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Dose of *Azospirillum brasilense* in single maize and intercropped with brachiaria in different soils

Amanda Gonçalves Guimarães[1*], Gessí Ceccon[2], Denise Prevedel Capristo[3], Odair Honorato de Oliveira[3], Ricardo Fachinelli[3]

[1] Universidade Federal de Santa Catarina, Curitibanos, SC, Brasil. E-mail: amandagguimaraes@yahoo.com.br
[2] Embrapa Agropecuária Oeste, Dourados, MS, Brasil. E-mail: gessi.ceccon@embrapa.br
[3] Universidade Federal da Grande Dourados, Dourados, MS, Brasil. E-mail: denise_prevedel@hotmail.com; odairhonorato2020@gmail.com; rfachinelli@hotmail.com

ABSTRACT: The intercropped of cultures and biological organisms are sustainable ways to produce due to the benefits for the environment, soil, and plant, in addition to reducing costs in cultivation. The objective was to evaluate the influence of doses of *Azospirillum brasilense* in two soil classes in the cultivation of single maize and in intercropping with *Brachiaria ruziziensis*. The experimental design was in randomized block, in a 2 × 2 × 3 factorial scheme, with six replications. First factor being two soils: Latossolo Vermelho Eutrófico (clayish) and Latossolo Vermelho Distrófico (sandy); the second factor two types of cultivation: single maize and intercropped with *B. ruziziensis*; the third inoculation factor: without inoculant, with one dose (120 mL per 50 kg of seed), and with two doses of *A. brasilense*. The potential of clayey soils had high values in relation to sandy soil for the trait hundred grain weight, and, also when associated in intercrop of maize with brachiaria for plant height, number of grains per ear had higher values than in sandy soils. One or two doses of *A. brasilense* in clayey soil and intercropped of maize with brachiaria resulted in higher maize grain yield.

Key words: cultivation systems; growth-promoting bacteria; *Zea mays*

Doses de *Azospirillum brasilense* em milho solteiro e consorciado com braquiária em diferentes solos

RESUMO: O consórcio de culturas e organismos biológicos são formas sustentáveis de produzir devido aos benefícios para o meio ambiente, solo e planta, além de reduzir custos no cultivo. Objetivou-se avaliar a influência de doses de *Azospirillum brasilense* associadas a duas classes de solo no cultivo de milho solteiro e em consórcio com *Brachiaria ruziziensis*. O delineamento experimental foi em blocos ao acaso, em esquema fatorial 2 × 2 × 3, com seis repetições, sendo o primeiro fator dois solos: Latossolo Vermelho Eutrófico (argiloso) e Latossolo Vermelho Distrófico (arenoso); o segundo fator dois tipos de cultivo: milho solteiro e milho consorciado com *B. ruziziensis*; o terceiro fator foi de inoculação: sem inoculante, com uma dose (120 mL por 50 kg de semente) e com duas doses de *A. brasilense*. O potencial dos solos argilosos apresentou valores elevados em relação ao solo arenoso para a característica massa de cem grãos, e, também quando associado em consórcio de milho com braquiária para altura de plantas, o número de grãos por espiga apresentou valores superiores aos dos solos arenosos. A inoculação com uma ou duas doses de *A. brasilense* em solo argiloso e consorciado de milho com braquiária resultou em maior produtividade de grãos de milho.

Palavras-chave: sistemas de cultivo; bactérias promotoras de crescimento; *Zea mays*

* Amanda Gonçalves Guimarães - E-mail: amandagguimaraes@yahoo.com.br (Corresponding author)
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Introduction

Maize (*Zea mays* L.) plays an important role in crop rotation and intercropping. In intercropping with forages, it is a good alternative to protect the soil against erosion, brings benefit to the chemical and biochemical parameters of the soil and to mycorrhizal symbiosis, allowing a greater cycling of nutrients (Méndez al., 2019).

The intercropping of maize with brachiaria has a high carbon/nitrogen in the soil ratio and may have nitrogen deficiency (N) and thus lower grain yields, as N is one of the main nutrients linked to crop growth (Sugihara et al., 2014). To supply this need, the use of nitrogen fertilizers has been an adopted practice, however, the industrial process of production of these fertilizers has high costs, which has been driving the search for natural sources of nitrogen (Ferreira et al., 2020; Guimarães et al., 2020).

The use of inoculants based on plant growth-promoting bacteria, such as those of the genus *Azospirillum*, has been an option to mitigate the use of N in the form of chemical fertilizers (Oliveira et al., 2018). These bacteria fix atmospheric N and transform it into ammonium, facilitating its assimilation by plants, producing, and releasing growth-regulating substances such as auxins, gibberellins and cytokinins (Repke et al., 2013).

In maize, the inoculation of *Azospirillum*, showed increases in grain yield of approximately 26% (Hungria et al., 2010; Hungria, 2011; Novakowisk et al., 2011), or reduced need for nitrogen fertilization by 50% (Guimarães et al., 2017). However, some questions about the efficiency of the use of *Azospirillum* in maize are: the type of maize genotype, strains of bacteria, doses of inoculant and soil and climate conditions (Quadros et al., 2014; Oliveira et al., 2018; Chaves et al., 2019; Galeano et al., 2019; Oliveira et al., 2022).

Chaves et al. (2019), in studies on the genetic diversity of various isolates of endophytic bacteria in maize and wheat, showed that the plant genotype can limit or induce the endophytic microbiota and that the plant microbiota is highly influenced by soil fertility. Galeano et al. (2019), in studies with inoculation of the isolates MAY1, MAY12, and BR-11001, and of the commercial inoculum (strains AbV5 and AbV6) for the initial development in maize, at 23 days after sowing, found that the dry mass of the shoot was higher with inoculation of MAY1 and the commercial inoculant.

As for the type of soil the use of bactérias, shown efficiency, as those in soils 'Argissolo Vermelho distrófico típico', with the inoculation of a mixture of three species of *Azospirillum* (*A. brasilense* (EL-S), *A. lipoferum* (LG1-R and L-S), and *A. oryzae* (MS)) in peaty medium with 200 mL of inoculum per kilogram increased the shoot dry matter yield of the AS 1575 and SHS 5050 (Quadros et al., 2014). Also, in 'Latossolo Amarelo distrófico', with the inoculation of *A. brasiliense* (Ab-V5 and Ab-V6 in concentration of 108 cells mL^{-1}, applied at a rate of 100 mL on 60,000 seeds), increased maize yield in BRS Caimbé and AG 1051 maizes (Oliveira et al., 2018).

Studies have mentioning that certain grasses, such as brachiaria, in successive crops can be hosts of diazotrophic bacteria native to the soil, which requires studies of intercropped maize and brachiaria crops (Hungria, 2011). Oliveira et al. (2022) concluded that the annual reinoculation with *A. brasilense* (AbV5 and AbV6 strains, with 2 × 10^{8} CFU mL^{-1}) results in a higher AG8480 maize grain yield in the intercropping with *Brachiaria ruziziensis* in the soil 'Latossolo Vermelho Eutrófico' (clayish).

However, there are few studies on the inoculant dose, soil types and cultivation system, whether single maize or intercropped with brachiaria. Thus, the objective of this study was to evaluate the influence of doses of *A. brasilense* in two soil classes in the cultivation of single maize and in intercropping with *B. ruziziensis*.

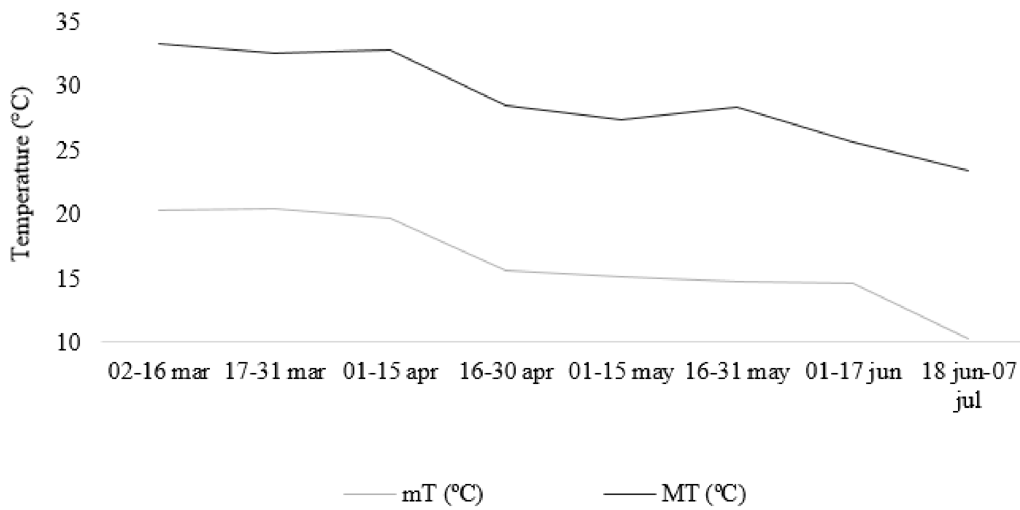
Materials and Methods

The experiment was carried out at Embrapa Agropecuária Oeste, in the municipality of Dourados, State of Mato Grosso do Sul, Brazil, located at coordinates 22° 16' 33.1" S, 54° 112.48' 55.6" W, and altitude of 430 m, with a humid mesothermal climate with hot summers and dry winters according to Köppen-Geige (Fietz et al., 2017). The experiment was carried out in the autumn-winter of 2021 in a non-air-conditioned screened house, with glass cover, in order to allow ambient light, and with sides coated with galvanized wire, allowing free air circulation, with temperatures recorded at the climatological station (Figure 1).

The experimental design used was randomized block, in a 2 × 2 × 3 factorial arrangement with six replications, in 60 cm high × 40 cm diameter vases, with 60 kg of soil. The first factor was composed of the two soils classified according to Santos et al. (2018) for clayish texture (16.0% sand and 72.3% clay - 'Latossolo Vermelho eutrófico') and sandy texture (72.6% sand and 22.3% clay - 'Latossolo Vermelho distrófico'); the second factor by cultivation: single maize and intercropped with *B. ruziziensis*; and, the third factor for the inoculations: without inoculant, with one dose, and with two doses of *A. brasilense*.

The implantation of the experiment was carried out on March 3, 2021, with fertilization of 2.5 g vase^{-1} (equivalent to 200 kg ha^{-1}) of the NPK 8-20-20 formula. Six KWS9606 VIP3 single hybrid maize seeds were sown. In the intercropped of maize with brachiaria plots, twenty seeds were sown in the vase, ten on each side. The inoculation treatments, A. brasilense, strains AbV5 and AbV6 with 2 × 10^{8} CFU mL^{-1} (MAPA, 2011), were carried out in the sowing furrow of maize and *Brachiaria*, with a solution equivalent to 120 mL per 50 kg of seed (one dose), and, the treatment two doses, this solution multiplied by two.

Soil moisture was kept close to 80% of the field capacity, applying rainwater, by dripping captured by gutters in the screened house. At 15 days after sowing, thinning was performed, leaving four maize plants and five plants of each side. After 30 days a top-dressing urea fertilization in the same proportion, in all vases.



Source: Guia Clima (2021).

Figure 1. Maximum temperature (MT, °C) and minimum temperature (mT, °C) obtained by the experimental station Embrapa Agropecuária Oeste, located 500 m from screened greenhouse. Dourados, MS, Brazil, 2021.

The maize harvest was carried out at 126 days after sowing and two plants were collected per plot. The following traits were evaluated: plant height (PH, cm), stem diameter (SD, mm), number of grains per ear (NGE), hundred grain weight (HGW, g), and grain yield (GY, kg ha^{-1}).

The results obtained were submitted to analysis of variance and when the difference was detected by the F test, the means of the treatments of the inoculation factor were compared by the Tukey test ($p < 0.05$) using the statistical program Genes (Cruz, 2016).

Results and Discussion

In the maize harvest, in the characteristics evaluated plant height, stem diameter and number of grains per ear there was a significant interaction between soil types and the types of cultivation. For hundred grain weight, there was an effect of soil types, but without significant interaction between the other treatments. There was a significant triple interaction between soil types, cropping systems and inoculation only for grain yield (Table 1).

The potential of clayey soils had high values in relation to sandy soil for the trait hundred grain weight (26.5 g), and, when associated in intercrop of maize with brachiaria for plant height (215.1 cm), number of grains per ear (200.3) had higher values than in sandy soils (Table 2). In sandy soil, the presence of brachiaria plants reduced the number of ear grains and plant height (Table 2).

Quadros et al. (2014) in studies in the municipality of Eldorado do Sul, State of Rio Grande do Sul, Brazil, in soils Argissolo Vermelho distrófico típico, tested P32R48, SHS 5050, and AS 1575 maize, and, the inoculation of a mixture of three species of *Azospirillum* (*A. brasilense* (EL-S), *A. lipoferum* (LG1-R and L-S), and *A. oryzae* (MS)) in peaty medium with 200 mL of inoculum per kilogram. These authors concluded that inoculation increased the shoot dry matter yield of the AS 1575 and SHS 5050 maizes, the weight of 1000 grains of the P32R48 maize and the plant height of the AS 1575 maize. However, in the present study, the inoculation of one or two doses had no influence on plant height or hundred-grain weight, which may be that in the study of Quadros et al. (2014) in addition to having mixtures

Table 1. Summary of the analysis of variance of the characteristics evaluated in the maize harvest in two soil classes (‘Latossolo Vermelho eutrófico’ – clayish and ‘Latossolo Vermelho distrófico’ – sandy), two types of cultivation (single maize and intercropped with *Brachiaria ruziziensis*) and three inoculations (without inoculant, one dose, and two doses of *Azospirillum brasilense*) in 2021. Embrapa, Dourados, MS, Brazil.

Source of variation	Traits				
	Plant height	Stem diameter	Number of grains per ear	Hundred grain weight	Grain yield
Soil (S)	*	ns	*	*	*
Cultivation (C)	*	ns	*	ns	*
Inoculation (I)	ns	ns	ns	ns	ns
S × C	*	*	*	ns	*
S × I	ns	ns	ns	ns	ns
C × I	ns	ns	ns	ns	ns
S × C × I	ns	ns	ns	ns	*
CV (%)	8.28	4.76	22.25	14.67	12.18

CV (%) - Coefficient of variation; ns - Not significant; * Significant ($p < 0.05$).

Table 2. Plant height (PH), stem diameter (SD), number of grains per ear (NGE) and hundred grain weight (HGW), as a function of two soil classes ('Latossolo Vermelho eutrófico' – clayish and 'Latossolo Vermelho distrófico' – sandy) and two types of cultivation (single maize and intercropped with *Brachiaria ruziziensis*) in 2021. Embrapa, Dourados, MS, Brazil.

Treatments	PH (cm)		SD (mm)		NGE		HGW (g)
	Single maize	Intercropping	Single maize	Intercropping	Single maize	Intercropping	
Sandy	214.06 aA	178.9 bB	24.8 aA	24.2 aA	231.3 aA	114.2 bB	22.84 b
Clayish	220.1 aA	215.1 aA	24.1 aB	25 aA	229.8 aA	200.3 aB	26.5 a
CV (%)	8.28		4.76		22.25		14.47

Means with the same lower-case letters in the column and upper case in the row do not differ from each other by the F test (p ≥ 0.05). CV (%) - Coefficient of variation.

of the bacteria of the genus, the maize genotypes used were also different.

Maize showed higher grain yield in clayey soil compared to sandy soil, regardless of the cultivation or dose of *A. brasilense* (Table 3). The inoculation of one or two doses of *A. brasilense* in maize acted beneficially in the clayey soil, either for single maize (4,440 kg ha^{-1} one dose, 5,196 kg ha^{-1} two doses) or intercropped with *B. ruziziensis* (4,016 kg ha^{-1} one dose, 3,907 kg ha^{-1} two doses) in relation to the sandy soil and the highest dose of inoculant in clayey soil with the single maize managed to express its productive potential of grains (5,196 kg ha^{-1}) (Table 3).

This higher maize grain yield in clayey soils is due to the fact that clay soils are more fertile than the sandy ones (Niranjana et al., 2018). When associated with intercrop of maize with brachiaria, the benefits are intensified due to the accumulation of straw in the soil, forming an insulating barrier, decreasing the surface temperature, protecting the soil against the weather conditions and the loss of water by evaporation (Costa et al., 2012). Oliveira et al. (2022) have found similar results in studies with maize AG8480 grown in pots in the municipality of Dourados, MS, Brazil, obtained a higher grain yield is obtained with the anual reinoculation of *A. brasilense* onto maize intercropped with B. ruziziensis in the clayey soil.

In sandy soil, single maize, inoculated with a dose (3,780 kg ha^{-1}) or two doses (3,896 kg ha^{-1}) of *A. brasilense* showed higher grain yield compared to intercrop of maize with brachiaria, while in clayey soil, only with two doses of *A. brasilense*, higher grain yield was observed in single maize (5,196 kg ha^{-1}) compared to intercrop of maize with brachiaria (3,907 kg ha^{-1}) (Table 3).

The double dose of bacteria caused greater productive potential in types of cultivation in these traits, probably increased the production of growth-regulating substances such as auxins, gibberellins and cytokinins that stimulate plant development (Repke et al., 2013). Hungria et al. (2010) established proportions of at least, 10^8 cells of *Azospirillum* mL^{-1} of the liquid product and used the dose of 150 mL per 50 kg of seed and verified that increased grain yields of maize by 24-30%, in relation to non-inoculated control. However, although in the present study, we used twice the proportion, that is, of 2×10^8 cells mL^{-1}, and with inoculations with a dose of 120 mL per 50 kg and two doses of 120 mL per 50 kg we did not verify twice the grain yield of the inoculated treatments compared to the treatment without inoculation. But in relation to one dose and two doses of inoculants in cultures in clayey soils in intercropping, it presented almost twice the productivity with the cultivation of maize in sandy soils in intercropping. Furthermore, double the inoculant dose in single maize in clayey soil was 0.731 kg higher compared to the treatment without inoculate. However, the change in results with different doses of inoculant (high number of colony-forming units) may be related to the competitiveness of the inoculant with a population of microorganisms native to the soil (Moreira et al., 2010).

Already Oliveira et al. (2018), used the same strains of bacteria (Ab-V5 and Ab-V6), but with different doses (concentration of 10^8 cells mL^{-1}, applied at a rate of 100 mL on 60,000 seeds) in different maize genotypes (BRS Caimbé and AG 1051) and soil ('Latossolo Amarelo distrófico'), concluded that the inoculation of *A. brasiliense* in increased grain yield compared to without inoculated plants for both maize cultivars. However, in the present study, it was not possible to observe the double of grain yield.

However, Silva Júnior et al. (2021), in an experiment in Anápolis, State of Goiás, Brazil, in soils 'Vermelho Distrófico' of the LG 3040 PRO2 maize, with inoculant containing *A.*

Table 3. Maize grain yield as a function of two soil classes ('Latossolo Vermelho eutrófico' – clayish and 'Latossolo Vermelho distrófico' – sandy), two types of cultivation (single maize and intercropped with *Brachiaria ruziziensis*) and three inoculations (without inoculant, one dose and two doses of *Azospirillum brasilense*) in 2021. Embrapa, Dourados, MS, Brazil.

Treatments	Grain yield (kg ha^{-1})			
	Sandy		Clayish	
	Single maize	Intercropping	Single maize	Intercropping
Without inoculant	4,164 aAα	1,923 aBβ	4,465 bAα	4,202 aAα
One dose	3,780 aAβ	2,154 aBβ	4,440 bAα	4,016 aAα
Two doses	3,896 aAβ	2,249 aBβ	5,196 aAα	3,907 aBα
CV (%)	12.18			

Means followed by the same letter dont differ by Tukey test significant (p ≥ 0.05). Lower case letters in the column compare inoculations within a cultivation and each soil. Upper case letters in the row compare the cultivation within each inoculation and each soil. Greek letters (α – β) in the row compare soils within each inoculation and each cultivation. CV (%) - Coefficient of variation.

Brasilense (strains AbV5 and AbV6, concentration of 2 × 10^8 CFU mL^{-1}) applied in seed treatment, observed different results. Although the inoculation with *A. brasilense* and a 15% reduction in nitrogen fertilization in coverage, showed efficiency as a promoter of growth and vegetative development, significant effect on plant height and on the green and dry mass of the inflorescence, concluded that inoculation with *A. brasilense* did not present significant results in maintaining yield in the face of reduced nitrogen fertilization, with a 21% reduction in yield. In the present study, we used the same strains of bacteria and concentrations, and similar soils, but different maize genotypes and we did not have differences in the same soil in terms of grain yield, regardless of the cultivation system single or in intercropping. However, it would be necessary to carry out another research involving the bacteria and the doses of nitrogen fertilizers in the same maize genotype present to verify the results.

Thus, given the above it is necessary to study the activity of these bacteria and the applied dose, which are dependent on the plant/bacteria ratio, that is, on the maize genotype, its strains and the cultivated edaphoclimatic regions (Quadro et al., 2014; O'Neal et al., 2020) to maintain or increase yields when inoculated into plants.

Conclusions

The doses of *Azospirillum brasilense* influence maize grain yield as a function of soil and maize cropping system.

Inoculation of *A. brasilense* with one or two doses in clayey soil and intercropped of maize with *Brachiaria ruziziensis* resulted in higher maize grain yields.

Compliance with Ethical Standards

Author contributions: Conceptualization: GC, RF; Data curation: AGG, DPC, OHO; Formal analysis: AGG, GC; Investigation: GC, RF; Methodology: AGG, DPC, OHO; Project administration: GC; Resources: GC, RF; Supervision: GC; Validation: AGG, GC, DPC, OHO, RF; Visualization: AGG, GC, DPC, OHO; Writing – original draft: AGG, DPC, OHO, GC; Writing – review & editing: AGG, GC.

Conflict of interest: The authors declare that are no conflict of interest.

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