker®, which estimates the content of chlorophyll indirectly, using units SPAD (Soil Plant Analysis Development) (FIORINI et al., 2019).

According to Argenta et al. (2001), a precise estimate of the chlorophyll content in the leaves is an efficient parameter for monitoring plant nitrogen levels. The chlorophyll meter readings indicate

proportional values of chlorophyll on the sheet, which are calculated based on the amount of light transmitted, and absorbed through the sheet at two wavelengths with different chlorophyll absorbances.

The height of plants was obtained, still in the field, using a measuring tape, measuring the six plants of the soil until the last expanded leaf of the corn (flag leaf). The stem diameter was obtained with the digital caliper close to the ground, in the first internode visible above the soil (FIORINI et al., 2019).

The characteristics of yield corn were evaluated after the ears were harvested manually on July 15, 2018, approximately 150 days after corn germination. They had a grain moisture in harvest at level of approximately 240.0 g kg^{-1} , were the samples were packed in plastic bags containing the respective plot markings and taken to UFMT (Sinop campus), with subsequently drying in full sun, until they reached a moisture level of 130.0 g kg^{-1} .

After harvesting, the analyzed six ears of the plants representative in each sample were evaluated according to characteristics components of productivity number of rows of grains, number of grains per row, length of ears, diameter of ears and the weight of a thousand grains obtained from each useful portion of samples were the treatments counted. The number of rows of grains and the number of grains per row were obtained by counting. The length of ears and diameter of ears were measured with a tape measure. After threshing with the aid of a manual thresher, the grain yield was quantified by weighing the grains from the useful area of each plot being calculated in kg ha^{-1} . Subsequently, the samples were threshed with the aid of a manual thresher, with subsequent counting of the mass of a thousand grains, where the weight was measured on a precision scale of brand Fillizola®, and the grain yield was quantified through the weight of the grains of each parcel in the area, of the useful portion of the plot, being calculated in kg ha^{-1} .

Tests for additivity of the model, normality of errors and homogeneity of variances were performed as well. No restrictions on the assumptions for the analysis of variance, the data were subjected to analysis of variance (ANOVA) at 5% probability through the F test, using the Sisvar® statistical analysis system (FERREIRA, 2011). For qualitative variables, means were compared using the Scott-Knott test at 5% probability. For quantitative variables, the models were selected based on the significance of the regression coefficients using the t Student-test, adopting a 5% probability of determination as the r^2 value (SQRegression/SQtotal).

Results and discussion

During the field crop cycle, water availability was ideal for the development of the simple corn hybrid, and there were excellent conditions to express its productivity, without interference from water stress (Figure 1). Temperatures during the period of conducting the experiment ranged between $19.0 \text{ }^\circ\text{C}$ and $35.0 \text{ }^\circ\text{C}$ with an average of 22.84°C , and the monthly average rainfall of the accumulated values of 395.0 mm in February, 180.0 mm in March and 62.0 mm in April, that is, they were not limiting the growth, development and productivity of the second crop corn crop (Figure 1).

For Albuquerque et al. (2010), a medium cycle hybrid, aiming at grain production, consumes 380.0 to 550.0 mm of water in its cycle. According to Sangoi et al. (2007) the sowing of second crop corn is extremely dependent on temperature variations, solar radiation and especially precipitation, since second crop corn is sown in late summer, where it is more susceptible to climatic limitations.

The characteristics evaluated in the full bloom of stage R2 corn, such as total chlorophyll content, plant height and stem diameter were not influenced by the different methods of application of *Azospirillum* (Table 1).

Table 1 – Means of the variables analyzed in full bloom stage R2: total chlorophyll content total (CLO), plant height (PH) and stem diameter (SD), under conditions of off-season corn crop according to the application methods. UFMT. 2017/2018 agricultural year, Sinop/MT.

Application methods:	Variables analyzed		
	CLO (units SPAD)	PH (m)	SD (mm)
Foliar	55.74 a	2.38 a	22.03 a
Foliar with Bovine Gelatin	55.64 a	2.36 a	22.14 a
Roller	54.99 a	2.34 a	21.28 a
C.V.(%)	4.57	2.87	4.35
General Averages	55.46	2.36	21.82

*The averages followed by the same letters did not differ at the 5% probability level by the Scott-Knott test.

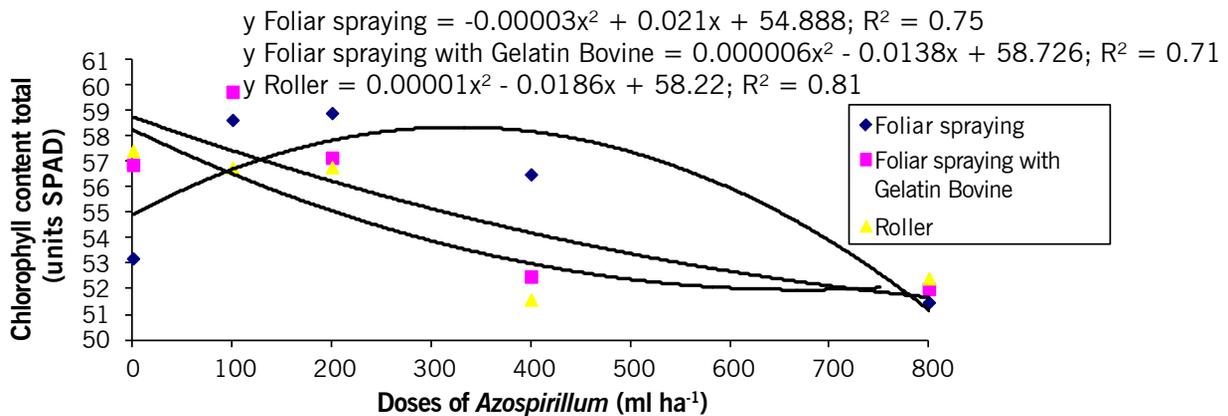
Source: Elaborated by the authors (2017).

The average chlorophyll content of the plant of the corn obtained in this study for various application methods was to 55.46 units SPAD, and are similar obtained by other authors at the flowering stage R2 on the opposite lower leaf of the main ear in corn crop (ARGENTA et al., 2001; AMARAL FILHO et al., 2005; CADORE et al., 2016; FIORINI et al., 2017; FIORINI et al., 2019).

For the variable plant height variable, there was no significant effect for the treatments tested, and the values obtained ranged from 2.34 to 2.38 m. The same occurred for the stem diameter, in which the values varied from 21.28 to 22.14 mm, with no statistical differences between treatments with residual fertilization of soy in corn. These values are similar to those obtained by Cadore et al. (2016) for the same corn hybrid, with no response regarding plant height and stem diameter as a function of N doses in coverage and application of inoculation with *Azospirillum*. Corroborating, until then, with the work of Valderrama *et al.* (2011), who evaluated application of 0, 40, 80 and 120 kg ha⁻¹ of N in the form of urea for the same corn hybrid and found no difference in this variable. Probably, the residual of the N fertilization of the soy with the application of the treatments with 10.0 kg ha⁻¹ of N was not enough to obtain answers in the vegetative characteristics of the growth of the corn and in the levels of total chlorophyll.

The chlorophyll content still varied according to the methods of application, where there were interactions between the methods of application and the bio stimulant doses with the highest values observed for the methods of application in the 100.0 mL ha⁻¹ dose of bio stimulant. For the leaf application method, the quadratic equation was the best fit of the data with the point of maximum estimated value for the dose 350.0 mL ha⁻¹. As for the foliar application methods with bovine gelatin and application via roller between the lines of the corn, as the dose of bio stimulant was increased, according to the quadratic regression equations there were reductions in chlorophyll contents with the points of minimum values estimated by the equations, 1150.0 and 930.0 mL ha⁻¹ (Figure 2).

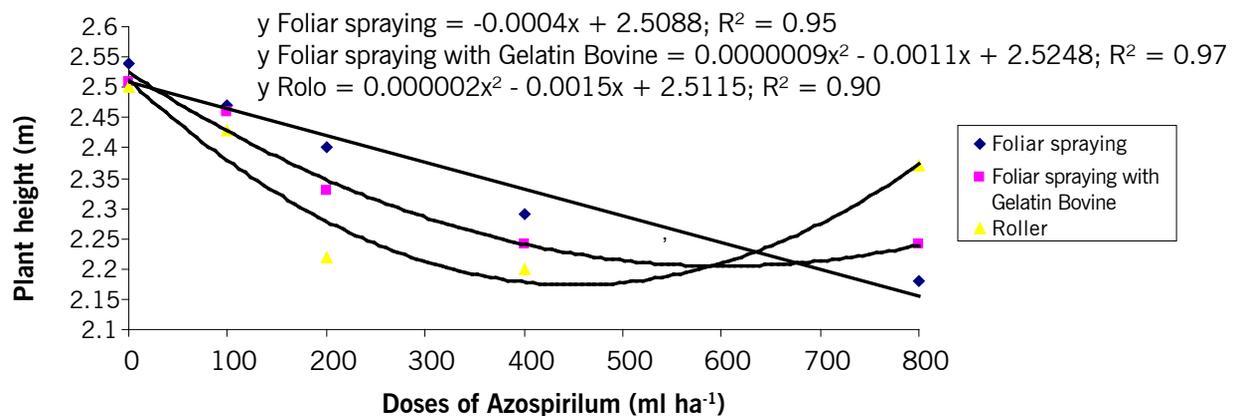
Figure 2 – Chlorophyll content total as a function of application methods and inoculant doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop/MT.



Source: Elaborated by the authors (2017).

The plant height varied according to the application method. There were interactions between the methods of application and the doses of bio stimulant with the highest values observed for the control without bio stimulant. For the foliar application method, there was a linear reduction according to the bio stimulant doses. The quadratic model was the best fit for the foliar application methods with bovine gelatin and via roller between the corn lines with the minimum values estimated by the equations for doses 611.0 and 375.0 mL ha⁻¹, respectively (Figure 3).

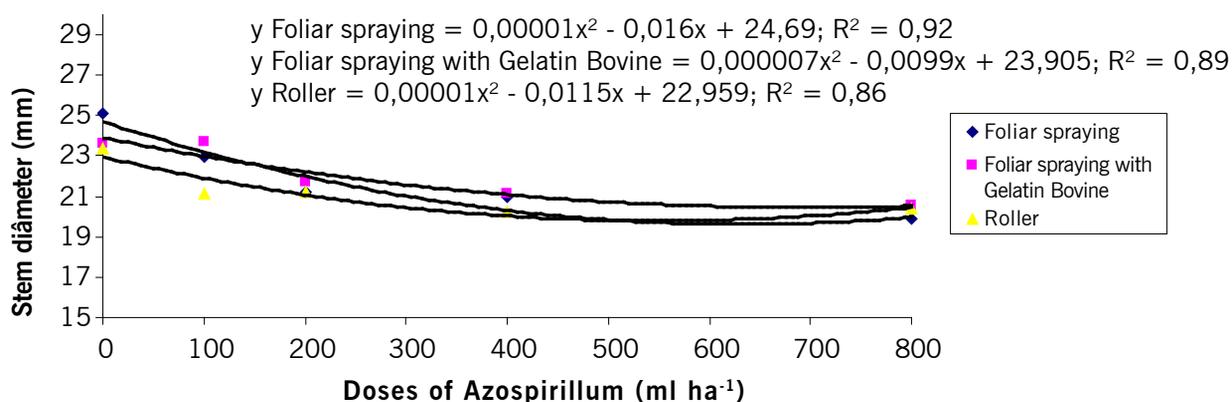
Figure 3 – Plant height (m) as a function of application methods and inoculants doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop/MT.



Source: Elaborated by the authors (2017).

For the stem diameter, there were reductions according to the increase in doses, where the control without the application of bio stimulant showed the highest values for this variable. The quadratic model was the best fit for the foliar application, foliar application with bovine gelatin and via roller between the corn lines with the minimum values estimated by the equations for the doses 800.0, 707.14 and 575.0 mL ha⁻¹, respectively (Figure 4). The stem diameter, as well as the plant height, showed higher values in the controls without the application of bio stimulant with differences of low magnitude in relation to the other doses in the application methods, despite that there was no damage to the productivity of off-season corn crop.

Figure 4 – Stem diameter (mm) as a function of application methods and inoculant doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop/MT.



Source: Elaborated by the authors (2017).

The application of treatments in the *Azospirillum* application methods did not statistically influence the component characteristics of corn yield: number of rows of grains, number of grains per row, length of ears, diameter of ears, weight of a thousand grains; except for grain yield, where the mode via application roller between the lines of the crop was statistically inferior to the other methods of application (Table 2).

Table 2 – Averages of the variables analyzed at harvest in the period of physiological maturity: number of rows of grains (NRG), number of grains per row (NGR), length of ears (LE), diameter of ears (DE), weight of a thousand grains (WTG) and grain yield (GY), under conditions of off-season corn crop according to the application methods. UFMT. 2017/2018 agricultural year, Sinop/MT.

Application methods:	Variables analyzed					
	NRG	NGR	LE (cm)	DE (cm)	WTG (g)	YG (kg ha ⁻¹)
Foliar	16.95 a	34.40 a	17.20 a	5.40 a	325.99 a	6892.00 a
Foliar with Bovine Gelatin	17.05 a	33.95 a	16.75 a	5.35 a	320.16 a	6781.00 a
Roller	17.10 a	34.15 a	17.03 a	5.35 a	317.58 a	6272.50 b
C.V.(%)	7.15	6.02	5.90	9.29	3.55	9.14
General Averages	17.03	34.16	16.99	5.16	321.24	6648.50

*The averages followed by the same letters did not differ at the 5% probability level by the Scott-Knott test.

Source: Elaborated by the authors (2017).

For the number of rows of grains (NRG), there was no significant effect for the methods of application of the *Azospirillum* tested, and the average values obtained ranged from 16.95 to 17.10 rows of grains on the ear of corn. The same occurred with the number of grains per row (NGR), where the values ranged from 33.95 to 34.40 grains per row, with no statistical differences between the methods of application of *Azospirillum* in corn. Biscaro et al. (2011) also found no difference in the number of rows evaluating N doses for off-season corn. Cadore et al. 2016, obtained different results, as the nitrogen coverage levels generated significant increases in relation to the non-application of N. However, there was no significant difference in the number of grains per row corroborating with the results obtained in the present study.

In a study carried out by Kappes et al. (2009), it showed that there was no difference between the treatments for the variable NRG, as it is a genetic characteristic controlled by several genes and little influenced by edapho-climatic and management factors. According to Ohland et al. (2005), evaluating the management of nitrogen fertilization in corn under no-tillage system did not find any difference for the ear diameter and length. Gomes et al. (2007) also did not find an effect on the weight of a thousand grains with the increase of N doses applied in the culture.

Among the nutrients required by corn, nitrogen (N) is the one that limits the most its growth, in addition to being required in greater quantity. N is a constituent of proteins, nucleic acids and other cellular constituents, as well as membranes and various plant hormones. Thus, its deficiency can cause generalized chlorosis of the older leaves, and decreased plant growth. It's most common absorption by corn is in the form of nitrate (NO_3^-), due to the nitrification process of N that occurs in the soil. According to Malavolta et al. (2006), N is an element involved in the synthesis of chlorophylls and protein compounds, showing the potential to increase the capacity of plants to produce reproductive buds. In the present study, the low dose of N applied at different stages of soybean 10.0 kg ha^{-1} of N was not sufficient to generate increases in NRG and NGR characteristics, compared to the control without N.

For the length of ears (LE), there was no significant effect for the *Azospirillum* application methods tested, and the mean values obtained ranged from 16.75 to 17.20 cm in the corn ear. The diameter of ear varied from 5.35 to 5.40 cm without statistical differences between the methods of application of *Azospirillum* in off-season corn crop. The same fact occurred for the number of grains per row (NGR), where the values varied from 5.35 to 5.40 cm in the ear, with no statistical differences between the methods of application of *Azospirillum* in corn. For the variable weight of a thousand grains (WTG), it was found that the application of N in different methods of application varied from 317.58 to 325.99 grams and did not differ statistically in the methods of application in the second crop corn crop. Most of the studies involved with nitrogen doses associated or not with inoculation with *Azospirillum* did not find differences for these characteristics of maize productivity (Ohland et al., 2005; Gomes et al., 2007; Barros Neto et al., 2008; Kappes et al., 2009; Vorpapel et al., 2010; Biscaro et al., 2011; Valderrama et al., 2011; PORTUGUAL et al., 2012; DARTORA et al., 2013; CUNHA et al., 2014; QUADROS et al., 2014; CICILIATO et al., 2015; CADORE et al., 2016; MUMBACH, 2017).

For the grain yield, the application via foliar with or without bovine gelatin ($6,892.0$ and $6,781.0 \text{ kg ha}^{-1}$) in the corn of the bacterium *A. brasilense* provided greater grain productivity that varied from in relation to the method of application via roller between the line of corn ($6,272.50 \text{ kg ha}^{-1}$). Portugal et al. (2012) found that corn productivity with the use of the bacterium *A. brasilense* was increased by 868 kg ha^{-1} , that is, an increase of 14.75%.

Probably, leaf inoculation of *A. brasilense* provided greater productivity due to the biological N fixation (BFN), indicated by the increase in N leaf content, that promoted greater growth of the root system, causing the roots to explore greater soil volume, increasing the absorption of nutrients and water. The data obtained agree with those of Barros Neto et al. (2008), who, using *A. brasilense* in an experiment with corn, obtained an increase in grain yield from $9,021.0$ to $9,814.0 \text{ kg ha}^{-1}$, that is, average productivity statistically 9.0 % higher than the control not inoculated. The predecessor crop of the present work was soy, a legume efficient in biological nitrogen fixation, in which it allows the availability of a residual N in the soil. This may have increased the efficiency of *Azospirillum*

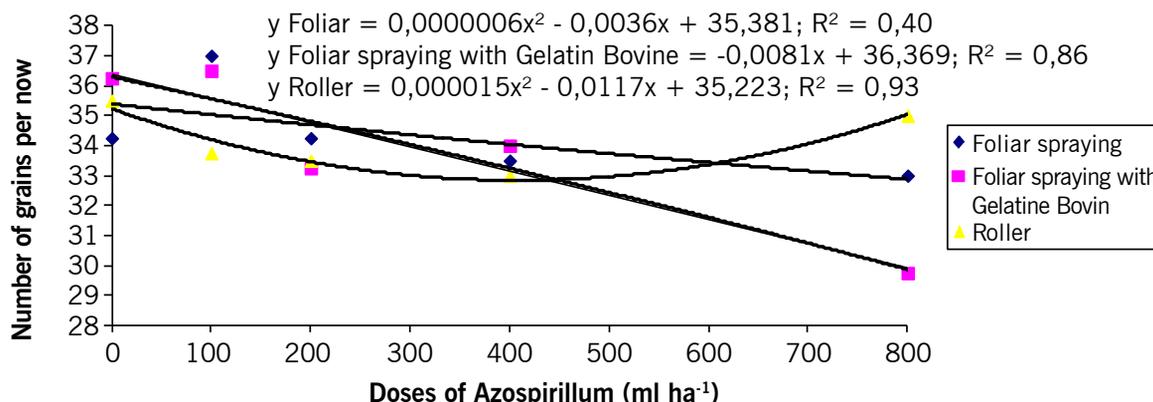
and increased the availability of N according to the doses applied in coverage reflecting significant differences in productivity.

These high values in the productivity of the off-season corn crop can also be explained by the high capacity of the soil to supply N to the plants, due to the residual N left by the predecessor crop of soybeans, as well as by the appropriate climatic conditions during the crop cycle, mainly good rainfall during the critical period of grain productivity (grain filling), fertility conditions of the soil that had good levels of nutrients and the fertilization of planting and corn cover.

According to Ritchie et al. (2003), when defining the number of grains and the size of the ear (stage V12), moisture and nutrient deficiencies can seriously reduce the potential number of seeds and the size of the ears harvested, which may explain the shorter lengths of ears and lower yields of off-season crop corn compared to first sown crop corn.

The number of grains per row of corn cob varied according to the application methods, where there were interactions between the application methods and the bio stimulant doses with the highest values observed for the application methods at the 100 ml ha⁻¹ dose, except for the application method via roller between the lines of the corn, where the control was of greater value. For the foliar application methods with bovine gelatin and application roller between the lines as the dose of bio stimulant was increased, there was a linear reduction according to the regression equation. For the leaf application method, the quadratic equation was the best fit of the data with the point of minimum value calculated for the dose 400.0 mL ha⁻¹ (Figure 5).

Figure 5 – Number of grains per row as a function of application methods and doses of *Azospirillum* inoculant under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop/MT.

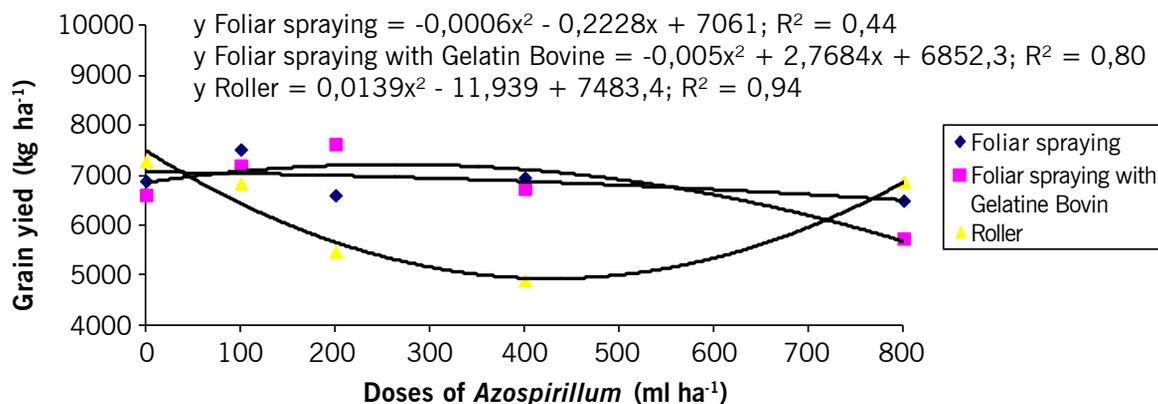


Source: Elaborated by the authors (2017).

For the number of rows of grains, length of ears, diameter of ears and weight of thousand grains, no statistically significant differences were found between the methods of application, doses and also did not show any interaction between the factors studied.

Grain yield varied according to the application method utilized, and there were interactions between the application methods used and the doses levels of bio stimulant. The quadratic model was the best fit for all the three application methods. The maximum values for the foliar spray and foliar application with bovine gelatin methods were at doses of 185.0 and 261.0 ml ha⁻¹, respectively. For the roller method, the minimum point was at 429.0 mL ha⁻¹ (Figure 6).

Figure 6 – Grain yield (kg ha^{-1}) as a function of application methods and doses of *Azospirillum* inoculant under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop/MT.



Source: Elaborated by the authors (2017).

Grain yield was greater when the plants were inoculated at doses of 100.0 mL ha^{-1} and 200.0 mL ha^{-1} for the foliar spray and foliar gelatin application methods, respectively. The inoculation with *Azospirillum* generated increases in the analyzed variables. For the roller application method, the control (0 ml ha^{-1}) provided the highest grain yield, with subsequent reduction up to the 400.0 mL ha^{-1} . The treatments differed statistically, and foliar inoculation with 100.0 mL ha^{-1} resulted in an increase in grain yield of 632.5 kg ha^{-1} , which corresponds to $10.54 \text{ bags ha}^{-1}$. With foliar bovine gelatin, the grain yield gain reached $1,017.5 \text{ kg ha}^{-1}$, or $16.95 \text{ bags ha}^{-1}$. Therefore, foliar inoculation of *Azospirillum brasiliense* may be a viable option for farmers who cannot perform it via seed.

Vorpagel et al. (2010) obtained a gain in corn grain yield of the order of 227.0 kg ha^{-1} ; however, this was not statistically different when compared with other treatments. Vorpagel also states that the use of *Azospirillum*, whether or not associated with the nitrogen fixation, did not show any statistically significant difference for any of the evaluated performance characters when compared with the control. According to Reis (2007), there has been great variability in the results for most diverse cultures that have been tested, with the average increase in grain yield being approximately 20.0 to 30.0 %. Portugal et al. (2012), observed an increase of 14.7% in corn grain yield through foliar application of *Azospirillum* at V6 stage.

Several studies demonstrate that corn is dependent on nitrogen fertilization, regardless of whether or not nitrogen-fixing bacteria are inoculated. However, authors such as Mumbach et al. (2017) state that inoculation can contribute up to 40.0 % of the nitrogen needed for the development of the crop. According to Hungria (2011), the nitrogen fixed by the bacteria varies from 30.0 to $50.0 \text{ kg per hectare year}^{-1}$.

Conclusions

The application of *Azospirillum* doses did not increase the chlorophyll content, plant height, stem diameter, number rows of grain, number of grains per row, length of ears, diameter of ear, weight of a thousand grains, except for grain yield.

The application of *Azospirillum* showed statistical differences between doses and application methods, as well as the interaction between doses with the application methods for chlorophyll and grain yield, highlighting the methods foliar application and foliar application with bovine gelatin.

The foliar application of *Azospirillum* with the dose of 100.0 mL ha⁻¹ showed an increase in grain yield, in relation to the control and other treatments, regardless of the reduction of chlorophyll content, stem diameter and number of grains per row.

An inoculant dose of 100.0 mL ha⁻¹, as recommended by the manufacturer, appears to be the most appropriate dose for the crop for increasing grain yield.

Foliar application with bovine gelatin at a dose of 200.0 mL ha⁻¹ also showed an increase in grain yield (control versus and other treatments), irrespective of the reduction in chlorophyll content, stem diameter and number of grains per row.

Modos de aplicação e doses de inoculação com *Azospirillum* no desempenho agrônômico do milho segunda safra

Resumo

Para alcançar altas produtividades para o milho de segunda safra semeado após a colheita da soja, são necessárias altas doses de fertilizantes nitrogenados. A inoculação de bactérias associativas de vida livre fixadoras de nitrogênio, como as do gênero *Azospirillum*, auxilia na diminuição do uso de fertilizantes nitrogenados. Objetivou-se avaliar características relacionadas ao desempenho agrônômico do milho híbrido DKB390Y em resposta a diferentes modos de aplicação, associadas a diferentes doses de inoculante à base de *Azospirillum brasiliense*. O experimento foi conduzido em uma área próxima a UFMT Campus Sinop, entre os meses de janeiro e junho de 2018. O delineamento experimental foi em blocos casualizados, em arranjo fatorial 3 x 5 (3 modos de aplicação: pulverização foliar, aplicação foliar com gelatina bovina e com auxílio de um rolo de tinta (Rapid Roller Bdpr 400-lã Black & Decker®) e 5 doses de inoculante: 0, 100, 200, 400 e 800 mL ha⁻¹) com 4 repetições. Para os modos de aplicação, não foram obtidas diferenças significativas nas variáveis clorofila, altura das plantas, diâmetro de colmos, número de fileiras de grãos, número de grãos por fileira, comprimento de espigas, diâmetro de espigas e peso de mil grãos, exceto para a produtividade de grãos. A produtividade de grãos foi superior quando se inoculou 100 mL ha⁻¹ no modo via foliar e 200 mL ha⁻¹ no modo foliar com gelatina bovina.

Palavras-chave: *Zea mays* L. Bioestimulante. Clorofila. Aplicação foliar. Aplicação por rolo.

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