Maize Yield Response to Nitrogen Rates and Sources Associated with Azospirillum brasilense

Fernando S. Galindo, Marcelo C. M. Teixeira Filho,* Salatiér Buzetti, Paulo H. Pagliari, José M. K. Santini, Cleiton J. Alves, Marcio M. Megda, Thiago A. R. Nogueira, Marcelo Andreotti, and Orivaldo Arf

ABSTRACT
New studies are needed to optimize the nitrogen amount that can be applied to utilize the Azospirillum brasilense benefits in maize cropping systems. In addition, information regarding the interaction between the urease inhibitor N-(n-buty1) thiophosphoric triamide (NBPT) and biological nitrogen fixation (BNF) and how they affect the crop development and yield is also needed. The objective of this study was to evaluate the effect of rates and sources of N in combination with A. brasilense, on leaf chlorophyll index (LCI), N leaf concentration, production components, and maize grain yield in the Brazilian Cerrado region. The study was performed in a Typic Rhodic Hapludox under no-tillage system. The experimental design was a randomized complete block design with four replications arranged in a 2 × 5 × 2 factorial scheme: two N sources (urea and urea with NBPT) and five N rates (0, 50, 100, 150, and 200 kg ha–1), with and without A. brasilense inoculation. The increase in N rates significantly enhance LCI, N leaf concentration, plant height, ear diameter, mass of 100 grains, and grain yield. No significant differences were observed among the N sources. Inoculation with A. brasilense increased LCI, stem diameter, ear length, and nitrogen use efficiency (NUE), with a positive effect on grain yield. The increase in N rate up to 200 kg ha–1 with A. brasilense inoculation increased grain yield, independently of the N source. The application of 100 kg ha–1 of N as urea, with inoculation of A. brasilense produced the highest profit in maize production.

Core Ideas
• Urea with N-(n-buty1 thiophosphoric) triamide provided similar effects to conventional urea in tropical edaphoclimatic conditions.
• Azospirillum brasilense increased maize grain yield, independently of the nitrogen source.
• Seed inoculation increase maize grain yield even with high nitrogen rates.
• Inoculation with A. brasilense increases profit in maize production.

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ammonia volatilization, leading to economic and environmental concerns (Linquist et al., 2013; Abalos et al., 2014). Lara Cabezas et al. (2000) estimated that there may be a 10 kg ha⁻¹ reduction in maize grain yield as a result of the volatilization of 1% of applied N as NH₃. One strategy that has been used to reduce urea hydrolysis and reduce NH₃ losses is by using urease inhibitors, such as of N-(n-butyl) thiophosphoric triamide (NBPT) (Prando et al., 2013). In contrast to results observed in countries located under temperate climatic conditions, the use of NBPT in Brazil has shown no significant improvements in grain yield when compared with conventional urea (Cantarella et al., 2008). Another strategy is the use of diazotrophic bacteria that can fix atmospheric N leading to better crop development, nutrition, and increased grain yield (Díaz-Zorita and Fernandez-Canigia, 2009; Hartmann and Bashan, 2009). The utilization of these plant growth-promoting bacteria are also being increasingly adopted in several countries, in particular Brazil, for crops such as maize, rice, and wheat (Marks et al., 2015; Galindo et al., 2018). Brazil has a long track record for research on biological nitrogen fixation by *Azospirillum* in associations with grasses (Dobereiner and Day, 1975; Hungria et al., 2010; Fukami et al., 2016, 2017; Galindo et al., 2016, 2017a,b,c).

Many researchers have reported that *Azospirillum* produces phytohormones that stimulate root growth in several plant species (Tien et al., 1979; Dardanelli et al., 2008; Pankievicz et al., 2015). Inoculation with *Azospirillum* spp. also has been reported to increase proline content in shoots and roots, improved water potential, increased apoplastic water content, greater cell wall elasticity, increased chlorophyll content, photosynthesis, stomatal conductance, and higher biomass production (Bashan et al., 2006; Barassi et al., 2008); water and mineral absorption, greater stress tolerance (such as drought and salinity), plant vigor, and productivity (Dobbelare et al., 2001); plant growth induction by the production and secretion of phytohormones (Bottini et al., 1989); increased nutrient availability (Hungria et al., 2010); and N content as a result of BNF (de-Bashan et al., 2012). The addition of *A. brasilense* to plants used in the study of Pankievicz et al. (2015) with *Setaria viridis* showed that plants grown in a N-depleted environment developed similarly to plants grown under sufficient N conditions. It has been reported that due to the wide array of mechanisms proposed for the stimulation of plant growth by *Azospirillum* spp., this bacteria might act either in a cumulative or sequential pattern, in a multiple mechanisms theory (Bashan and de-Bashan, 2010). In addition, *Azospirillum* spp. has been reported to increase plant resistance to pathogens by many mechanisms. For example, inhibiting development of bacterial diseases in *Solanum lycopersicum* var. cerasiforme (Romero et al., 2003) and acting as an antifungal against *Colletotrichum acutatum* in strawberry (Tortora et al., 2011).

Although several benefits may be observed when seeds are inoculated with *Azospirillum* spp., an increase in maize grain yield may not be necessarily the case. Analysis of field trials conducted worldwide for over 20 yr where various non-legume crops were inoculated with *Azospirillum* spp under different weather and soil conditions demonstrated that yield can increase up to 30% (Okon and Labanera-Gonzalez, 1994; Fukami et al., 2016, 2017). More studies involving *Azospirillum* spp. are needed to maximize its influence on plant development and productivity. In addition, studies are still lacking to define how much N mineral needs to be applied in combination with *Azospirillum* spp. to achieve maximum maize grain yield. In addition, it would also be valuable to determine the potential of using *Azospirillum* spp. in combination with NBPT in maize evaluating NUE. The hypothesis of this study was that inoculation with *Azospirillum brasilense* would increase NUE even when associated with high N rates. Therefore, the objective of this research was to evaluate the effect of different N rates and sources on maize productivity parameters in association with *A. brasilense* in the Brazilian Savannah (Brazilian Cerrado).

**MATERIAL AND METHODS**

**Location and Management History**

This research was conducted for two consecutive years during the 2013–2014 and 2014–2015 cropping seasons at an experimental area located in Selvíria, MS, Brazil (20°22’S, 51°22’W, 335 m altitude). The experiment was set up on a Typic Rhodic Haploxudox, according to the USDA (2010), or Latossolo Vermelho distrófico, according to Embrapa (2013). This experimental site had been used for annual crop production for more than 27 consecutive years, and for the last 10 yr the experimental site was managed in a no-tillage system. In 2013, the experimental site was cultivated with oats and wheat in succession prior to planting maize at the beginning of the study. The long-term average temperature in this location was 23.5°C, rainfall was 1370 mm, and the relative humidity was between 70 and 80%. The data related to the climatic conditions during the period when the study was conducted is reported in Fig. 1.

A composite soil sample was collected from the entire experimental field for initial assessment of the soil properties. Soil particle size analysis (depth of 0–0.20 m) showed that the particle distribution was 433, 90, and 471 g kg⁻¹ for clay, silt, and sand, respectively. Soil chemical properties were determined in the surface layer (0–0.20 m) according to methodology proposed by van Raij et al. (2001) and total N also was determined by the regular Kljeldahl method using a block digester and determined, followed by diffusion with NaOH (Bremer and Keeley, 1966; Stevens et al., 2000) (Table 1). Based on the soil analysis and to increase the base saturation to 70%, as recommended by Cantarella et al. (1997), 2.5 t ha⁻¹ of dolomitic limestone (total neutralizing power of 88%) was applied on the experimental area 65 d before maize sowing in 2013 without incorporation. At planting, 400 kg ha⁻¹ of a formulated fertilizer (08–28–16, N-P₂O₅–K₂O) was applied, which supplied 32 kg ha⁻¹ of N (urea), 112 kg ha⁻¹ P₂O₅ (triple superphosphate), and 64 kg ha⁻¹ of K₂O (potassium chloride) to all treatments. This application was based on the soil analysis and maize requirements in the region and was performed in both the 2013–2014 and in 2014–2015 cropping seasons. Glyphosate (1.8 kg ha⁻¹ of a.i.) and 2,4-D (0.67 kg ha⁻¹ of a.i.) were applied 15 d before maize planting. The herbicides tembotrione (84 g ha⁻¹ of a.i.) and atrazine (1000 g ha⁻¹ of a.i.) along with the addition of an adjuvant oil (720 g ha⁻¹ of a.i.) were sprayed on 1 Jan. 2014 and 28 Dec. 2014 for the control of post-emergence weeds. Insect control was performed with methomyl (215 g ha⁻¹ of a.i.) and triflumuron (24 g ha⁻¹ of a.i.), on 15 Jan. 2014 and 11 Jan. 2015. The area was irrigated by a central pivot type sprinkler system, with a mean
water depth of 14 mm and irrigation time of approximately 72 h in both cropping seasons, when necessary.

**Experimental Design and Treatments Application**

The experiment was set up in a randomized complete block design (RCBD) with four replications arranged in a factorial scheme 2 × 5 × 2: two sources of N (conventional urea, with 45% of N, and Super N– urea with NBPT (45% N); five N rates (0, 50, 100, 150, and 200 kg ha–1) hand applied; with and without seed inoculation with *Azospirillum brasilense*. The experimental unit was composed of seven rows of maize measuring 6 m in length spaced at 0.45 m. However, to avoid interferences due to edge effect, the useful area for data collection was the five central rows excluding 0.5 m from each end of the plot. Maize seeds were inoculated with *Azospirillum brasilense* strains Ab-V5 and Ab-V6. Inoculants consisted of a mixture of strains CNPSo 2083 (= Ab-V5) and CNPSo 2084 (= Ab-V6) of *A. brasilense* from the Collection of Diazotrophic and Plant Growth Promoting Bacteria of Embrapa Soja (WFCC #1213, WDCM #1054, guarantee of 2 × 10⁸ CFU mL⁻¹) at the rate of 200 mL of inoculant [liquid] per hectare, 1 h before sowing the crop. Planting took place on 4 Dec. in 2013–2014 and 2 Dec. in 2014–2015, and plots were seeded to the triple hybrid DKB 350 VT PRO (resistant to *Spodoptera frugiperda*). Nitrogen treatments were manually applied without soil incorporation on 8 Jan. 2014 and 4 Jan. 2015, when the plants had six leaves completely unfolded (V6). Harvest took place on 28 Mar. 2014 and 4 Apr. 2015, or at 108 and 120 d after maize emergence, respectively.

**Data Collection**

Several growth parameters were measured during the growing season, including (i) N concentration in the ear leaf (the middle third of 20 leaves per plot were collected and analyzed according to Cantarella et al. [1997]); (ii) leaf chlorophyll index (LCI) (in

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**Table 1. Soil chemical attributes in the arable layer in Selvíria, MS, Brazil, 2016–2017.**

| P, resin | S, SO₄ | OM† | pH | K | Ca | Mg | pH+Al | Al | B | Cu | Fe | Mn | Zn | V† | Total N |
|---------|--------|-----|----|---|----|----|-------|----|---|----|----|----|----|----|-----|--------|
| mg dm⁻³ | g dm⁻³  |     |    |  |    |    | mmol dm⁻³ |   |   |    |    |    |    |    |     | g kg⁻¹  |
| 13      | 6      | 23  | 4.8| 2.6| 13.0| 8.0| 42.0   | 0.0| 0.24| 5.9 | 30.0| 93.9| 1.0| 36  | 1.04  |
| † OM, organic matter; V, base saturation. |
the middle third of ear leaf, evaluating 10 plants per plot were analyzed at flowering stage; (iii) stem diameter measured with a digital caliper at the second internode, in cm; and (iv) height at maturation (R6) measured from the soil surface to the apex of the tassel. In addition, 10 maize ears were collected at the time of harvest for the following evaluations: ear diameter; ear length, determined from the apex to the base of the ear; number of kernel rows in each ear; number of kernels per row; number of kernel per ear; 100 kernel weight determined using a scale with a precision of 0.01 g, at 13% moisture content (wet basis); NUE calculated with the following equation: (grain yield with fertilizer−grain yield without fertilizer)/(amount of N applied), according to Moll et al. (1982); and grain yield, determined by harvesting the plants contained in the useful area of each plot (5 maize rows measuring 5 m). An economic analysis was also performed, evaluating the total operating cost (TOC) of production according to Matsunaga et al. (1976), which consists in the sum of expense costs: operations, inputs (fertilizers, seeds, pesticides, etc.), labor, machinery, and irrigation, called effective operating cost (EOC). Besides EOC, the study considered other expenses and costing interest rates, representing 5% of EOC (Matsunaga et al., 1976), thus resulting in the TOC, which were extrapolated to 1 ha and the total operating profit (OP) of the two crops, calculated as the difference between gross revenue (GR) (in USD), as the product between produced quantity (in number of 60 kg sacks), and mean selling price (in USD) and TOC in (USD) according to Martin et al. (1998).

### Statistical Analysis

All data were initially tested for normality using the Shapiro and Wilk (1965) test. All data were distributed normally (W ≥ 0.90). Data were submitted to analysis of variance (F test) with repeated measures in the compound symmetry model for the covariance parameter. When a significant result was verified by the F test (p ≤ 0.05), the Tukey test (p ≤ 0.05) was used for comparison of means of N sources, inoculation with or without Azospirillum brasilense, and years of study, and adjusted to polynomial regression for the N rates using the SPSS program (SPSS IBM Version 22.0; IBM, Armonk, NY).

### RESULTS

#### Leaf Chlorophyll Index and Maize Leaf Nitrogen Concentration

There were no significant effects of N source on LCI and N leaf concentration (Table 2; Supplemental File S1). Similarly, the inoculation with A. brasilense did not influence N leaf concentration (Table 2; Supplemental File S1).

The interaction between rate × inoculation and inoculation × year was significant for the LCI (Supplemental File S1). Inoculation with A. brasilense improved LCI without N fertilization and with application of 50, 100, and 200 kg ha⁻¹ of N. The LCI increased linearly with increasing N rates when A. brasilense was inoculated, and increased until the rate of 190 kg ha⁻¹ of N in the uninoculated treatments (Fig. 2A). Inoculation with A. brasilense improved LCI compared to the uninoculated treatments in both crops. The LCI in 2014–2015 crop was higher than 2013–2014 crop, both for inoculated or uninoculated treatments (Fig. 2B).

In relation the interaction between N rate × year was significant for N leaf concentration (Supplemental File S1). In the absence of N fertilization and application of 100 kg ha⁻¹, the N leaf concentration in 2013–2014 was higher than 2014–2015 crop. N leaf concentration increased until the rates of 150 and 185 kg ha⁻¹ of N in 2013–2014 and 2014–2015 crops, respectively (Fig. 2C).

#### Nitrogen Use Efficiency, Maize Grain Yield, and Economical Analysis

The interaction between N rate × inoculation and N rate × N source × year was significant for NUE (Supplemental File S1). The inoculation with A. brasilense propitiated more NUE than non-inoculated treatments at the application rates of 50,
The NUE decreased linearly with increasing N rates when \textit{A. brasilense} was inoculated (Fig. 5A). Urea was more efficient than urea with NBPT at the application rate of 50 kg ha\(^{-1}\) of N in 2013–2014 crop, however, urea with NBPT was more efficient than urea at the application rates of 50 and 150 kg ha\(^{-1}\) of N in 2014–2015 crop (Table 3; Fig. 5B). Urea was more efficient at the application rate of 50 kg ha\(^{-1}\) of N in 2013–2014 than 2014–2015 crop, and urea with NBPT was more efficient at the application rate of 150 kg ha\(^{-1}\) of N in 2014–2015 than 2013–2014 crop (Table 3; Fig. 5B). The NUE decreased linearly with increasing N rates when urea was applied in topdressing in the 2013–2014 crop (Table 3; Fig. 5B).

Maize grain yield was significantly affected by the interactions of N rate \(\times\) inoculation and inoculation \(\times\) year (Supplemental File S1). The application of 100 and 150 kg ha\(^{-1}\) of N in topdressing associated with inoculation with \textit{A. brasilense} provided higher maize grain yield when compared to uninoculated treatments at the same N rates (Fig. 5C). Maize grain yield increased linearly with increasing N rates for both inoculated and uninoculated treatments, respectively (Fig. 5C). The inoculation with \textit{A. brasilense} provided higher maize grain yield in both crops, and in 2013–2014 crop the observed yield was higher than 2014–2015 crop for both inoculated and uninoculated treatments (Fig. 5D).

### Table 2. Means, least significant difference, and overall mean of leaf chlorophyll index, N leaf concentration, stem diameter, plant height, ear diameter, ear length, number of rows per ear, grains per row, grains per ear, mass of 100 grains, nitrogen use efficiency, and maize grain yield in function of N rates and sources, inoculation with \textit{Azospirillum brasilense}, and years of study in Selvíria, MS, Brazil.

<table>
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<tr>
<th>N rates (kg ha(^{-1}))</th>
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<th>Plant height</th>
<th>Ear diameter</th>
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<td>2.37</td>
<td>5.14</td>
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<td>0.02</td>
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<th>Mass of 100 grains</th>
<th>Nitrogen use efficiency</th>
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| Overall mean             | 15.31                  | 38.79                   | 595.10                  | 30.99               | 11.38                   | 7606.85    |

† Different letters correspond to a significant difference at 5% probability level (p ≤ 0.05).
The highest TOCs were verified using urea with NBPT (USD $1020.45 and $1027.73 on average, as a function of N rates, without and with inoculation with *A. brasilense* respectively), however the highest OPs were obtained with the use of urea and inoculation with *A. brasilense* (USD $157.09 on average, as a function of N rates) (Table 4). The application of 100 kg ha$^{-1}$ of N in topdressing associated with inoculation with *A. brasilense* provided higher OP, with a profit of USD $360.84 ha$^{-1}$ (Table 4).

**DISCUSSION**

**Leaf Chlorophyll Index and Maize Leaf Nitrogen Concentration**

The N rates applied were absorbed by maize as evidenced by increased N leaf concentration and LCI once N is one of the components of the chlorophyll molecule (Galindo et al., 2016). There have been several studies that reported the positive linear correlation with LCI and increasing N rates in maize crops. Kappes et al. (2013a) applied up to 90 kg ha$^{-1}$ of N as urea, and Kappes et al. (2014) used 0, 50, 100, and 150 kg ha$^{-1}$ of N as urea and both reported a linear relationship between N rate and LCI measurements. Although the LCI values are relatively high in our study, even in the control crops (ranging from 61 to almost 71, respectively), the results are comparable to those reported in the literature. Kappes et al. (2013a) reported LCI values ranging from 45.8 to 62.9, and Kappes et al. (2014) reported LCI values ranging from 55.3 to 62.1.

With respect to N leaf concentration, similar results were obtained by Costa et al. (2012), who observed a linear and positive effect of N rates on N concentration in leaf tissue. It is worth noting that, the N leaf concentration was in the range considered adequate (27–35 g kg$^{-1}$) (Cantarella et al., 1997), even in the control crops (27.21 g kg$^{-1}$). However, it is noteworthy the higher N requirement of maize hybrids of earlier cycle and greater production potential.

The similar result between the N sources for LCI can be attributed to the similar N concentrations of leaves obtained with urea and urea with urease inhibitor NBPT. This could be a result of poor efficiency of NBPT at neutralizing urease enzymes in the soil. Some of the reasons for the inefficiency of NBPT to control urease activity could be because some of the straw from the previous year’s wheat crop remained in the soil surface, and the year was exceptionally hot (Fig. 1). Similarly, other studies have reported no significant differences in yield between urea and improved N sources with slow-release polymers in maize (Queiroz et al., 2011, Valderrama et al., 2011, Galindo et al., 2016).

Regarding the positive results of inoculation in LCI, similarly, Müller et al. (2016) and Galindo et al. (2016) found that maize plants that were inoculated with *A. brasilense* had higher LCI than plants not inoculated. Positive responses to inoculation with this diazotrophic bacteria have been obtained even when the crops are grown under condition that provided adequate amounts of N for optimum growth (Galindo et al., 2017c). This suggests that the positive plant responses to inoculation occurs not exclusive because of BNF in grasses but also because of the production of plant growth hormones, including indoleacetic acid, gibberellin, and cytokinin (Galindo et al., 2017c), that can play an essential role in the promotion of plant growth (Bashan and de-Bashan, 2010). According to Pankievicz et al. (2015), the inoculation with *A. brasilense* can improve development and root system growth in *Setaria viridis* grass due to greater CO$_2$ fixation and lower accumulation.
of photoassimilated carbon in the leaves, which led to greater above ground growth, higher water content in tissue, and less stress. In addition, increased indoleacetic acid production may improve the uptake of nutrients by the higher growth of root system (Hungria et al., 2010).

Maize Biometric Evaluations and Productive Components

Castro et al. (2008) reported that plant height is influenced by the availability of N in the soil since this nutrient participates directly in photosynthetic process and cell division and
expansion. Gross et al. (2006) recommend that N should be applied in one or two applications during the season only, due to the positive effects on plant height and maize grain yield. However, it is worth noting that plant height does not always correlate with productivity since modern hybrids with high productive potential are mostly of lower height (Cruz et al., 2008). In our study, it was observed that increasing N rates led to increased N availability, which possibly increased the N grain accumulation and subsequently increased the plant height, ear diameter, number of grains per spike, mass of 100 grains, and maize grain yield.

With respect to the mass of 100 grains, grain mass is a characteristic influenced by genotype, climatic conditions, and nutrient availability during grain filling stages (Chen et al., 2012). Additionally, for Mello et al. (2017), this productive component is very dependent on N uptake by maize, which reaches the uptake peak during the period between the beginning of flowering and the beginning of grain formation. Nitrogen deficiency in this period may contribute to the formation of grains with lower specific mass due to the non-translocation of adequate amounts of nutrients, which justifies the increased mass of 100 grains observed in the present study with the increase in N rates applied.

Inoculation with *Azospirillum brasilense*, the increase in stem diameter with inoculation is interesting since this morphological characteristic is one that has been more related to the percentage of lodging or plant breakage in maize. In addition, stem diameter has been reported to be an important driver for high yield because the larger the diameter, the greater the capacity for plant to store photoassimilates which contribute to grain filling (Cruz et al., 2008; Lana et al., 2009), which also justifies the increase in LCI, ear length and grain yield verified in the present study as a function of the inoculation with *A. brasilense*. Inoculation with *A. brasilense* also increased ear length compared with uninoculated treatments. It is possible that inoculation with *A. brasilense* favored the development of an improved root system, leading to a higher uptake of water and nutrients, positively influencing the nutritional status of the plant. The amount of water and nutrients to be sent to the ear is directly related to the nutritional status of the plant, the better-nourished maize plant tends to better develop its spike, which is demonstrated in the length.

It is possible that the lack of response to N sources is due to the fact that when irrigating the area, a substantial reduction in N-NH₃ volatilization probably occurred as a result of increased contact between fertilizer and soil particles leading to higher NH₄⁺ adsorption by soil (Silva et al., 1995), and the effect of NBPT in reducing volatilization losses were reduced under high N rates (Silva et al., 2017). In addition, the magnitude of the positive effects associated with the use of urea with NBPT has varied greatly with soil characteristics, crop management, and climatic conditions that alter NH₃ volatilization at the time of fertilizer application and in the early days subsequent to this practice (Cantarella et al., 2008; Tasca et al., 2011). Several studies report that the addition of urease inhibitors to urea retards the NH₃ volatilization peak which, for conventional urea, is concentrated in the first week after the fertilizer application to the soil surface (Cantarella et al., 2008; Rochette et al., 2009; Tasca et al., 2011). In this way, irrigation of the experimental area soon after nitrogen fertilization associated to the rainfall that occurred in the week in which the fertilizers was applied in the first year (45 mm of rainfall between 11 to 16 Jan. 2014, 3 d after the application of nitrogen fertilizers; Fig. 1A) and in the second year (19 mm of rainfall on 14 Jan. 2015, and 21 mm on 21 Jan. 2015, 10 and 17 d after the application of nitrogen fertilizers, respectively; Fig. 1B) effectively contributed to minimize
the volatilization losses of urea, providing a similar effect to urea with NBPT. Therefore, the use of urea with NBPT in hot days and in weeks with dry conditions, a common climatic condition between April and September in the Brazilian Savannah could be very advantageous, elucidating new studies.

Studies involving the use of polymer-coated urea compared to conventional urea have shown similar effect (Queiroz et al., 2011; Mello et al., 2017). Additionally, Valderrama et al. (2011), comparing the effect of conventional urea and soluble polymer coated urea did not find advantages with the encapsulation of urea with polymers for maize grown in the Brazilian Cerrado. As the sources did not influence the main evaluations performed, urea becomes more advantageous due to its better cost-benefit ratio, in agreement with Queiroz et al. (2011) and Maestrelo et al. (2014).

On the other hand, unlike in the present study, Abalos et al. (2014) support the hypothesis that the use of the urea inhibitor NBPT is the most appropriate option if losses through NH₃ volatilization are expected to be high. According to Abalos et al. (2014), under conditions where high inputs of N fertilizer are applied and that favors high drainage, with irrigated systems the efficiency of the urea with inhibitor NBPT can be higher. However, the author concluded that new studies are needed to improve our understanding of the conditions under which the improved efficiency fertilizers are economically viable and to compare their efficiency with that of other options, such as improved water and N fertilizer management.

**Nitrogen Use Efficiency, Maize Grain Yield, and Economical Analysis**

The reduction in NUE as a function of N rates increasing can be attributed to the loss of N, as clearly described in the literature. Greater N rates result in greater losses and less utilization by

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Fig. 5. (A) Nitrogen rates and inoculation with *Azospirillum brasilense* interaction for nitrogen use efficiency (NUE); (B) N rates, sources and years of study interaction for NUE; (C) N rates and inoculation with *Azospirillum brasilense* interaction for maize grain yield; and (D) inoculation with *Azospirillum brasilense* and years of study interaction for maize grain yield in Selvíria, MS, Brazil. Error bars indicate the standard error of the mean (n = 4). Different letters indicate a difference between means, according to the Tukey test (p < 0.05).
the crops since plant nutritional demand is limited (Galindo et al., 2016). Plants are able to absorb a certain quantity of nutrients in a certain time; the N that is applied and is not absorbed can be lost, decreasing the efficiency of fertilization with higher N rates, as stated in the literature as the law of diminishing returns.

The increase in NUE due to the inoculation with *Azospirillum brasilense* was accentuated. On average, the NUE provided by the inoculation was 3.5 times higher than the NUE of the non-inoculated. According to Cormier et al. (2013), two strategies may be devised for NUE improvement: maintaining high yield when reducing N supply and/or increasing yield at a constant N supply. Based on the results obtained, the inoculation with *Azospirillum brasilense* is a very interesting strategy in N efficiency improvement and can be used to decrease N fertilizer rates applied, maintaining the same NUE and without affect negatively grain yield.

Concerning grain yield, in several studies increases in maize grain yield have been reported with the application of increasing N rates (Kitchen et al., 2009; Holland and Schepers, 2010; Venterea et al., 2011; Kappes et al., 2014; Galindo et al., 2016), supporting the results observed in the current study. Also, Galindo et al. (2016) verified that the highest maize grain yield was obtained when N was supplied in larger rates at the topdressing time, and that the NH₃ volatilization losses of urea that occur in irrigated maize crop did not reduce the grain yield. According to the authors, there was N available in the soil solution in the period in which the plant requires higher amounts of the nutrient. An explanation would probably be that the N applied at sowing is already in the soil solution and, when N is added, the plant has a larger amount of the nutrient to be absorbed.

It was verified an increase in maize grain yield as a function of inoculation compared to the non-inoculated treatments verified in the present study was 1012.05 kg ha⁻¹ (equivalent to 14.3%). In addition, based on the results obtained, even with the application of high N rates associated with inoculation with *Azospirillum brasilense* maize grain yield was not negatively affected, indicating that high N rates do not eliminate the benefits of inoculation with *Azospirillum brasilense*. Kappes et al. (2013b) reported that maize inoculated with *Azospirillum brasilense* had a 9.4% increase in yield. Cavallet et al. (2000) reported a 17% increase in maize yield when the seeds were inoculated with *Azospirillum* spp. Galindo et al. (2018) verified a 5.7% increase in maize grain yield by seed inoculation with *Azospirillum brasilense*, using five N rates in topdressing, with an average grain yield above 9826 kg ha⁻¹. Similar results were obtained by Müller et al. (2016), where maize yields were 3.8% higher with seed inoculation of *Azospirillum brasilense* when compared to the control, with an average grain yield above 11,000 kg ha⁻¹. Hungria et al. (2010) also obtained increases in maize yield on the order of 27%, corresponding to 743 kg ha⁻¹.

According to Hungria (2011), the effects of maize seed inoculation on grain yield depend on the genetic characteristics of plants and strains (bacteria) in addition to the environmental conditions. In this sense, the interaction between genotypes with efficient strains of bacteria is the key fact for the success of biological N fixation on grasses (Lana et al., 2012), and can explain the variation in the increase of maize grain yield verified in the literature.

The maize grain yield increased as a result of inoculation with *Azospirillum brasilense* have been commonly attributed to multiple mechanisms, including, but not restricted to, the synthesis of phytohormones (for example auxin, cytokinin and gibberellin), improvement in N nutrition and use, enhancement in leaf photosynthetic parameters, attenuation/minimization of stress, and biological control of some pathogenic agents (Bashan and de-Bashan, 2010). In the present study, the principal evaluations that were positive influenced by inoculation was LCI, stem diameter, ear length, and NUE, which reflected positively in the increase of maize grain yield, and consequently increasing profitability with maize production when inoculated with *Azospirillum brasilense*.

On the subject of the economic analysis, the highest TOCs of the treatments with urea application with NBPT and inoculation with *Azospirillum brasilense* are due to the cost of these agricultural inputs. The average price paid by the farmers was USD $599.33 and $673.40 per ton for urea and urea with NBPT, respectively. For the inoculation with *Azospirillum brasilense*, the expenditure was around USD $3.37 per dose, and two doses were used per hectare in both maize crops, totaling USD $6.74. As urea with NBPT did not lead to an increase in grain yield, the OP was not...
favored either; however, inoculation with *A. brasilense* increased grain yield by 15.7% in the average of the 2 yr of cultivation, and due to the low acquisition and application cost (only 0.71% of TOC; USD $6.74 per ha), resulted in an increase in OP with maize production, regardless N source and rate applied.

Taking into account the cost of N rates applied and the OP provided by them, the application of 100 kg ha⁻¹ of N as urea source associated with *A. brasilense* provided higher profitability in maize production (US$ 360.84), while in the absence of inoculation, the highest profitability was obtained without nitrogen fertilization (US$ 174.88), difference of 106.34% in the OP, reiterating the importance of inoculation with *A. brasilense* increasing NUE, grain yield, and profitability with maize crop.

Brazil is the third largest producer and second largest exporter of maize in the world, with about 16.5 million ha cultivated (CONAB, 2018). Thus, based on the increase in profitability obtained by inoculation with *A. brasilense* in maize crop, the adoption of this technology by the farmers and due to the large volume and area of production, it is possible to increase the profits obtained with this activity in the order of millions of dollars per year, positively impacting the Brazilian agricultural production system. This can be extrapolated to tropical conditions in the future and spread in several countries, benefiting the world agriculture.

The results obtained demonstrate a benefit in maize grain yield as a function of seed inoculation with *A. brasilense*. In function of low economic cost, ease of application, non-toxic to the environment, and with a high potential of response from the maize crop, even with the application of N rates considered high for BNF, the inoculation with *A. brasilense* likely to be a technology increasingly used by farmers.

**CONCLUSIONS**

Urea with and without the urease inhibitor NBPT had a similar effect on the biometric evaluations, productive components, and maize grain yield. Increasing N rates positively influenced LCI, N leaf concentration, plant height, ear diameter, number of grains per ear, mass of 100 grains, and grain yield. Inoculation with *Azospirillum brasilense* increased LCI, stem diameter, ear length, and NUE, with a positive effect on maize grain yield and operating profit, without increasing the N concentration in the leaf. Therefore, the application of 100 kg ha⁻¹ of N as urea source, with inoculation of *Azospirillum brasilense* is recommended to obtain the highest profitability in maize production.

Inoculation with *Azospirillum brasilense* is a technology that will be increasingly used by farmers due to the ease of application and acquisition, low cost, and non-polluting technique in addition to increased NUE, grain yield, and profitability to agricultural activity.

**REFERENCES**


