

## Inoculation methods and agronomic efficiency of *Azospirillum brasilense* Strain Az39 for corn crop under different Brazilian edaphoclimatic conditions

Anabel González Hernández<sup>1</sup> , Emanuela Pille da Silva<sup>1</sup> , Paulo Ademar Avelar Ferreira<sup>2</sup> ,  
Paulo Emílio Lovato<sup>1</sup> , Guilherme Pereira de Oliveira<sup>3</sup> , Cláudio Roberto Fonsêca Sousa Soares<sup>1\*</sup> 

<sup>1</sup> Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil. E-mail: [anabelgonzalezher@yahoo.es](mailto:anabelgonzalezher@yahoo.es); [manupille@gmail.com](mailto:manupille@gmail.com); [paulo.lovato@ufsc.br](mailto:paulo.lovato@ufsc.br); [crfsoares@gmail.com](mailto:crfsoares@gmail.com)

<sup>2</sup> Universidade Federal de Santa Maria, Cachoeira do Sul, RS, Brasil. E-mail: [paulo.ferreira@ufsm.br](mailto:paulo.ferreira@ufsm.br)

<sup>3</sup> Lallemand Plant Care, Patos de Minas, MG, Brasil. E-mail: [goliveira@lallemand.com](mailto:goliveira@lallemand.com)

**ABSTRACT:** In recent years, the research that look for biological technologies that guarantee sustainable agricultural production and application methods that maximize the benefits of adopting this practice has been increasing. This study aimed to assess, for the first time, the different application methods and agronomic efficiency of *Azospirillum brasilense* Az39 for corn cultivation in Brazil. Four trials were conducted under different soil and climate conditions in the 2017 - 2018 harvests (Santa Maria-RS and Ibirubá-RS, Brazil) and 2019 harvest (Londrina-PR and Primavera do Leste-MT, Brazil). The experimental design used was a randomized block, evaluating three inoculation treatments with 10 replicates each: control = non-inoculated ; TS = inoculation with *A. brasilense* Az39 via corn seed; and Foliar500 = foliar application of *A. brasilense* Az39 at the V4 stage of corn. The *A. brasilense* Az39 strain was effective in Brazilian soils and increased corn grain yield in all evaluated areas. Furthermore, foliar application of this strain at a dosage of 500 mL ha<sup>-1</sup> resulted in greater increases compared to seed treatment. Thus, the *A. brasilense* Az39 strain is a promising bio-input technology for Brazilian agriculture.

**Key words:** bio-inputs; foliar spraying; plant growth-promoting bacteria; seed treatment; *Zea mays* L.

## Métodos de inoculação e eficiência agrônômica da estirpe *Azospirillum brasilense* Az39 para a cultura do milho em diferentes condições edafoclimáticas brasileiras

**RESUMO:** Nos últimos anos tem sido crescente a busca por tecnologias biológicas que garantam uma produção agrícola sustentável e métodos de aplicação que maximizem os benefícios da adoção desta prática. O presente estudo teve como objetivo avaliar de forma inédita diferentes métodos de aplicação e a eficiência agrônômica de *Azospirillum brasilense* Az39 para a cultura do milho no Brasil. Para tanto, ensaios foram conduzidos em condições edafoclimáticas distintas nas safras 2017 - 2018 (Santa Maria-RS e Ibirubá-RS, Brasil) e safra 2019 (Londrina-PR e Primavera do Leste-MT, Brasil). O delineamento experimental utilizado foi em blocos casualizados, avaliando-se três tratamentos de inoculação com 10 repetições, sendo estes: Controle = não inoculado; TS = inoculação de *A. brasilense* Az39 via semente de milho; Foliar500 = aplicação foliar de *A. brasilense* Az39 no estágio V4 do milho. Foi verificado que a estirpe *A. brasilense* Az39 foi eficiente em solos brasileiros, proporcionando aumentos na produtividade do milho em todas as áreas avaliadas. A aplicação foliar desta estirpe na dosagem de 500 mL ha<sup>-1</sup> proporcionou incrementos superiores aos obtidos com o tratamento de sementes. Desta forma, a estirpe *A. brasilense* Az39 demonstrou ser uma tecnologia de bioinsumo promissora para a agricultura brasileira.

**Palavras-chave:** bioinsumos; pulverização foliar; bactérias promotoras do crescimento vegetal; tratamento de sementes; *Zea mays* L.



## Introduction

Since the Green Revolution, farmers have increasingly depended on industrial inputs for soil fertilization and phytosanitary control of their crops to achieve higher yields (Villarreal-Delgado et al., 2018). Over the past few decades there has been an increase in the consumption of nitrogen fertilizers; however, this did not result in a proportional increase in crops yield (Hirel et al., 2011). Moreover, there are economic and environmental limitations related to synthetic inputs, especially nitrogen fertilizers, which are expensive and have low crop utilization efficiencies.

Corn cultivation has shown significant growth in both Brazilian and global agricultural scenarios. The estimated production in Brazil for the 2023/24 harvest was 118.53 million tons (Conab, 2023). This crop is characterized by a high demand for nitrogen fertilizers. Owing to the high cost of nitrogen fertilizers and the environmental impacts associated with their agricultural application, the adoption of new technologies based on biological inputs is necessary to ensure sustainable agricultural production (Telles et al., 2023).

Bio-inputs based on plant growth-promoting microorganisms show potential to reduce the use of chemical fertilizers, improve soil quality, and increase crop yield through direct and indirect mechanisms (Ma et al., 2018). Direct mechanisms are related to improving the absorption and availability of nutrients for plants, while indirect mechanisms involve the control of pests and diseases (Glick, 2012; Soares et al., 2023). Owing to the wide range of benefits for plants, plant growth-promoting bacteria (PGPB) have been increasingly used as bio-input formulations in agriculture worldwide (Santos et al., 2021).

Among the best-known PGPB, *Azospirillum brasilense* was first isolated in Brazil from the rhizosphere of *Digitaria decumbens* (Dobereiner et al., 1976), placing Brazil as a pioneer and leader in studies of this genus (Ferreira et al., 2022). The first commercial inoculant based on *A. brasilense* was introduced into the Brazilian market in 2009. This inoculant mostly consisted of a combination of strains Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084), which are natural variants obtained from the Sp7 strain of *A. brasilense* (Santos et al., 2021). The benefits of this inoculation include gains in root growth, increased plant vigor, enhanced mineral nutrition, and induced tolerance to abiotic and biotic stressors. Such improvements are mainly associated with biological nitrogen fixation (BNF) and phytohormone synthesis (Scudeletti et al., 2023).

Bacteria of the *Azospirillum* genus has been used as inoculants for various crops worldwide. The *A. brasilense* Az39 strain was isolated from the rhizosphere of wheat in 1982 in Córdoba, Argentina (Díaz-Zorita & Fernández-Canigia, 2009), and was selected for its ability to increase yields of wheat and corn (Cassán & Díaz-Zorita, 2016). Perrig et al. (2007) have identified a multitude of bioactive molecules in this strain that can affect crops development,

including zeatin, gibberellic acid, abscisic acid, ethylene, putrescine, spermine, spermidine, and cadaverine. Recent taxonomic studies based on genome sequencing have proposed the reclassification of this strain as a new species, *A. argentinense* sp. (Ferreira et al., 2022). The *A. brasilense* Az39 strain has been successfully used for several decades in Argentina and is the active ingredient in more than 50% of the inoculants currently used in that country (Cassán & Díaz-Zorita, 2016).

*Azospirillum* sp. should be evaluated under multiple edaphoclimatic conditions for a comprehensive and robust understanding of the benefits of inoculation. Production systems should also consider management conditions that maximize the benefits of inoculation. Seed treatment is the most commonly used method of *A. brasilense* inoculation in corn cultivation. However, recent studies have shown a positive effect when the inoculant is applied to the planting furrow or via foliar application (Santini et al., 2018). Foliar application of *A. brasilense*, unlike to other inoculation methods, allows for more intimate contact between bacteria and plant, enabling colonization of the inside of the leaf blade and more effective involvement in biological nitrogen fixation (BNF) and phytohormone production (Preininger et al., 2018). Other benefits associated with the foliar application of *Azospirillum* sp. include the absence of competition with soil microbiota and negative interactions with physicochemical soil factors that interfere with bacterial survival and symbiotic efficiency. Besides, this application method avoids incompatibility of the inoculant with agrochemicals used in seed treatment (Bashan et al., 1995; Fukami et al., 2016; Cardozo et al., 2022).

Considering that the Az39 strain of *A. brasilense* has not yet been evaluated for its potential benefits in agricultural production under Brazilian edaphoclimatic conditions, the present study aimed to assess the application methods and agronomic efficiency of *A. brasilense* Az39 for corn cultivation in Brazil. The hypothesis of the present study is that inoculation methods significantly impact the agronomic efficiency of the *A. brasilense* Az39 strain in corn cultivation in different Brazilian edaphoclimatic conditions.

## Materials and Methods

The field experiments were conducted at four experimental stations, located in the municipalities of Santa Maria-RS (2017-2018 harvest), Ibirubá-RS (2017-2018 harvest), Londrina-PR (2019 harvest) and Primavera do Leste-MS (2019 harvest) to evaluate different inoculation methods of a commercial biological product (LaRise Azos<sup>SC</sup>) based on *A. brasilense* strain Az39 ( $1.0 \times 10^9$  CFU mL<sup>-1</sup>) for corn cultivation. Agronomic validation of this inoculant followed the protocols established by the Ministry of Agriculture, Livestock, and Food Supply (MAPA) for the registration of products based on plant growth-promoting microorganisms, as outlined in IN SDA 13, of 25/03/2011, and IN SDA 53, of 24/10/2013. Table 1 lists the experimental implementation

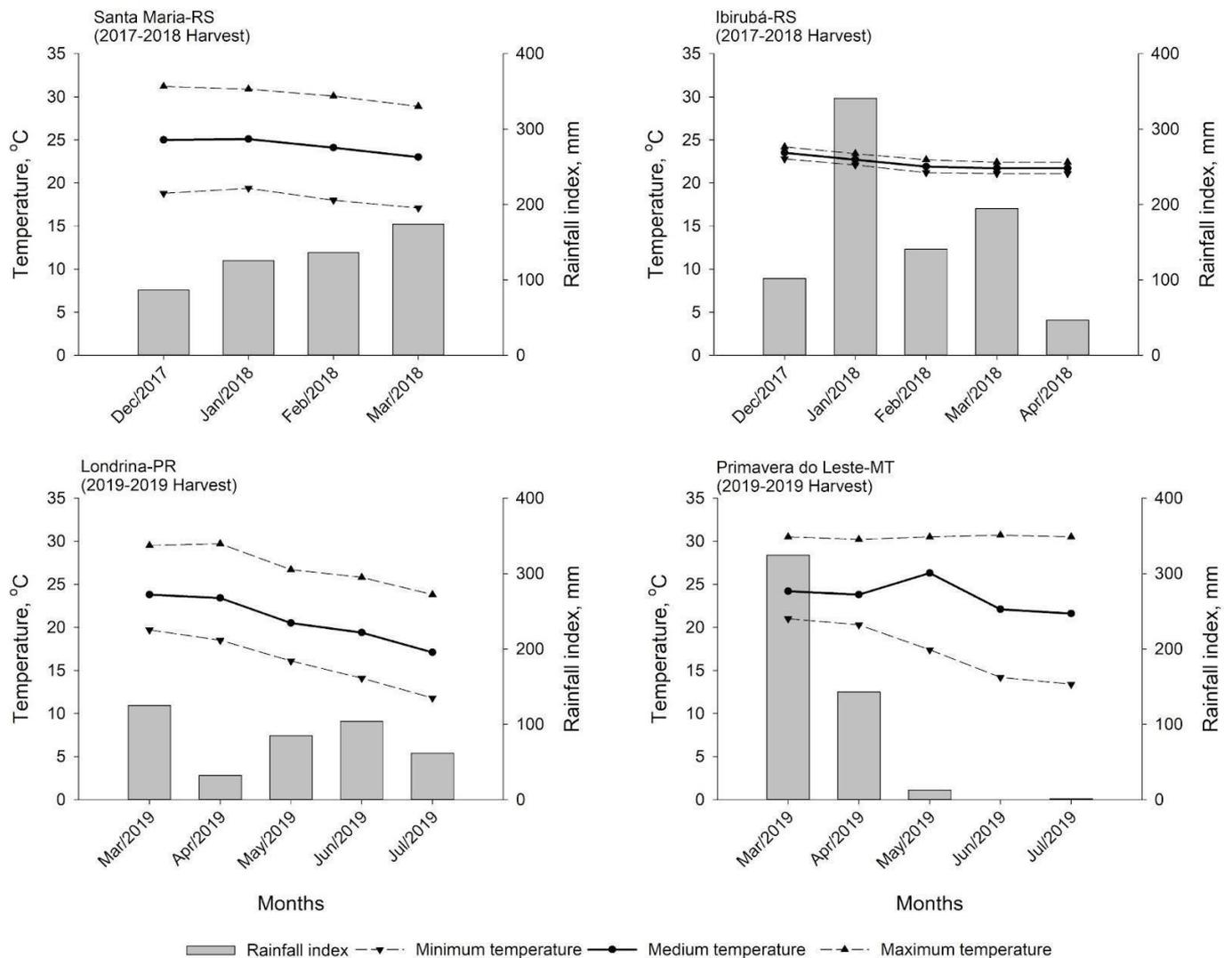
**Table 1.** Physico-chemical soil characterization of experimental areas for agronomic validation of the *Azospirillum brasilense* Az39 inoculant for corn crops.

Location/Harvest	Coordinates	pH H <sub>2</sub> O	Ca	Mg (cmol <sub>c</sub> dm <sup>-3</sup> )	K	Al	P (mg dm <sup>-3</sup> )	C <sub>org</sub> (g dm <sup>-3</sup> )	Clay (g dm <sup>-3</sup> )	Soil classification (FAO; Soil Taxonomy)
Santa Maria-RS (2017-2018)	29°43'39.76"S; 53°33'20.08"W	5.5	14.1	5.6	0.28	0.0	23.8	19.1	280	Arenic - Profondic - Rhodic Acrisol; Rhodic Paleudalf
Ibirubá-RS (2017-2018)	28°66'24.52"S; 53°10'44.75"W	5.4	13.6	7.2	0.32	0.2	18.4	21.5	360	Rhodic Ferralsol; Rhodic Hapludox
Londrina-PR (2019-2019)	23°15'22.98"S; 51°06'05.82"W	5.5	8.0	2.3	0.37	0.0	4.1	19.4	562	Rhodic Ferralsol; Rhodic Hapludox
Primavera do Leste-MT (2019-2019)	15°25'52.17"S; 54°12'44.09"W	6.0	2.8	1.4	0.10	0.0	29.7	15.5	390	Umbric - Humic - Chromic Acrisol; Typic Haplohumult

coordinates and soil physicochemical characteristics. Information on climatic conditions (temperature and rainfall) during the experimental period is shown in [Figure 1](#). The corn cultivars used in the trials were those recommended for each region, with the Dekalb 290 VT PRO3 hybrid used in the Santa Maria-RS, Ibirubá-RS, and Londrina-PR trials and the NS 77 PRO 2 hybrid from Nidera used in the Primavera do Leste-MT trial. Planting and topdressing fertilization were

performed according to the recommended systems for each region; the quantities applied are presented in [Table 2](#).

A randomized block design trial was conducted at each location to evaluate three inoculation treatments with 10 replicates each: control = non-inoculated control; TS = inoculation of *A. brasilense* Az39 via corn seed; and Foliar500 = foliar application of *A. brasilense* Az39 at the V4 stage of corn. For this purpose, experimental plots of 28.0 m<sup>2</sup> (7.0 ×

**Figure 1.** Climatological data of temperature (°C) and rainfall (mm) during the period of conducting trials to evaluate the inoculation of *Azospirillum brasilense* Az39 in corn crops.

**Table 2.** Sowing and midcycle top dressing fertilization in trials conducted for corn crops to evaluate inoculation of *Azospirillum brasilense* strain Az39.

	Planting fertilizer	Top dressing fertilization*
Santa Maria-RS	15 kg ha <sup>-1</sup> N 60 kg ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> 45 kg ha <sup>-1</sup> K <sub>2</sub> O	40 kg ha <sup>-1</sup> N (V5)
Ibirubá-RS	15 kg ha <sup>-1</sup> N 60 kg ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> 45 kg ha <sup>-1</sup> K <sub>2</sub> O	40 kg ha <sup>-1</sup> N (V6)
Londrina-PR	30 kg ha <sup>-1</sup> N 100 kg ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> 30 kg ha <sup>-1</sup> K <sub>2</sub> O	50 kg ha <sup>-1</sup> N (applied in V6 and V10) 30 kg ha <sup>-1</sup> K <sub>2</sub> O (V6)
Primavera do Leste-MT	50 kg ha <sup>-1</sup> N 105 kg ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> 30 kg ha <sup>-1</sup> K <sub>2</sub> O	50 kg ha <sup>-1</sup> N (applied in V6 and V10) 25 kg ha <sup>-1</sup> K <sub>2</sub> O (V6)

\* V5, V6 and V10 correspond to corn phenological stages at five, six and ten fully expanded leaves.

4.0 m) were established, spaced 1.0 m apart (laterally and frontally), consisting of 8 rows of 7.0 m in length spaced 0.50 m apart. Plots from the four central rows were considered the useful plots, disregarding 1.0 m from each end.

For the TS treatment, corn seeds were initially treated with the insecticide CropStar (3.5 mL kg<sup>-1</sup> seeds) and, after drying the product, the liquid inoculant of *A. brasilense* Az39 was added to seed at a dosage of 2 mL kg<sup>-1</sup> seeds combined with the Protector Add-It bioprotector (1 mL kg<sup>-1</sup> seeds). For the Foliar500 treatment, sprays of the inoculant *A. brasilense* Az39 were carried out at a dosage of 500 mL ha<sup>-1</sup> at the V4 stage of corn, using a backpack research sprayer pressurized at CO<sub>2</sub> (12 lbs in<sup>-2</sup>) with a four-nozzle boom (XR 110.015/TEEJET) spaced 0.5 m apart, and application spray volume of 120 L ha<sup>-1</sup>. The adjuvant Break Thru (1 mL L<sup>-1</sup> solution) was added to the spray solution to improve droplet distribution spectrum.

The following biometric and productivity variables were evaluated throughout the corn crop development:

- i) Dry biomass production was assessed at the V4 and R1 stages: the aboveground parts of three plants per experimental plot were collected and cut 5 cm above the soil surface. Subsequently, the samples were dried in an oven at 60 °C until constant weight, with results expressed in g plant<sup>-1</sup>;
- ii) Stem diameter was measured at the R1 stage by taking measurements of five plants from each plot 10 cm above the soil surface using a caliper, with results expressed in millimeters.
- iii) Tassel insertion height was assessed at the R1 stage on five plants per plot by measuring the height from the ground to the tassel insertion point using a tape measure, with results expressed in centimeters.
- iv) SPAD index was determined at the R1 stage using ClorofiLOG CFL 1030 equipment (Falker) based on readings taken in the middle third of the plant (ear leaf) from five plants per plot.
- v) Thousand-grain weight was estimated after plant harvesting, expressed in g

vi) Grain Yield: The harvest involved 70 ears from the central rows of the useful plot, and grain weight was measured. The grain mass was weighed, and the moisture content was estimated, with the results expressed in kg ha<sup>-1</sup> at a 13% moisture basis.

Statistical analyses were conducted using R Studio version 5.12.8. Initially, the data were subjected to the Grubbs test to identify possible outliers, which were removed if identified. Subsequently, analysis of variance (ANOVA) was performed of the means of statistical differences compared using Tukey test. A significance level of 5% was used for all the statistical analyses.

## Results

Compared to the control, *A. brasilense* Az39 inoculation treatments had a significant effect on most of the analyzed biometric variables in all edaphoclimatic regions (Table 3). The dry matter production of the aboveground parts at the V4 stage of plants grown in Santa Maria-RS and Ibirubá-RS showed no statistical differences among the treatments. On the other hand, in the Primavera do Leste-MT and Londrina-PR trials, the TS treatment resulted in higher aboveground dry matter production at the V4 stage, with increases ranging from 14 to 29% compared with the non-inoculated treatment.

At the R1 stage, plant dry matter showed no statistical differences among the treatments in the Ibirubá-RS, Primavera do Leste-MT, and Londrina-PR regions. In contrast, in the trial conducted in Santa Maria-RS, the Foliar500 treatment resulted in a significant 15% increase in dry matter production at the R1 stage compared to the control.

Stem diameter, showed no significant differences among the treatments in the experiments conducted in Ibirubá-RS, Primavera do Leste-MT, and Londrina-PR. However, in the trial conducted in Santa Maria-RS, both the TS and Foliar500 treatments resulted in larger stem diameters. Regarding tassel insertion height, there were no significant differences in the trials conducted in Ibirubá-RS and Primavera do Leste-MT. However, in the trial conducted in Santa Maria-RS, the foliar application of *A. brasilense* Az39 resulted in a greater tassel insertion height. In Primavera do Leste-MT, tassel insertion height was higher in both the control and TS treatments.

Significant differences in the relative chlorophyll content (SPAD index) were observed among the treatments in all the regions where the trials were conducted (Table 3). In Santa Maria-RS, the TS treatment resulted in increases of 29% and 22% in the relative chlorophyll content compared to that of the control and Foliar500 treatments, respectively. In contrast, in Ibirubá-RS, Primavera do Leste-MT, and Londrina-PR foliar application of *A. brasilense* Az39 resulted in the highest values of relative chlorophyll content.

The 1000-grain weight showed no significant differences among treatments in the trials conducted in Santa Maria-RS and Londrina-PR. However, in the trials conducted in Ibirubá-RS and Primavera do Leste-MT, the Foliar500 treatment

**Table 3.** Effect of different inoculation methods of *Azospirillum brasilense* strain Az39 in maize shoot dry matter at V4 and R1, stalk diameter, tassel insertion height, SPAD index and thousand-grain weight.

Variables analyzed/ Evaluation stage	Inoculation Methods / Locations		
	Control	Seed treatment	Foliar500
	Santa Maria – RS		
Shoot dry matter - V4 (g planta <sup>-1</sup> )	2.70a*	3.28a	2.70a
Shoot dry matter - R1 (g planta <sup>-1</sup> )	368.12b	389.26ab	423.09a
Stalk diameter - R1 (mm)	21.23b	23.10a	23.10a
Tassel insertion height - (cm)	231b	237ab	244a
SPAD index - R1	54.16b	70.03a	57.22b
Weight of 1000 seeds (g)	323.34a	324.86a	328.67a
	Ibirubá – RS		
Shoot dry matter - V4 (g planta <sup>-1</sup> )	2.46a	2.56a	2.48a
Shoot dry matter - R1 (g planta <sup>-1</sup> )	338.45a	347.09a	379.41a
Stalk diameter - R1 (mm)	26.86a	27.80a	26.89a
Tassel insertion height - (cm)	233a	233a	237a
SPAD index - R1	54.17b	57.13ab	58.42a
Weight of 1000 seeds (g)	313.88b	318.75ab	332.23a
	Primavera do Leste – MT		
Shoot dry matter - V4 (g planta <sup>-1</sup> )	5.11b	6.58a	5.25b
Shoot dry matter - R1 (g planta <sup>-1</sup> )	167.53a	180.83a	164.26a
Stalk diameter - R1 (mm)	19.6a	19.6a	19.9a
Tassel insertion height - (cm)	81.10a	77.64a	63.62b
SPAD index - R1	57.91c	60.58b	61.90a
Weight of 1000 seeds (g)	289.34b	299.09ab	301.45a
	Londrina – PR		
Shoot dry matter - V4 (g planta <sup>-1</sup> )	4.01b	4.67a	3.94b
Shoot dry matter - R1 (g planta <sup>-1</sup> )	153.37a	165.48a	154.76a
Stalk diameter - R1 (mm)	19.0a	19.0a	19.3a
Tassel insertion height - (cm)	71.31a	72.55a	77.81a
SPAD index - R1	57.03c	59.71b	61.02a
Weight of 1000 seeds (g)	239.75a	241.26a	245.63a

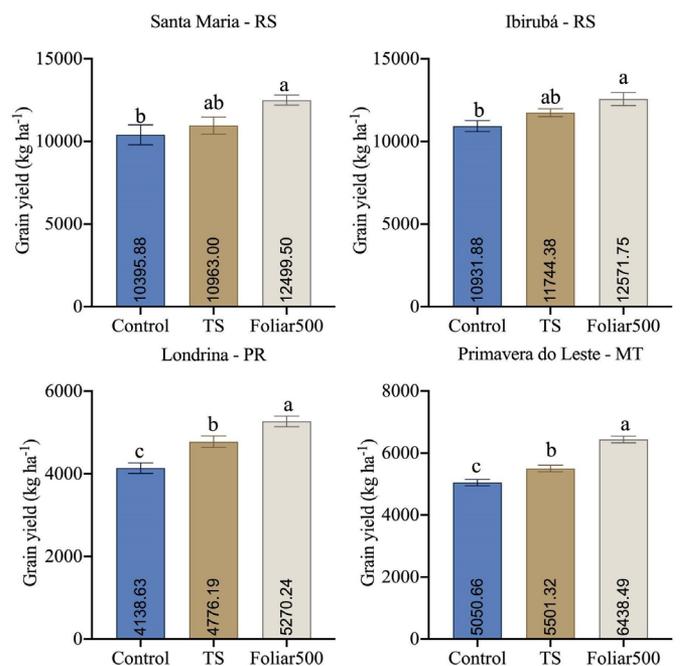
\* Means followed by the same letter do not differ by the Tukey test at 5% probability.

resulted in increases of 5% and 3% in the 1000-grain weight, respectively, compared with the control treatment.

In terms of grain yield, the Foliar500 treatment stood out in all four regions where the trials were conducted (Figure 2). On average, this treatment resulted in 23% higher yield than the control treatment. In trials conducted in Santa Maria-RS and Ibirubá-RS, the foliar application of *A. brasilense* Az39 was superior to the control treatment, increasing corn yield by 20% and 15%, respectively (Figure 2). Both TS and Foliar500 treatment significantly increased corn yield in the trials conducted in Londrina-PR and Primavera do Leste-MT (Figure 2). In Londrina-PR, more specifically, TS and Foliar500 inoculation treatments increased productivity by 15% and 27%, respectively, compared to the control. While, in the Primavera do Leste-MT region, inoculation with *A. brasilense* Az39 in the seeds and foliar resulted in a 8% and 27% increase in corn yield, respectively, compared with the control treatment.

## Discussion

This study presents pioneering results on the agronomic efficiency of the *A. brasilense* Az39 strain under different Brazilian edaphoclimatic conditions. Studies of this nature are essential because of the influence of climatic conditions



**Figure 2.** Effect of *Azospirillum brasilense* (strain Az39) inoculation on corn grain yield under different edaphoclimatic conditions in Brazil. TS = Seed treatments (2 mL kg<sup>-1</sup> seeds); Foliar500 = (500 mL ha<sup>-1</sup> applied at stage V4). Means followed by the same letter do not differ by the Tukey test at 5% probability.

(temperature and rainfall) and physical, chemical, and biological soil conditions on the survival of PGPB in the soil, the establishment of symbiosis, and the potential benefits for symbiotic plants (Lopes et al., 2021). Although various studies have demonstrated the agronomic effectiveness of *A. brasilense* for corn cultivation, adopting this practice is not as effective as inoculating rhizobia for soybean cultivation. This is partly due to the inconsistency in research results, which can vary depending on i) the cultivar or hybrid, ii) climatic conditions, iii) competition with autochthonous strains, and iv) the heterogeneous soil conditions that bacteria are exposed (Bartchechen et al., 2010). Those aspects underscore the importance of conducting field trials under different edaphoclimatic conditions to better understand the agronomic implications of *Azospirillum* in corn cultivation, particularly in Brazil, which has a continental dimension.

Although *Azospirillum* species have been successfully isolated in various parts of the world, their ability to survive in soil has been inconsistent (Bashan & Vazquez, 2000). In some soils, *Azospirillum* species survive satisfactorily for extended periods (Baldani et al., 1986; Oliveira & Drozdowicz, 1988), while in other soils, this has not been the case (Bashan & Levany, 1988; Bashan et al., 1991). The effects of individual soil variables such as texture and clay content on bacterial survival are well known (Van Elsas, 1992; Hartel et al., 1994), and it has been demonstrated that clay and organic matter contents, as well as water retention capacity, are positively correlated with the viability of *A. brasilense* (Bashan et al., 1995). Therefore, the inoculation with *Azospirillum* via seed treatment may result in different responses under specific edaphoclimatic conditions.

*A. brasilense* Az39 TS treatment showed different responses to growth variables in the early stages of corn growth in the present study. In soils with lower clay content (Santa Maria-RS and Ibirubá-RS) inoculated plants had shoot biomass similar to that of the non-inoculated control at the V4 stage. However, even under conditions of lower rainfall, such as in the trials conducted in Londrina-PR and Primavera do Leste-MT, the clayey soil texture in these locations might have contributed to higher soil water retention, leading to the survival of *A. brasilense* Az39 applied via TS. This may explain, at least in part, the benefits of seed treatment for corn shoot biomass production at the V4 stage compared with the non-inoculated control. Based on these results, inoculation with *A. brasilense* Az39 via TS resulted in intermediate responses in relation to corn grain yield.

In general, foliar application of *A. brasilense* Az39 benefited corn growth and relative chlorophyll content (SPAD index) at the R1 stage. It has been demonstrated that *Azospirillum* foliar sprays enable the formation of bacterial aggregates on the surface of trichomes and junctions of epidermal cell walls, as well as on the outer periclinal wall and near the stomatal complexes. It has been suggested that bacterial penetration into leaves occurs passively through the stomata, and foliar colonization begins in the substomatal chamber and progresses through the intercellular spaces of

the spongy chlorophyll tissue of the leaf mesophyll (Fukami et al., 2016), benefiting plant growth, development, and productivity under controlled and field conditions. Puente et al. (2018) demonstrated that the positive effects on plant growth through foliar application of the Az39 strain are due to both the presence of the Az39 microorganism as part of the colonization process and the *in situ* production of IAA. Genomic analyses of the *A. brasilense* Az39 strain have revealed biological nitrogen fixation and biosynthesis of plant hormones such as IAA, the most representative auxin, cytokinins, gibberellins, and polyamines, as mechanisms of action that promote plant growth (Coniglio et al., 2019).

The potential of Az39 in promoting growth of agriculturally relevant crops has been increasingly reported in Argentina. Díaz-Zorita & Fernández-Canigia (2009) found a positive response to the inoculation of wheat with *A. brasilense* in approximately 70% of the experiments conducted (297 sites). The authors observed an average increase in wheat grain yield of 260 kg ha<sup>-1</sup>, equivalent to 8.0% of the average wheat yield achieved under cultivation conditions in the Argentine Pampas region. Studies have also indicated that the inoculation of Az39 in soybean crops led to positive responses in terms of nodulation and crop yield, with higher levels of nitrogen and proteins observed when the strain was applied via foliar spraying (Puente et al., 2019). The benefits of foliar application have also been observed in corn. Cardozo et al. (2022) reported an increase in corn yield following foliar inoculation with *A. brasilense* Az39 under the management of an appropriate combination of herbicides used in the field.

*Azospirillum* inoculants in Brazil are predominantly formulated with the Ab-V5 and Ab-V6 strains, either individually or in combination, as commercial inoculants. Seed inoculation and spraying in planting furrows are the methods most commonly used by agricultural producers in Brazil (Barbosa et al., 2021). Previous studies carried out in Brazil using peat inoculant containing pure culture of Ab-V4, Ab-V5, Ab-V6 or Ab-V7 strains of *A. brasilense* in seed treatment resulted in corn yield ranging from 3,408 to 3,569 kg ha<sup>-1</sup>, with an increase of 24 to 30% compared to the control treatment (2,746 kg ha<sup>-1</sup>) (Hungria et al., 2010). In the present study, the average grain yield (average of the four evaluated locations) in the control treatment was 7,629 kg ha<sup>-1</sup> (127 sacks ha<sup>-1</sup>), and in the Foliar500 treatment was 9,195 kg ha<sup>-1</sup> (153 sacks ha<sup>-1</sup>). Considering the current average price of corn sack (60 kg) at US\$ 14.50, the adoption of this technology can represent an average gain of US\$ 377.00 ha<sup>-1</sup> for farmers. This revenue is much higher than the average cost of \$7.20 required for the acquisition of *Azospirillum* inoculant for foliar application at a dosage of 500 mL ha<sup>-1</sup>, highlighting the economic viability of adopting this Az39 strain-based technology in corn cultivation in Brazil.

## Conclusion

The *A. brasilense* Az39 strain is a promising technology for commercial products to be applied in corn production

in Brazil. Foliar application maximized the benefits of this strain, resulting in a higher grain yield than non-inoculated plants under different Brazilian edaphoclimatic conditions.

## Compliance with Ethical Standards

**Author contributions:** Conceptualization: AGH, EPS, PAAF, CRFSS; Formal analysis: EPS, PAAF, GPO; Investigation: GPO; Writing – original draft: PEL, CRFSS; Writing – review & editing: AGH, EPS, PAAF, CRFSS.

**Conflict of interest:** The authors do not have any conflict and competing of interest (professional or financial) that may influence the article.

**Funding source:** The Coordination for the Improvement of Higher (CAPES) – Finance Code 001 and the National Council for Scientific and Technological Development (CNPq) - 152109/2022-6, 307410/2021-8, and 308397/2022-3.

## Literature Cited

- Baldani, V. L. D.; Alvarez, M. A. de B.; Baldani, J. I.; Döbereiner, J. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. *Plant and Soil*, v. 90, n. 1, p. 35–46, 1986. <https://doi.org/10.1007/BF02277385>.
- Barbosa, J. Z.; Hungria, M.; Sena, J. V. D. S.; Poggere, G.; Dos Reis, A. R.; Corrêa, R. S. Meta-analysis reveals benefits of co-inoculation of soybean with *Azospirillum brasilense* and *Bradyrhizobium* spp. in Brazil. *Applied Soil Ecology*, v. 163, e103913, 2021. <https://doi.org/10.1016/j.apsoil.2021.103913>.
- Bartchechen, A.; Fiori, Cláudia Cristina Leite; Watanabi, S. H.; Guarido, R. C. Efeito da inoculação de *Azospirillum brasiliense* na produtividade da cultura do milho (*Zea mays* L). *Revista Campo Digital*, v. 5, n. 1, p.56-59, 2010. <https://revista.grupointegrado.br/revista/index.php/campodigital/article/view/982>. 04 Jan. 2024.
- Bashan, Y.; Levanony, H. Adsorption of the rhizosphere bacterium *Azospirillum brasilense* Cd to soil, sand and peat particles. *Microbiology*, v. 134, n. 7, p. 1811–1820, 1988. <https://doi.org/10.1099/00221287-134-7-1811>.
- Bashan, Y.; Mitiku, G.; Whitmoyer, R. E.; Levanony, H. Evidence that fibrillar anchoring is essential for *Azospirillum brasilense* Cd attachment to sand. *Plant and Soil*, v. 132, n. 1, p. 73–83, 1991. <https://doi.org/10.1007/BF00011014>.
- Bashan, Y.; Puente, M. E.; Rodriguez-Mendoza, M. N.; Toledo, G.; Holguin, G.; Ferrera-Cerrato, R.; Pedrin, S. Survival of *Azospirillum brasilense* in the bulk soil and rhizosphere of 23 soil types. *Applied and Environmental Microbiology*, v. 61, n. 5, p. 1938–1945, 1995. <https://doi.org/10.1128/aem.61.5.1938-1945.1995>.
- Bashan, Y.; Vazquez, P. Effect of calcium carbonate, sand, and organic matter levels on mortality of five species of *Azospirillum* in natural and artificial bulk soils. *Biology and Fertility of Soils*, v. 30, n. 5–6, p. 450–459, 2000. <https://doi.org/10.1007/s003740050023>.
- Cardozo, P.; Di Palma, A.; Martin, S.; Cerliani, C.; Esposito, G.; Reinoso, H.; Travaglia, C. Improvement of maize yield by foliar application of *Azospirillum brasilense* Az39. *Journal of Plant Growth Regulation*, v. 41, n. 3, p. 1032–1040, 2022. <https://doi.org/10.1007/s00344-021-10356-9>.
- Cassán, F.; Diaz-Zorita, M. *Azospirillum* sp. in current agriculture: From the laboratory to the field. *Soil Biology and Biochemistry*, v. 103, p. 117–130, 2016. <https://doi.org/10.1016/j.soilbio.2016.08.020>.
- Companhia Nacional de Abastecimento - Conab. Acompanhamento da Safra Brasileira 2023. <https://www.conab.gov.br/info-agro/safras>. 25 Set. 2023.
- Coniglio, A.; Mora, V.; Puente, M.; Cassán, F. *Azospirillum* as biofertilizer for sustainable agriculture: *Azospirillum brasilense* AZ39 as a model of PGPR and field traceability. In: Zúñiga-Dávila, Doris; González-Andrés, Fernando; Ormeño-Orrillo, Ernesto (Eds.). *Microbial probiotics for agricultural systems: advances in agronomic use*. (Book series: Sustainability in Plant and Crop Protection). Cham: Springer International Publishing, 2019. p. 45–70. [https://doi.org/10.1007/978-3-030-17597-9\\_4](https://doi.org/10.1007/978-3-030-17597-9_4).
- Díaz-Zorita, M.; Fernández-Canigia, M. V. Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. *European Journal of Soil Biology*, v. 45, n. 1, p. 3–11, 2009. <https://doi.org/10.1016/j.ejsobi.2008.07.001>.
- Dobereiner, J.; Marriel, I. E.; Nery, M. Ecological distribution of *Spirillum lipoferum* Beijerinck. *Canadian Journal of Microbiology*, v. 22, n. 10, p. 1464–1473, 1976. <https://doi.org/10.1139/m76-217>.
- Ferreira, F., Natália Dos Santos; Coniglio, A.; Puente, M.; Sant’anna, F. H.; Maroniche, G.; García, J.; Molina, R.; Nievas, S.; Volpiano, C. G.; Ambrosini, A.; Passaglia, L. M. P.; Pedraza, R. O.; Reis, V. M.; Zilli, J. É.; Cassan, F. Genome-based reclassification of *Azospirillum brasilense* Az39 as the type strain of *Azospirillum argentinense* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, v. 72, n. 8, e005475, 2022. <https://doi.org/10.1099/ijsem.0.005475>.
- Fukami, J.; Nogueira, M. A.; Araujo, R. S.; Hungria, M. Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. *AMB Express*, v. 6, n. 1, e3, 2016. <https://doi.org/10.1186/s13568-015-0171-y>.
- Glick, B. R. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, v. 2012, e 963401, 2012. <https://doi.org/10.6064/2012/963401>.
- Hartel, P. G.; Fuhrmann, J. J.; Johnson, W. F.; Lawrence, E. G.; Lopez, C. S.; Mullen, M. D.; Skipper, H. D.; Staley, T. E.; Wolf, D. C.; Li, A. G. W.; Zuberer, D. A. Survival of a lacZY-containing *Pseudomonas putida* strain under stressful abiotic soil conditions. *Soil Biology & Biochemistry*, v. 58, n. 3, p. 770–776, 1994. <https://doi.org/10.2136/sssaj1994.03615995005800030019x>.
- Hirel, B.; Tétu, T.; Lea, P. J.; Dubois, F. Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, v. 3, n. 9, p. 1452–1485, 2011. <https://doi.org/10.3390/su3091452>.
- Hungria, M.; Campo, R.J.; Souza, E.M.; Pedrosa, F.O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil*, v.331, p. 413-25, 2010. <https://doi.org/10.1007/s11104-009-0262-0>.

- Lopes, M. J. D. S.; Dias-Filho, M. B.; Gurgel, E. S. C. Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems*, v. 5, e606454, 2021. <https://doi.org/10.3389/fsufs.2021.606454>.
- Ma, M.; Jiang, X.; Wang, Q.; Guan, D.; Li, L.; Ongena, M.; Li, J. Isolation and identification of PGPR strain and its effect on soybean growth and soil bacterial community composition. *Int. J. Agric. Biol.*, v. 20, n. 6, p.1289-1297, 2018. <https://iarrp.caas.cn/en/docs/2021-07/20210718143428041173.pdf>. 18 Jan. 2024.
- Oliveira, R. G. B.; Drozdowicz, A. Are *Azospirillum* bacteriocins produced and active in soil? In: Klingmüller, W. (Ed.). *Azospirillum IV: Genetics, Physiology and Ecology*. Berlin: Springer-Verlag, 1988. p.101-108. [https://doi.org/10.1007/978-3-642-73072-6\\_13](https://doi.org/10.1007/978-3-642-73072-6_13).
- Perrig, D.; Boiero, M. L.; Masciarelli, O. A.; Penna, C.; Ruiz, O. A.; Cassán, F. D.; Luna, M. V. Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied Microbiology and Biotechnology*, v. 75, n. 5, p. 1143–1150, 2007. <https://doi.org/10.1007/s00253-007-0909-9>.
- Preininger, C.; Sauer, U.; Bejarano, A.; Berninger, T. Concepts and applications of foliar spray for microbial inoculants. *Applied Microbiology and Biotechnology*, v. 102, n. 17, p. 7265–7282, 2018. <https://doi.org/10.1007/s00253-018-9173-4>.
- Puente, M. L.; Gualpa, J. L.; Lopez, G. A.; Molina, R. M.; Carletti, S. M.; Cassán, F. D. The benefits of foliar inoculation with *Azospirillum brasilense* in soybean are explained by an auxin signaling model. *Symbiosis*, v. 76, n. 1, p. 41–49, 2018. <https://doi.org/10.1007/s13199-017-0536-x>.
- Puente, M. L.; Zawoznik, M.; De Sabando, M. L.; Perez, G.; Gualpa, J. L.; Carletti, S. M.; Cassán, F. D. Improvement of soybean grain nutritional quality under foliar inoculation with *Azospirillum brasilense* strain Az39. *Symbiosis*, v. 77, n. 1, p. 41–47, 2019. <https://doi.org/10.1007/s13199-018-0568-x>.
- Santini, J. M. K.; Buzetti, S.; Teixeira Filho, M. C. M.; Galindo, F. S.; Coaguila, D. N.; Boleta, E. H. M. Doses and forms of *Azospirillum brasilense* inoculation on maize crop. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 22, n. 6, p. 373–377, 2018. <https://doi.org/10.1590/1807-1929/agriambi.v22n6p373-377>.
- Santos, M. S.; Nogueira, M. A.; Hungria, M. Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: Lessons that farmers are receptive to adopt new microbial inoculants. *Revista Brasileira de Ciência do Solo*, v. 45, e0200128, 2021. <https://doi.org/10.36783/18069657rbcS20200128>.
- Scudelletti, D.; Crusciol, C. A. C.; Momesso, L.; Bossolani, J. W.; Moretti, L. G.; De Oliveira, E. F.; Tubaña, B. S.; Silva, M. D. A.; De Castro, S. G. Q.; Hungria, M. Inoculation with *Azospirillum brasilense* as a strategy to enhance sugarcane biomass production and bioenergy potential. *European Journal of Agronomy*, v. 144, e126749, 2023. <https://doi.org/10.1016/j.eja.2023.126749>.
- Soares, C. R. F. S.; Hernández, A. G.; Da Silva, E. P.; De Souza, J. E. A.; Bonfim, D. F.; Zobot, G. L.; Ferreira, P. A. A.; Brunetto, G. Applications and Market of Micro-Organism-Based and Plant-Based Inputs in Brazilian Agriculture. *Plants*, v. 12, n. 22, e3844, 2023. <https://doi.org/10.3390/plants12223844>.
- Telles, T. S.; Nogueira, M. A.; Hungria, M. Economic value of biological nitrogen fixation in soybean crops in Brazil. *Environmental Technology & Innovation*, v. 31, e103158, 2023. <https://doi.org/10.1016/j.eti.2023.103158>.
- Van Elsas, J. D. Environmental pressure imposed on gemmos in soil. In: Stewart-Tull, D. E. S.; Sussman, M. (Eds.). *The release of genetically modified microorganisms - REGEM 2*. Boston: Springer US, 1992. (Federation of European Microbiological Societies Symposium Series). p. 1-14. [https://doi.org/10.1007/978-1-4613-0493-7\\_1](https://doi.org/10.1007/978-1-4613-0493-7_1).
- Villarreal-Delgado, M. F.; Villa-Rodríguez, E. D.; Cira-Chávez, L. A.; Estrada-Alvarado, M. I.; Parra-Cota, F. I.; Santos-Villalobos, S. De Los. The genus *Bacillus* as a biological control agent and its implications in the agricultural biosecurity. *Mexican Journal of Phytopathology*, v. 36, n. 1, p.95-130, 2018. <https://doi.org/10.18781/R.MEX.FIT.1706-5>.