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AGRONOMIC PERFORMANCE OF CORN IN FUNCTION OF METHODS OF APPLICATION AND DOSES INOCULATED WITH *Azospirillum brasilense*

Abstract - Nitrogen supplementation becomes necessary when trying to increase yields of off-season corn crop grown in rotation with early-maturing soybean crop. 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 as the 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 differences in chlorophyll content at the bottom of the plant, plant height, and leaf area index were observed when varying the application methods. However, leaf nitrogen level, dry mass and grain yield changed when the application method used for inoculation changed. When evaluating doses, the control was found to be superior to the other treatments based on chlorophyll content at the bottom of the plant, plant height, leaf area, leaf nitrogen, and dry mass values. Grain yield was found to be superior with foliar inoculation at 100 mL ha⁻¹ method foliar spray and 200 mL ha⁻¹ methods foliar application with bovine gelatin.

Keywords: biostimulant, chlorophyll, foliar application, vegetative development.

DESEMPENHO AGRONÔMICO DO MILHO EM FUNÇÃO DE MODOS DE APLICAÇÃO E DOSES DE INOCULAÇÃO COM *Azospirillum brasilense*

Resumo – A suplementação com altas doses de nitrogênio torna-se necessária para aumentar a produtividade do milho segunda safra semeado em rotação com a soja de maturação precoce. A fixação biológica de nitrogênio ajuda a reduzir o uso de fertilizantes nitrogenados através da inoculação de bactérias associativas fixadoras de nitrogênio, como as do gênero *Azospirillum*. Este estudo teve como objetivo avaliar o desempenho agrônomo do híbrido de milho DKB390Y em resposta a diferentes modos de aplicação e diferentes doses de inoculante a base de *Azospirillum brasilense*. O experimento foi conduzido em uma propriedade próxima à Universidade Federal de Mato Grosso (campus de Sinop), entre fevereiro e julho de 2018. O delineamento experimental foi em blocos casualizados, em arranjo fatorial 3x5 (3 modos de aplicação: pulverização foliar, pulverização foliar com gelatina bovina e com auxílio de um rolo de tinta [Rapid Roller Bdpr400-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 no teor de clorofila da parte de baixo da planta, altura das plantas, índice de área foliar e nitrogênio foliar, já para a massa seca e produtividade de grãos foram obtidas diferenças significativas. Quanto às doses de inoculante, a testemunha se mostrou superior aos demais tratamentos na avaliação de clorofila da parte de baixo da planta, altura de planta, área foliar, nitrogênio foliar e massa seca. 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: bioestimulante, clorofila, aplicação foliar, desenvolvimento vegetativo.

Corn (*Zea mays* L.) is among 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 (2018), the overall production of the second corn crop of 2017/2018 in Mato Grosso, Brazil, reached 26,201,200 tons, with an average productivity of 5,860 kg ha⁻¹. However, the growing use of cereal for industries and human consumption has made it necessary to adopt new methods and techniques to increase productivity. These new methods and techniques include improvements in soil fertility, management and application technologies of pesticides, higher yielding varieties, and phytosanitary products with a better spectrum of action (CHIQUITO et al, 2012). However, the increase in productivity needs to be achieved without considerably affecting the ecosystem, that is, sustainably.

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. This nutrient can be made available to plants in different manners, for example, via a chemical fertilizer, organic matter from the soil, and an organic fertilizer of animal or vegetable origin. The nutrient 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 CAMPOS et al. (2001), the annual gain in soybean productivity due to inoculation varies from 80 to 291 Kg ha⁻¹, corresponding to an increment from 4 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. However, the use of BNF played a key role in solving this problem 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, 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, 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, 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 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.

Consequent to the above discussion, it is necessary to deepen our understanding 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 next to Federal University of Mato Grosso (UFMT), Sinop campus, between February and July 2018. The experiment site is located at latitude 11°86'32"S and longitude 55°47'89"O and at an altitude of approximately 380 m with a flat topography. The climate according to Koppen (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 and 27 °C. The average annual rainfall in the region is approximately 2100 mm (SOUZA et al., 2013).

Soil was collected six months before soybean sowing with the aid of a Dutch auger in the 0 to 20 cm depth layer and subsequently a chemical analysis of the soil was conducted at the Soil Laboratory of UFMT (Sinop campus); 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: 00; 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; silt: 125 and clay: 378. 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”) 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 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 each applied three times during the development stages.

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 after emergence 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 February 19, 2018, the corn hybrid DKB 390Y was sown, with a final stand of 60,000 plants per hectare. The hybrid’s seeds received industrial chemical treatment from BASF®. For sowing fertilization, a dose of 40 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% N) at a dose of 30 kg ha⁻¹ (SOUZA and LOBATO, 2004).

The experimental design included randomized blocks in a 3×5 factorial arrangement, replicated four times, thereby totaling 60 plots. A commercial inoculant (Ab-V5 and Ab-V6 of *Azospirillum brasilense* [108 UFC mL⁻¹]) was applied in three different manners: foliar spray, foliar application with bovine gelatin, and with the aid of a size 23 paint roller (Black & Decker Rapid Roller BDPR400-®) size 23 cm, material (sheep wool) with 9 mm application in the soil between the corn lines. For each application method, five different inoculant doses were used: 0, 100, 200, 400 and 800 mL ha⁻¹. The treatments were applied in the V4 development stage (RITCHIE et al., 2003). The experimental plots consisted of four cultivation lines five meters in length and half a meter of spacing between lines. The usable area of the plot for samples was considered to be between the two central lines and four meters in length, thereby disposing off half a meter at each end of the plots and the two lateral lines.

Next, the following characteristics were evaluated: chlorophyll content at the bottom of the plant, leaf area, total dry mass, and leaf nitrogen content. The measurements were obtained at the full flowering stage (R2) from six plants selected at random from each useful plot area. The chlorophyll content at the bottom of the plant was determined using the bottom

third leaf, below the main ear, 60 days after corn germination, with the aid of a chlorophyll meter (Falker ClorofiLOG CFL-1030), which estimates the content of chlorophyll indirectly, using SPAD/Falker units. 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, are calculated based on the amount of light transmitted, and absorbed through the sheet at two wavelengths with different chlorophyll absorbances. After collecting the aerial part of the plants representing their plots in the field, the samples were taken to the UFMT (Sinop campus) Viveiro laboratory. In the laboratory, the leaves were detached and the leaf area in cm^2 was obtained with the aid of a leaf area integrator (LICOR LI-3010). To obtain the dry mass, the samples were packed in paper bags and placed in a forced circulation oven at a temperature of $65\text{ }^\circ\text{C}$ where they were dried until they reached a constant weight. For measuring the leaf nitrogen content, the first opposite leaf below the ear of representative plants from each plot was collected. The leaves were then dried at $65\text{ }^\circ\text{C}$ for 24 h. The actual lab test was outsourced and performed by Perfil Agroanalysis ME.

After the grains were harvested manually on July 15, 2018, they had a moisture level of approximately 240 g kg^{-1} ; the plants were packed in plastic bags containing the respective plot markings and taken to UFMT (Sinop campus) with subsequent drying in full sun until they

reached a moisture level of 130 g kg^{-1} . 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} .

Tests for additivity of the model, normality of errors and homogeneity of variances were performed as well. With no restrictions on the assumptions for the analysis of variance, the data were subjected to analysis of variance (ANAVA) at 5% probability through the F test, using the Sisvar statistical analysis system (FERREIRA, 2011). For qualitative variables, means were compared using the Skott-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/SQtreatments).

Results and discussion

The results of our experiments are summarized in Table 1. The chlorophyll content at the bottom of the plant, leaf area index, and leaf N content were not influenced by the application method selected. However, there were significant differences in total dry mass and grain yield for different application methods, wherein foliar spray and foliar application with bovine gelatin were found to be statistically superior to application roller (Table 1).

The average chlorophyll content at the bottom of the plant of the corn leaves at the

flowering stage obtained in this study for various application methods was 53.72 SPAD/ClorofiLOG units, reaching values slightly lower than those obtained by other authors on the opposite lower leaf of the main ear in corn crop with an average of 55 SPAD units (ARGENTA et al., 2001; AMARAL FILHO et al., 2005; FIORINI et al., 2017). This may be because the measurement in this study was made on the third leaf below the ear (half of the lower middle of the plant), where there is greater self-shading by upper leaves, lesser photosynthesis and possibly lower N levels in the lower leaves.

The leaf area index measurements showed no significant difference ($p > 0.05$) between different methods of application, which demonstrates that the conditions for plant development were the same even when the application method was

changed. Müller et al. (2013) and Cunha et al. (2014) also found no significant difference with the inoculation of corn in their study where different doses of N with and without inoculation were evaluated. However, further studies should be conducted in the area to consolidate the results, considering that in Battistus's study (2015) the opposite effect was observed. In the latter study, the agronomic performance of corn in response to leaf inoculation and the associated sowing of seeds treated with Tiametoxan showed an increase in leaf area in response to inoculation with *Azospirillum*.

For total dry mass, leaf nitrogen content, and grain yield, the foliar spray and foliar application with bovine gelatin methods did not differ significantly ($p > 0.05$) and were found to be superior to roller application (Table 1). Dartora

Table 1. Averages of the variables analyzed in flowering stage (R2): chlorophyll content from the bottom (CLO), leaf area (LA), total dry mass (TDM), leaf nitrogen content (N LEAF) and grain yield (GY), in corn crop as a function of *Azospirillum* application methods. UFMT. 2017/2018 agricultural year, Sinop - MT.

Application methods:	Averages:				
	CLO (units SPAD/ClorofiLOG)	LA (cm ²)	TDM (grams)	N LEAF (%)	GY (Kg ha ⁻¹)
Foliar application	53.82 a	5688.35 a	112.45 a	25.85 a	6892.00 a
Foliar application with bovine gelatin	53.52 a	5408.40 a	102.10 a	24.30 a	6781.00 a
Application roller	53.83 a	5356.20 a	91.30 b	23.75 b	6272.50 b
C.V. (%)	3.23	15.53	21.88	10.20	9.14
General Averages	53.72	5484.31	101.95	24.66	6648.50

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

(2013) found that there was no significant effect of inoculation on grain and dry matter productivity; however, average productivity values reached 10,916 Kg ha⁻¹, achieving an average increase of 12% with inoculation, in that study.

The application methods did not influence the chlorophyll content or the leaf area of plants (Table 1). For total dry matter, leaf nitrogen content, and grain yield, the foliar and foliar application methods with bovine gelatin also showed almost no difference; however, they were superior to the roller application method.

The leaf N content ranged from 23.75 to 25.85 g kg⁻¹ dry matter (Table 1). Therefore, the presence of *Azospirillum brasilense* was observed to increase the N content in the plant, especially with foliar spray and foliar application with bovine gelatin methods, which were found to be superior to the roller application method. This increase must be due to BNF and increase

in the volume of the root system promoted by the bacteria, thereby allowing the plant to explore a larger volume of soil, and consequently, a higher concentration of N in the leaf.

The chlorophyll content of the lower part of the corn plant varied with the application method. We also observed interactions between the application methods and the dose levels of biostimulant. For foliar spray and foliar application with bovine gelatin methods, as the dose of biostimulant was increased, there was a linear behavior according to the regression equation with reductions in chlorophyll contents, with the highest dose of biostimulant providing the lowest values in relation to other doses. For the roller application method, the quadratic equation was the best fit for the data, with the point of minimum value calculated at a dose of 692.5 mL ha⁻¹ (Figure 1).

Although it did not differ statistically, the

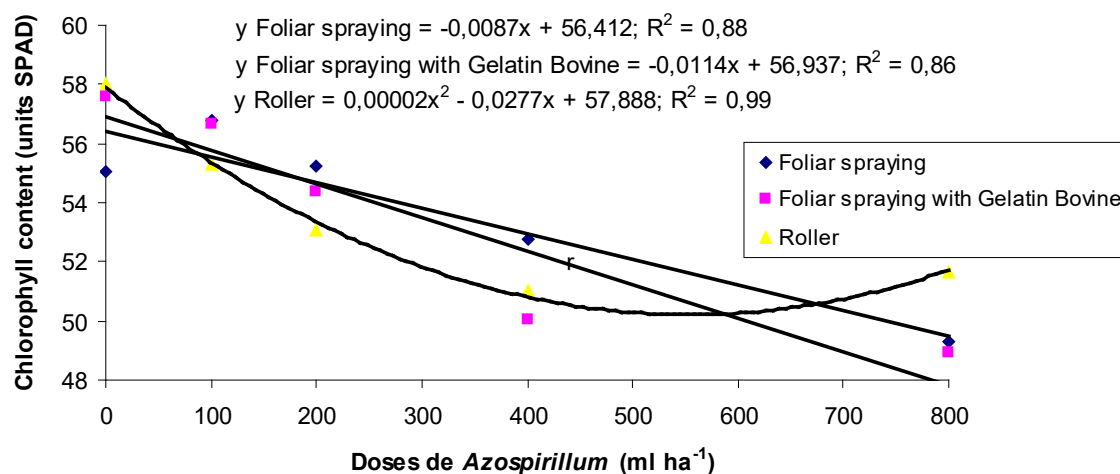


Figure 1. Chlorophyll content from the bottom (units SPAD) as a function of application methods and doses of *Azospirillum* inoculant under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop-MT.

foliar application methods at a dose of 100 mL ha⁻¹ showed higher values than other treatments and control (0 mL ha⁻¹). Peres et al. (2013) found that an increase in the inoculant dose reduced the chlorophyll content between concentrations of 0 mL ha⁻¹, 100 mL ha⁻¹ and 200 mL ha⁻¹, exhibiting agreement with the results of the present study, as shown in Figure 2.

The results obtained with the application of *Azospirillum brasilense* are inconsistent. Further, there are often limited available results for comparison, particularly when the experiments are conducted under field conditions. Bartchechen et al. (2010) demonstrate that the main barrier to research with *Azospirillum spp.* is the inconsistency of results due to variations in the existing research conducted thus far. Possible causes for this are the complexity of the interactions involved between the plant, the introduced bacteria and the other components

of the rhizospheric microbiota, among other factors. Another relevant factor mentioned by Reis et al. (2000) is that inoculation efficiency is dependent on several factors and among them, the compatibility between the species and the plant's genotype is a factor that limits it the most. At low levels of nitrogen fertilization, the response to BNF inoculation is superior.

Another factor that may have influenced the results was the selection of cultivar. Quadros et al. (2014) observed different responses to *Azospirillum* inoculation in three hybrids and suggested that the plant genotype plays a fundamental role in the colonization by bacteria. They also proposed that the interaction between the bacteria and the genotypes available in the market should be further researched, in addition to the comparison in different regions, considering that the type of soil and microclimates are factors that affect the plant/bacterium reaction as well.

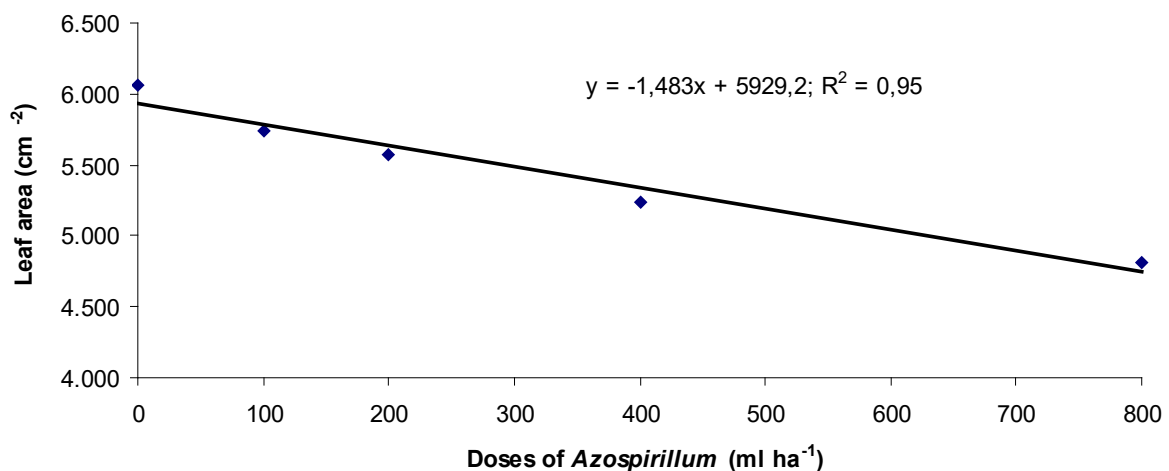


Figure 2. Leaf area index (cm²) as a function of *Azospirillum* inoculant doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop-MT.

Regarding leaf area, no differences were found between the various application methods, with the linear model being the best fit and there being a linear reduction with increase in doses. The control (dose of 0 mL ha⁻¹) presented the highest values for this variable (Figure 2). The application of *Azospirillum brasilense*—irrespective of the application method—changed the leaf area, reducing it from 6100 cm² in the control to 5500 cm² in the 800 mL ha⁻¹ dose, a reduction of 600 cm².

Cunha (2014), in his study wherein he inoculated *Azospirillum* associated with nitrogen fertilization, evaluated IAF, where he did not observe any significant difference between various treatments. However, he obtained values higher values during treatment intervals: 5750 cm² without inoculant and 5540 cm² with inoculant, showing similarity with the results presented here.

The success results found in the literature of the association plant-*Azospirillum* are related, in most cases, to factors related to the bacterium itself, such as the selection of the strain, the ideal number of cells per seed and its viability. Arsac et al. (1990) found that the concentration of bacteria in the inoculant solution is more important than the dose. For the authors, the optimal bacterial concentration that promotes the growth of corn plants is 10 million viable cells mL⁻¹, that is, approximately 17,000 seed⁻¹ colony forming units. An increase in dose had an inhibitory effect on the growth of the inoculated plants, while the control showed higher values of leaf area.

For total dry mass of plants, there were no differences between the various application methods, with the quadratic model being the best fit for the data, with the point of minimum value calculated at a dose of 742 ml ha⁻¹ (Figure 3).

Verona et al. (2010) report that there was no significant difference for height, that is, dry mass of aerial part of inoculated corn plants. In the same manner, the application of different doses of *Azospirillum*-based inoculant in corn seeds did not promote an increase in the fresh mass of the root system or in the accumulation of dry matter in the aerial part (SILVA et al., 2015).

According to Vorpagel (2010), there is substantial evidence that the inoculation of corn seeds with *Azospirillum* is responsible for increasing the rate of accumulation of dry matter, particularly in the presence of high doses of nitrogen, which seems to be related to the increase in activity photosynthetic enzymes and nitrogen assimilation.

In addition, several authors have observed variations in response to inoculation with *Azospirillum spp.* depending on the type and form of application of the inoculant. In fact, the biggest obstacle to the use of BNF would be the inconsistency of results in field experiments (REIS, 2007).

The leaf N content did not differ statistically for different doses. There were reductions in the values where the control showed the highest value of 25.41% and the highest dose showed a value of 24.85% (Figure 4).

Nunes et al. (2015) evaluated the

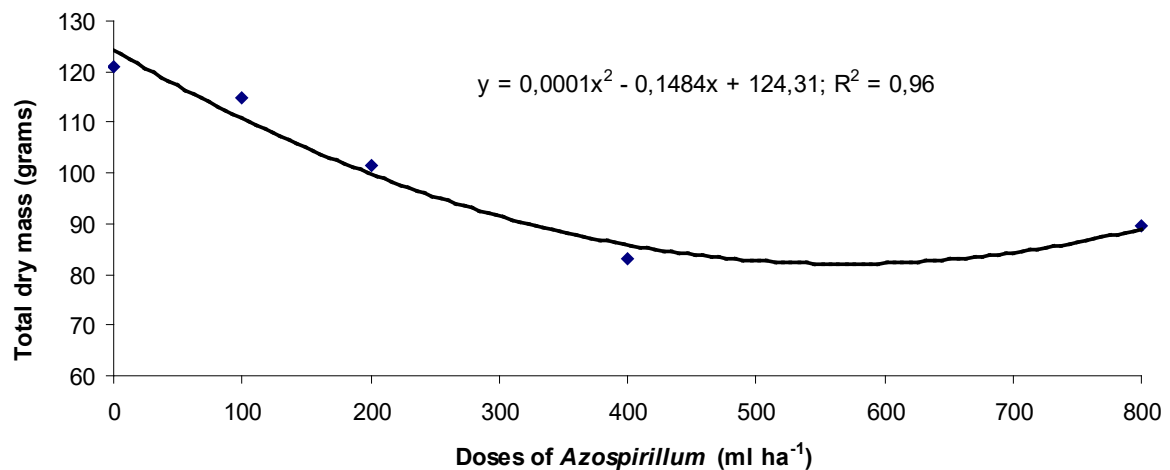


Figure 3. Total dry mass (grams) as a function of *Azospirillum* inoculant doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop-MT.

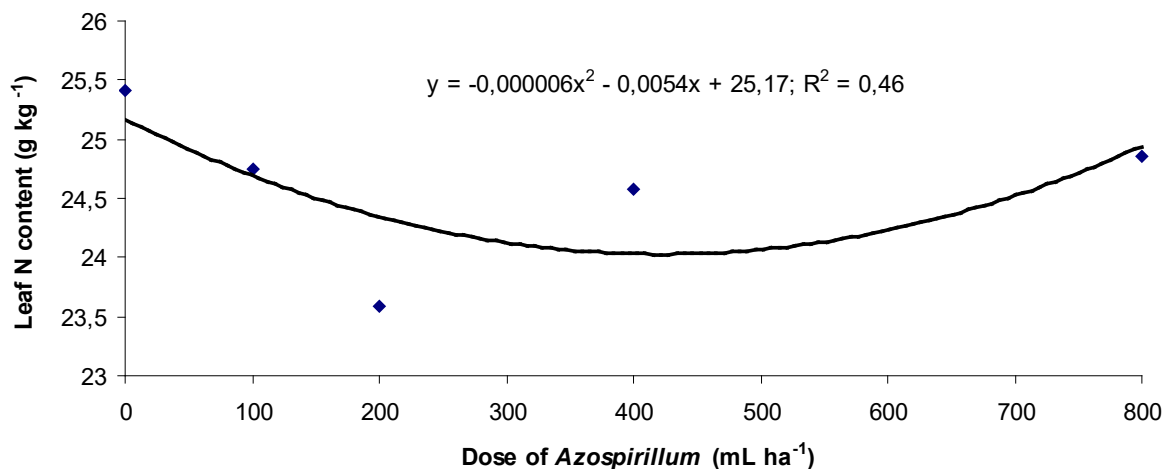


Figure 4. Leaf N content (g kg⁻¹) as a function of *Azospirillum* inoculant doses under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop-MT.

application of nitrogen in wheat plants, whether or not they were associated with the inoculation of *Azospirillum*, they then obtained an increase in leaf nitrogen levels with the application of *Azospirillum*. According to the author, treatment

with a higher level of residual nitrogen and inoculation shows a decrease in grain yield.

The results obtained by Araujo et al. (2013), who evaluated the effects of inoculation associated with nitrogen fertilization, showed

a significant effect on the N content of leaves upon inoculation with *Azospirillum* and *Herbaspirillum*.

Several studies show that corn is dependent on nitrogen fertilization, irrespective of whether nitrogen-fixing bacteria are inoculated. However, some research studies, such as Mumbach (2017), state that inoculation can contribute up to 40% of the nitrogen needed for the development of the crop. According to Hungria (2011), the nitrogen fixed by bacteria varies from 30 to 50 kg hectare⁻¹ year⁻¹.

Grain yield varied according to the application method utilized, and there were interactions between the application methods used and the doses levels of biostimulant. 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 and

261 ml ha⁻¹, respectively. For the roller method, the minimum point was at 429 ml ha⁻¹ (Figure 5).

Grain yield was greater when the plants were inoculated at doses of 100 mL ha⁻¹ and 200 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 ml ha⁻¹. The treatments differed statistically and foliar inoculation with 100 mL ha⁻¹ resulted in an increase in grain yield of 632.5 kg ha⁻¹, which corresponds to 10.54 bags ha⁻¹. With foliar bovine gelatin, the grain yield gain reached 1,017.5 kg ha⁻¹ or 16.95 bags ha⁻¹. Therefore, foliar inoculation of *Azospirillum brasilense* may be a viable option for farmers who cannot perform it via seed.

Vorpagel (2010) obtained a gain in

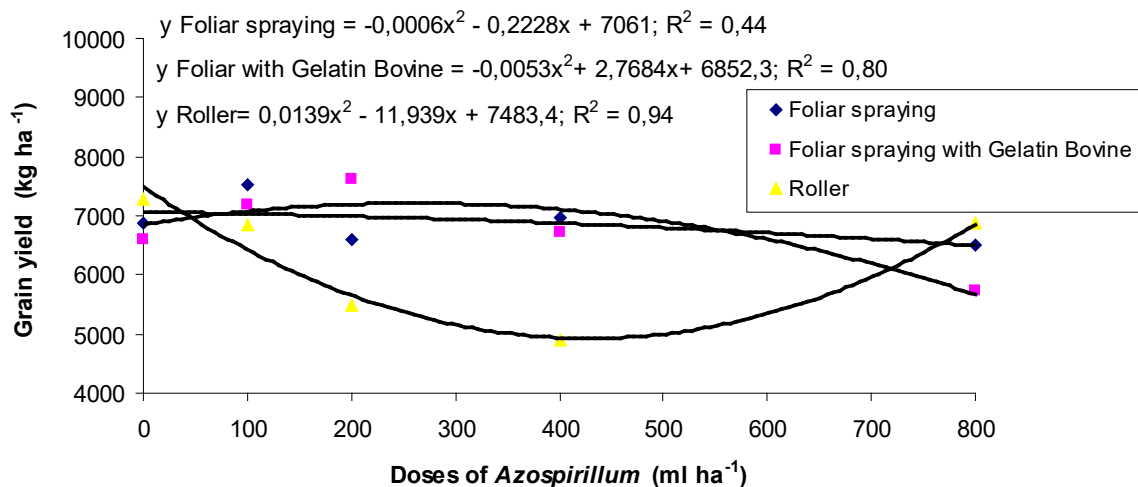


Figure 5. Grain yield (kg ha⁻¹) as a function of application methods and doses of *Azospirillum* inoculant under conditions of off-season corn crop in the 2017/2018 agricultural year. Sinop-MT.

corn grain yield of the order of 227 kg ha⁻¹; however, this was not statistically different when compared with other treatments. Vorpágel 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 to 30%. Portugal et al. (2012), observed an increase of 14.7% in corn grain yield through foliar application of *Azospirillum* at V6 estage.

Conclusions

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 content at the bottom and grain yield, highlighting the methods foliar application and foliar application with bovine gelatin.

The application of *Azospirillum* did not increase the chlorophyll content at the bottom, plant height, leaf area index, N content in the leaves, or the total dry mass. Foliar application with a dose of 100 mL ha⁻¹ showed an increase in grain yield (versus control and other treatments), irrespective of the reduction in chlorophyll content at the bottom of the plant, leaf area, total dry mass, and leaf nitrogen content. The

measurements were obtained at, plant height, leaf area index, N content in leaves or total dry mass.

Foliar application with bovine gelatin at a dose of 200 mL ha⁻¹ also showed an increase in grain yield (versus control and other treatments), irrespective of the reduction in chlorophyll content, plant height, leaf area index, N content in the leaves or total dry mass.

An inoculant dose of 100 mL ha⁻¹, as recommended by the manufacturer, appears to be the most appropriate dose for the crop for increasing grain yield.

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