Renewable Agriculture and Food Systems

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Azospirillum brasilense increases corn growth and yield in conventional low input cropping systems

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Research Paper

Cite this article: Coelho SP *et al* (2021). *Azospirillum brasilense* increases corn growth and yield in conventional low input cropping systems. *Renewable Agriculture and Food Systems* **36**, 225–233. https://doi.org/10.1017/S1742170520000241

Received: 7 August 2019
Revised: 19 June 2020
Accepted: 13 July 2020
First published online: 13 August 2020

Key words:
Conventional agriculture; diazotrophic bacteria; nitrogen; organic agriculture; *Zea mays*

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Abstract

The supplementation of nitrogen can be increased by the use of nitrogen-fixing, diazotrophic bacteria such as *Azospirillum brasilense*. These bacteria can act to promote plant growth in various plant species, including corn (*Zea mays* L.). However, there is a need to understand the behavior of these bacteria in different agricultural systems. The objective of this study was to evaluate the effect on the growth and yield of corn inoculated with *A. brasilense*, and to identify the type of farming system which benefited most from the use of *A. brasilense*-based inoculants. The experiment conducted over two corn crop seasons was arranged in a 6 × 2 factorial scheme, consisting of six farming systems and the presence or absence of inoculation with the bacteria *A. brasilense*. The farming systems were derived from a long-term experiment with different fertilization systems that has been conducted since 1984. Among these systems, there were three conventional systems (CNT1: conventional no-till without fertilizer; CNT2: conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3: conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea), and three organic systems (ONT1: organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2: organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3: organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*). Although the *Azospirillum* population in the soil before planting was the same for all six systems, the count in the rhizospheric soil was higher in the organic systems, and there was no increase in that count due to inoculation. In this study, the only difference observed was within the CNT1 system, between the inoculated (CNT1-I) and uninoculated (CNT1-NI) treatments. In this system, inoculation resulted in an increase in plant height, in addition to higher concentrations of foliar N and P, and a higher plant survival rate, which culminated in higher yield. Corn inoculated with *A. brasilense* in the CNT1-I treatment showed a significant increase in yield—2839 kg ha^{-1} higher than that recorded for CNT1-NI. This study shows that, in the conventional low input treatment CNT1-I, inoculation with *A. brasilense* resulted in an increase in corn growth and yield.

Introduction

Concerns about food production are increasing with the growth of the world population. One of the processes that greatly influenced food production was the synthesis of ammonia by the Haber–Bosch process (Erisman *et al*., 2008). However, the availability of reactive nitrogen from industrial processes varies in different parts of the world (Galloway *et al*., 2008).

In marginal agricultural areas, the use of nitrogen fertilizers is limited by the lack of financial resources required for investment, or the lack of investment owing to the high chance of yield losses due to climate risk. Low input systems are more dependent on soil organic matter and plant residues (Gijsman *et al*., 2002). However, organic production systems, which range from closed systems (also considered low input) to simpler systems that only meet certification, also depend on the production of organic compost and green manure (Reganold and Wachter, 2016).

Organic corn (*Zea mays* L.) production in the Zona da Mata region of Minas Gerais in Southeastern Brazil is restricted to only one crop per year because of its rainfall regime, and

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the additional restriction of low availability of mechanizable area for corn farming makes it more difficult for rotation with a leguminous crop. Therefore, alternative means of improving nitrogen management in the crops are needed. One of them is the consortium of corn with legumes, including jack bean (*Canavalia ensiformis*), which are associated with rhizobia nitrogen-fixers and can keep growing after the corn harvest, to be used as green manure in the successor crop (Corrêa *et al.*, 2011). However, it is preferable to grow black oat (*Avena strigosa*) during the off-season, in order to obtain a higher production of straw with a high C/N ratio for both increased soil cover and weed control (Coelho *et al.*, 2016).

Another alternative is the inoculation of corn plants with selected strains of diazotrophic bacteria. Since 2019, the strains Ab-v5 and Ab-v6 of the species *Azospirillum brasilense* have been registered for use in corn. Inoculation with these strains has resulted in a 27% increase (842 kg ha^{-1}) in corn yield (Hungria *et al.*, 2010). Although this bacterium only establishes an associative relationship with the host plant, it can still partially supply the plant's demand for nitrogen. Furthermore, it is recommended for the co-inoculation of several species (Santos *et al.*, 2019), and allows for reductions between 25 and 50% of nitrogen fertilizer inputs, to obtain the same yield of corn (Hungria *et al.*, 2010; Piccinin *et al.*, 2013; Fukami *et al.*, 2016). The use of nitrogen-fixing bacteria represents a promising means of reducing the need for nitrogen fertilizers, making it an alternative technology for the development of more sustainable agriculture (Santos *et al.*, 2019).

However, it is necessary to understand the behavior of this bacterium in different agricultural systems, especially in organic and other low input systems. Thus, this study aimed to evaluate the effect of *A. brasilense* inoculation on corn yield and to identify the agricultural production systems that can be most benefited by the use of *A. brasilense*-based inoculants.

Material and methods

Description of location and soil

The experiment was carried out in the agricultural years 2015/2016 and 2016/2017, at the Coimbra agricultural experimental station of the Federal University of Viçosa (UFV), located in Coimbra, in the Zona da Mata region, Minas Gerais, Brazil (20°45′S, 45°51′W, altitude 650 m). The soil of that region is classified as Argisolo vermelho-amarelo (Ultisol) (Embrapa, 2013). Due to the use of fertilizers since 1984, this soil exhibited different chemical characteristics between the production systems as shown in Table 1.

The present study was conducted over a long-term experiment that started in 1984, comprising 12 treatments and four blocks. In the first phase of the long-term experiment, from 1984 to 2003, the soil management was conventional, using mineral and organic fertilizers incorporated into the soil and herbicides for weed control. The treatments consisted of a 3 × 2 × 2 factorial structure, with three levels of mineral fertilization, with and without the application of an organic compost, using corn in monoculture or associated with common beans (*Phaseolus vulgaris*) (Galvão, 1994). The fertilizer rates used in this experiment are shown in Table 2. A few years later, to conduct the 3 × 2 × 2 factorial experiment, the co-cultivation of corn and common beans was replaced by a second corn variety monocropped.

From 2003, the experiment was converted to a no-till system. The plots that were receiving the organic compost were converted to an organic system in which mineral fertilizers and herbicides were no longer used. In this system, the organic compost was applied on the soil surface, close to the corn plants, and weed control was carried out using a mechanical brush cutter. The cultivation of cover crops in the off-season, mainly black oat, began and the ONT2 and ONT3 treatments were modified to a consortium of corn with the Fabaceae jack bean (*C. ensiformis*) at densities of 30,000 and 60,000 plants ha^{-1}, respectively. Two corn cultivars with similar yield levels were used (Corrêa *et al.*, 2011).

In the third and current phase of the experiment, the treatments were set up in a factorial 6 × 2, with six cultivation systems, with and without seed inoculation with *A. brasilense* (I = inoculated and NI = not inoculated). The production systems are described in Table 3, and outlined in Figure 1.

The results of the chemical analysis of the organic compost according to the methodology described by Kiehl (1985) were: 10.61% organic carbon; 1.10% total N; 0.38% P; 1.20% K; 0.94% Ca; 0.42% Mg; 0.53% S; 158 mg kg^{-1} Zn; 37,686 mg kg^{-1} Fe; 239 mg kg^{-1} Mn; 68 mg kg^{-1} Cu; 13.1 mg kg^{-1} B; 18% Na; and pH 8.83.

Field management

During the off-season, black oat was sown at a seed density of 80 kg ha^{-1} and was incorporated into the soil by light harrowing. After flowering, the oat crop was cut using a mechanical brush cutter and left on the ground.

After 14 days, the open pollinated corn variety AL Bandeirante was manually sown, and a population density of 50,000 plants ha^{-1} was maintained after thinning. In the ONT3 treatment, the consortium of corn with the Fabaceae *C. ensiformis* was established. The Fabaceae was sown on the same planting line and on the same day as the corn, at a population density of 62,500 plants ha^{-1}. In the organic no-tillage systems, the organic compost was applied on the soil surface, close to the sowing line, after the emergence of the corn. In the conventional systems, the fertilizer was introduced into the furrow and the urea fertilizer was applied by broadcast when the corn presented four expanded leaves.

In the plots CNT1, CNT2, and CNT3, weed control was performed using a mixture of the herbicides Atrazine and Nicosulfuron (1.50 and 0.030 kg ha^{-1}, respectively) at 20 days after emergence of the corn. In the ONT plots, weed control was carried out using a mechanical brush cutter between the planting lines, at the stages of two and four expanded leaves.

Seed inoculation

The corn seeds were inoculated with a commercial inoculant, AZOTOTAL®, containing strains Ab-v5 and Ab-v6 of the bacteria *A. brasilense* at a minimum concentration of 2×10^8 viable cells mL^{-1}, using the recommended dose of 100 mL ha^{-1} and in accordance with the manufacturer's procedure. The inoculation of the 2015 harvest-year seeds was performed with a liquid inoculant whereas for the 2016 harvest-year seeds, a peat-based inoculant was used. The liquid inoculant was replaced by the peat because the latter ensures a more uniform seed coverage. Seed inoculation was carried out in the morning under shade, using the peat-based inoculant, mixed with a 10% sugar solution. The seeds were then kept in the shade for 30 min to dry.

Colonization of plants by Azospirillum spp

In the 2016/17 harvest, the *Azospirillum* population in the soil was determined before planting, and later in the rhizospheric

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

Table 1. Chemical composition of the soil: pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), organic matter (OM), organic carbon (OC), potential acidity (H + Al), sum of bases (SB), effective CTC [CTC(t)], CTC pH 7 [CTC(T)], exchangeable aluminum (Al^{3+}) and percent base saturation (BS)

		P	K	Ca	Mg	OM	OC	H + Al	SB	CTC[t]	CTC [T]	Al^{3+}	BS
System[a]	pH	mg dm^{-3}				dag kg^{-1}		$cmol_c$ dm^{-3}					%
CNT1	5.8	1.60	175	2.14	1.14	3.29	1.91	4.13	4.05	4.05	8.17	0.0	45.33
CNT2	5.3	4.97	216	1.76	0.75	3.60	2.09	5.16	3.09	3.16	8.22	0.0	37.00
CNT3	5.2	11.18	245	1.66	0.68	3.26	1.89	5.78	2.97	3.12	8.74	0.0	33.75
ONT1	6.5	25.43	515	4.10	1.85	4.10	2.38	3.02	7.27	7.27	10.28	0.0	71.00
ONT2	6.6	23.42	450	4.07	1.61	4.12	2.39	2.96	6.83	6.83	9.80	0.0	69.75
ONT3	6.6	33.22	497	4.11	1.69	4.10	2.38	2.89	7.07	7.07	9.84	0.0	71.50

[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*. OC was estimated by: OM = 1724 × OC.

Table 2. Quantity of nutrients applied with mineral fertilizer (kg ha^{-1} of N, P_2O_5, and K_2O, respectively) and organic compost applied for each fertilization system depending on the phase of the long-term experiment

	Phase 1—1984–2003		Phase 2—2003–2015		Phase 3—2015–2017	
Systems	Fertilizer [N-P_2O_5-K_2O] (kg ha^{-1})	Organic compost (m^3 ha^{-1})	Fertilizer [N-P_2O_5-K_2O] (kg ha^{-1})	Organic compost (m^3 ha^{-1})	Fertilizer [N-P_2O_5-K_2O] (kg ha^{-1})	Organic compost (m^3 ha^{-1})
CNT1	00-00-00	–	00-00-00	–	00-00-00	–
CNT2	30-35-20	–	34-42-24	–	34-42-24	–
CNT3	60-70-40	–	88-84-48	–	88-84-48	–
ONT1	00-00-00	40	–	40	–	40
ONT2	30-35-20	40	–	40	–	20
ONT3	60-70-40	40	–	40	–	40

soil, roots, and leaves as well. The soil samples, taken before planting, were collected up to a depth of 20 cm using disinfected PVC probes. Five sampling points were selected randomly in each plot.

The rhizospheric soil was collected at the silking (R1) stage from two plants per plot, using a spade (20 × 20 × 20 cm). The excess soil was discarded, leaving only the soil that adhered to the roots to be used for the samples. These roots were also kept for further analysis. The middle third of the leaf below and opposite the ear was also collected from ten corn plants at the R1 stage. The ribs were excluded from these leaf samples.

The soil samples were homogenized and weighed. A mixture of 10 g of soil and 90 mL of saline solution was submitted to agitation at 150 rpm for 1 h. This mixture was then diluted to 10^{-3} before plating. The soil moisture was measured by expressing the results as the weight of dry soil. This analysis was performed by placing the samples in an oven at 105°C for 24 h.

The root and leaf samples were washed in tap water, sectioned and divided into sample portions each weighing 1 g. The samples were then disinfected by immersion in 70% alcohol for 10 s, and 1% sodium hypochlorite for 30 s, followed by two washes in autoclaved distilled water. They were then immersed in 9 mL of saline solution, crushed using POLITRON® and diluted to 10^{-2} before plating. The culture medium used was NFb (Döbereiner *et al.*, 1995) and the count was performed using the spiral plater technique (Gilchrist *et al.*, 1973). The plates were stored in a climatized chamber at 28°C and after 5 days, were counted using a magnifying glass.

Table 3. Description of treatments for conventional and organic farming systems

Systems	Description
CNT1	Conventional no-till without fertilizer
CNT2	Conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea
CNT3	Conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea
ONT1	Organic no-till with 40 m^3 ha^{-1} of organic compost
ONT2	Organic no-till with 20 m^3 ha^{-1} of organic compost
ONT3	Organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*

Mineral fertilizer contains 8% N, 28% P_2O_5, and 16% K_2O; urea contains 44% N.

Sampling, plant analysis, and harvest

In the 2015 and 2016 harvests, growth of the corn plants was evaluated at the fourth leaf collar (V4) stage and at R1 by measuring the following parameters: plant height, diameter at the base of the stem and aerial dry matter. The Soil Plant Analysis Development (SPAD) index was also evaluated at these same two phenological stages using SPAD model Minolta 502 DL PLUS. The last

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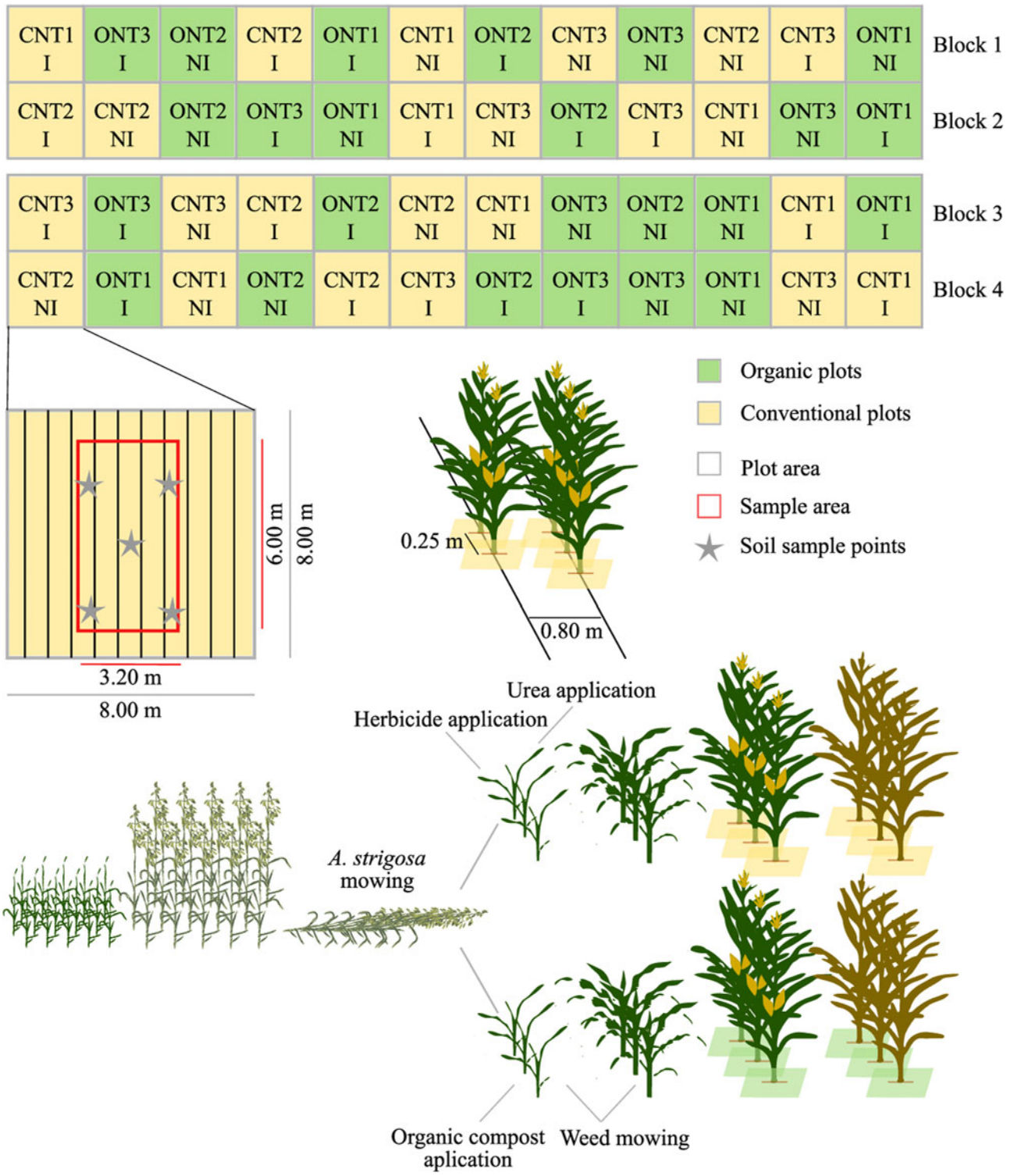


Fig. 1. Scheme of the field experiment. CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg $ha^{-1}$of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg $ha^{-1}$of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*. Non-inoculated (NI); inoculated (I).

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

completely expanded leaf was evaluated at the V4 stage and the first leaf below and opposite the ear was evaluated at the R1 stage.

The leaf nitrogen content was evaluated in the first leaf opposite and below the first ear at the R1 stage, for this, ten leaves per plot were collected. The samples were washed with tap and distilled water, then dried for 72 h at 70°C. The material was milled using a Willey/Star FT80 knife mill before being submitted to analysis of total nitrogen (N) (dag kg^{-1}) according to the Kjeldahl method.

To evaluate the components of corn productivity, the following variables were quantified: final plant stand (including the number of broken and lodged plants at the time of grain harvest); prolificacy (total number of cobs divided by the number of plants per plot); number of rows of grains per ear; number of grains per row, thousand grain weight, and yield.

The harvest was carried out manually, and the ears threshed in a stationary electric thresher. The grain weight and yield was corrected to 13% moisture.

Statistical analysis

The experiment was performed over two harvest seasons. The average of the two harvests was used for the analysis of variance, except for the data from the *Azospirillum* count, which was performed only in the 2016 harvest. The means were compared by the Tukey test, at 5% probability. The log (x) transformation was used for counting the data. When there was an interaction between factors, its effect was evaluated; otherwise, only the individual effect was tested. All the analyses were performed using SAEG software (Saeg 2007).

Results

The average population density of *Azospirillum* spp. in the soil before planting was 1.5×10^7 CFU g^{-1} and was not influenced by the previous fertilization management system (Table 4). However, it was observed that the fertilization treatment affected the *Azospirillum* spp. population during the flowering stage. The ONT3 treatment presented a population of 7.18×10^7 CFU g^{-1} in the rhizospheric soil, which was similar to the other organic treatments, but much higher compared to 2.0×10^7 CFU g^{-1} presented in the CNT3 treatment. All the other conventional treatments also presented a population similar to that of CNT3. The inoculation of the seeds did not affect the population of *Azospirillum* spp. in the rhizospheric soil. The population present in roots and leaves was not affected either by inoculation or by the fertilization management system.

Regarding plant growth, the first variable to present an interaction between the factors was plant height at the V4 stage (Table 5). Plant height in the CNT1-NI treatment was 10 cm, and 15 cm in the CNT1-I treatment. The latter was similar to the other treatments, except ONT3-I that presented the greatest plant height. At R1, plant height and stem diameter of CNT1-NI were 105 cm and 13 mm, respectively. These values were different from those found in the CNT1-I treatment, which were 143 cm and 16 mm, respectively (Table 6). However, at this stage, CNT1-I only presented values similar to other conventional treatments, whereas the organic treatments ONT1 and ONT2 presented similar values to ONT3. This pattern was also observed for the variable, stem diameter.

Regarding plant nutrition, seed inoculation increased the concentrations of N and P in CNT1 (Table 7). In CNT1, inoculation increased the concentration of N in the leaves from 10 to 14 g kg^{-1}. The non-inoculated treatment (CNT1-NI) presented the lowest concentration, while the inoculated one (CNT1-I) showed the same level of N concentration as the other treatments, except ONT3, which presented the highest N concentration. The other treatments were not affected by inoculation.

The concentration of nutrient P in the leaves was similarly affected by inoculation. CNT1 presented an increase in P concentration from 2.8 g kg^{-1} (CNT1-NI) to 4.2 g kg^{-1} (CNT1-I). CNT2-NI showed similar P concentration to CNT1-NI. CNT1-I, however, showed higher P concentrations when compared to the conventional treatments CNT2-I and CNT3-I. This was similar to what was observed for N concentration; the inoculation did not affect P concentration in the other treatments. The concentration of K was not influenced by any factor. The SPAD index and aerial dry matter were not affected by inoculation (Table 5). However, in the ONT3 system, the SPAD index was higher in corn leaves during the flowering stage, compared to ONT2 and the conventional systems (Table 6).

The plant population, grains per row and thousand grain weight were also influenced by inoculation in the CNT1 system. CNT1-NI showed values of 44.075 plants ha^{-1}, 20 grains per row and 245 g of thousand grain weight, compared to 51,532 plants ha^{-1}, 29 grains per row and 274 g of thousand grain weight presented by CNT1-I. The inoculation did not affect these production components in the other systems. The number of ears per plant and number of rows of grains per cob showed less variability among the treatments. Regarding these variables, the CNT1 system differed only from ONT3 (Tables 8 and 9).

Thus, the corn inoculated with *A. brasilense* presented an increase in yield in the CNT1 system (Table 9). The corn yield in CNT1-I was 4079 kg ha^{-1} which is an increase of 2839 kg ha^{-1} or 229% compared to the uninoculated treatment (1240 kg ha^{-1}). The highest productivity in the experiment was achieved with the ONT3 treatment, which did not differ from the other inoculated organic treatments. The CNT1-NI treatment showed the lowest productivity; however, CNT1-I had similar levels to those presented by CNT2-I and CNT3-I.

Discussion

In this study, we found high populations of *Azospirillum* spp. in the soil before planting, even without inoculation. This could be explained by the wide distribution of this organism in tropical soils (Dobereiner *et al.*, 1976). In addition, the characteristics of this experiment, such as the absence of soil disturbance, clay content, organic matter content and the presence of live plants in the off-season, are important factors for the survival of *Azospirillum* in the soil (Bashan *et al.*, 1995).

The higher population of *Azospirillum* spp. found in the rhizospheric soil of organic systems is related to the higher biomass production of corn plants in these systems, where it is also expected to find a higher production of root exudates, which are considered a source of energy for the bacteria (D'Angioli *et al.*, 2017). It was observed that the higher C input in these organic systems positively influenced the population of the class Alphaproteobacteria, to which *Azospirillum* and rhizobia belong (Semenov *et al.*, 2020).

Fukami *et al.* (2016) also found high populations of *Azospirillum* spp. even in non-inoculated systems. These authors also observed higher populations of *Azospirillum* spp. in rhizospheric soil and in the internal tissues of inoculated plants.

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

Table 4. Population count of *Azospirillum* spp. (CFU g^{-1}) present in the soil before planting, in rhizospheric soil, in leaf and root tissue, and according to the fertilization system in the 2016/17 harvest

Systems[a]	Soil before planting	Rhizospheric soil	Leaf	Root
CNT1	1.99×10^7 a[b]	3.00×10^7 bc	1.76×10^5	0.99×10^5
CNT2	1.14×10^7 a	2.90×10^7 bc	2.36×10^5	1.50×105
CNT3	0.93×10^7 a	2.00×10^7 c	1.06×10^5	1.66×10^5
ONT1	1.62×10^7 a	5.88×10^7 a	2.02×10^5	2.63×10^5
ONT2	1.35×10^7 a	4.74×10^7 ab	1.97×10^5	1.26×10^5
ONT3	1.98×10^7 a	7.18×10^7 a	0.89×10^5	1.39×10^5
CV (%)	3.21	2.81	9.20	16.60

[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same lowercase letter in a column do not differ by the Tukey's test, at 5%.

Table 5. Average plant height, stem diameter, weight per plant, and SPAD index at the V4 stage of corn, according to the fertilization system and seed inoculation with *Azospirillum brasilense*, in the 2015/16 and 2016/17 harvests

	Height (cm)		Diameter (mm)	Weigh (g)	SPAD
Systems[a]	NI	I	–	–	–
CNT1	10 dB[b]	15 bA	8.1 c	3.0 a	32 b
CNT2	13 cdA	13 bA	8.1 c	2.8 a	34 b
CNT3	15 bcA	14 bA	8.7 bc	2.8 a	37 a
ONT1	18 abA	16 abA	10.9 a	5.4 a	37 a
ONT2	15 bcA	15 bA	9.0 bc	3.2 a	34 b
ONT3	20 aA	20 aA	11.9 a	4.8 a	38 a
CV (%)	12.20		14.69	53.52	5.30

Non-inoculated (NI); inoculated (I).
[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same uppercase letters in a line and by the same lowercase in a column do not differ significantly by the Tukey's test, at 5%.

However, in the present study, we did not find such higher populations in the inoculated treatments. This could be one of the reasons why an increase in the plant growth was not observed in the inoculated treatments in five of the six systems performed in this study.

The fact that inoculated plants showed increased growth in early stages is relevant, as plants from the CNT1-I treatment were taller than those from CNT1-NI, since the V4 stage. Thus, the increase in root growth is expected to be at least proportional to or even higher than the increase observed in aerial part, or the root biomass remains unchanged but it turns out more efficient, as reviewed by Bashan and Dubrovsky (1996).

The lack of fertilization for 34 years in the CNT1 system has led to a low availability of nitrogen and phosphorus in easily absorbable forms. However, as reported by Pii *et al.* (2019), inoculation led to a similar effect as to the availability of nitrate at the beginning of root development, which mainly affected the length of the secondary root branches and the biomass of the root system. This increase in root development may be the cause of the differences between CNT1-I and CNT-NI, leading the inoculated treatment (CNT1-I) recording similar levels of foliar N and P compared to systems CNT2 and CNT3, even with less availability of these nutrients.

Inoculation positively affected the vegetative growth of corn plants in the CNT1 system, resulting in taller plants with a larger stem diameter. Zeffa *et al.* (2019) studied plant growth and nitrogen absorption efficiency in corn inoculated with *A. brasilense*. They reported that the growth of corn plants was related to changes in the root system caused by *A. brasilense*. These bacteria stimulate root elongation (Dobbelaere *et al.*, 1999), as well as the development of secondary roots and root hair (Creus *et al.*, 2005; Molina-Favero *et al.*, 2008). These root modifications lead to greater nutrient absorption, resistance to abiotic stressors, and biomass gain in corn plants.

The results showed that the inoculation of corn with *A. brasilense* increased the absorption of nitrogen and phosphorus, and the growth of plants under nutrient-limiting conditions (CNT1). This is similar to the findings of Cassán and Diaz-Zorita (2016), who concluded that the response of plants to inoculation is often observed when nitrogen availability is a limiting factor for productivity. The inoculation efficiency of *A. brasilense* varies with the plant genotype, bacterial strain and edaphoclimatic conditions

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

Table 6. Averages of plant height, stem diameter, ear height and SPAD index at the R1 stage of corn, according to the fertilization system and seed inoculation with *Azospirillum brasilense*, in the 2015/16 and 2016/17 harvests

	Plant height (cm)		Diameter (mm)		Ear height (cm)	SPAD
Systems[a]	NI	I	NI	I	–	–
CNT1	105 cB[b]	143 cA	13.1 cB	16.2 bA	47 d	36 d
CNT2	139 bA	143 cA	16.0 bcA	15.9 bA	59 cd	34 cd
CNT3	152 bA	158 bcA	16.6 abA	17.2 bA	67 bc	36 bcd
ONT1	182 aA	176 abA	19.2 aA	18.6 abA	88 a	40 ab
ONT2	166 abA	162 abcA	19.3 aA	17.2 bB	79 ab	39 bc
ONT3	188 aA	187 aA	18.9 abB	21.1 aA	94 a	44 a
CV (%)	8.44		7.85		13.90	7.89

Non-inoculated (NI); inoculated (I).
[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same uppercase letters in a line and by the same lowercase in a column do not differ significantly by the Tukey's test, at 5%.

Table 7. Average concentration of nitrogen (N), phosphorus (P) and potassium (K) in corn leaves during flowering, according to the fertilization system and seed inoculation with *Azospirillum brasilense*, in the 2015/16 and 2016/17 harvests

	N (g kg^{-1})		P (g kg^{-1})		K (g kg^{-1})
Systems[a]	NI	I	NI	I	–
CNT1	10.1 dB[b]	14.1 bcA	2.8 dB	4.2 bA	19.8
CNT2	13.0 cA	12.0 cA	2.9 cdA	3.1 cA	19.6
CNT3	13.7 cA	13.9 bcA	3.7 bcA	3.2 cA	19.8
ONT1	17.2 abA	15.8 bA	4.9 aA	4.5 abA	19.9
ONT2	15.2 bcA	15.6 bA	4.5 abA	5.0 aA	18.9
ONT3	19.7 aA	19.3 aA	4.4 abA	4.1 bA	19.8
CV (%)	8.64		10.37		5.58

Non-inoculated (NI); inoculated (I).
[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same uppercase letters in a line and by the same lowercase in a column do not differ significantly by the Tukey's test, at 5%.

Table 8. Average number of rows of grain per ear, grains per row and thousand grain weight according to the fertilization system and seed inoculation with *Azospirillum brasilense*, in the 2015/16 and 2016/17 harvests

	Number of rows	Grains per row		1000 grain weight (g)	
Systems[a]	–	NI	I	NI	I
CNT1	14 b[b]	20 cB	29 abA	245 dB	274 aA
CNT2	14 ab	28 bA	27 bA	263 cdA	261 aA
CNT3	14 ab	30 abA	28 abA	281 bcA	265 aB
ONT1	14 ab	32 aA	29 abA	293 abA	277 aB
ONT2	14 ab	30 abA	30 abA	287 abA	276 aA
ONT3	15 a	31 abA	32 aA	307 aA	303 bA
CV (%)	4.92	7.55		3.75	

Non-inoculated (NI); inoculated (I).
[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same uppercase letters in a line and by the same lowercase in a column do not differ significantly by the Tukey's test, at 5%.

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

Table 9. Means of prolificacy (number of ears per plant), stand (plant population), and corn yield according to the fertilization system and seed inoculation with *Azospirillum brasilense*, in the 2015/16 and 2016/17 harvests

	Prolificacy	Stand (plants ha^{-1})		Yield (kg ha^{-1})	
Systems[a]	–	NI	I	NI	I
CNT1	0.83 b[b]	44,075 bB	51,562 aA	1240 eB	4079 bcA
CNT2	0.96 ab	52,408 aA	49,479 aA	2977 deA	3077 cA
CNT3	0.95 ab	54,231 aA	50,651 aA	3896 cdA	3748 bcA
ONT1	0.97 ab	52,343 aA	47,265 aA	5889 abA	5096 abA
ONT2	0.96 ab	50,065 abA	51,822 aA	4754 bcA	4948 abA
ONT3	1.09 a	52,929 aA	50,911 aA	6754 aA	6204 aA
CV (%)	13.67	7.40		18.73	

Non-inoculated (NI); inoculated (I).
[a]CNT1, conventional no-till without fertilizer; CNT2, conventional no-till with 150 kg ha^{-1} of mineral fertilizer + 50 kg ha^{-1} of urea; CNT3, conventional no-till with 300 kg ha^{-1} of mineral fertilizer + 100 kg ha^{-1} of urea; ONT1, organic no-till with 40 m^3 ha^{-1} of organic compost; ONT2, organic no-till with 20 m^3 ha^{-1} of organic compost; ONT3, organic no-till with 40 m^3 ha^{-1} of organic compost and intercropped with *Canavalia ensiformis*.
[b]Means followed by the same uppercase letters in a line and by the same lowercase in a column do not differ significantly by the Tukey's test, at 5%.

(Bashan *et al.*, 2014). However, nitrogen fertilization management has been shown to have a significant influence on inoculation efficiency (Veresoglou and Menexes, 2010). Similar results were reported by Zeffa *et al.* (2019), who observed a positive effect on nitrogen absorption efficiency, plant growth, and biochemical characteristics for inoculated corn grown in nitrogen-limiting conditions.

Although some strains of *Azospirillum* can solubilize P, this is not a characteristic of the genus, but it might be involved in niche-specific adaptation (Wisniewski-Dyé *et al.*, 2012). The increase in the concentration of P is mainly due to the greater absorption of nutrients due to greater root exploration of the soil (Rondina *et al.*, 2020).

Microbial inoculants improve the nutritional absorption capacity of cultivated plants and also increase the available form of nutrients close to the rhizosphere (Sammauria *et al.*, 2020). The present study demonstrated that the *A. brasilense* inoculation also favors the absorption of phosphorus, which was observed by Pereira *et al.* (2020). These authors found that inoculation, associated with P_2O_5 fertilization increased the phosphorus uptake and the absorption efficiency of this nutrient by 54.6%.

In the CNT1 system, the plant stand was affected by inoculation, leading to a positive effect on the plant population. The greater survival rate of plants and the resulting increased corn canopy has a direct impact on productivity since corn presents low phenotypic plasticity and low ability to compensate for stand failures. Corrêa (2009) experienced difficulties in establishing an adequate stand in the CNT1 system. To avoid that, our experiment was performed using a greater quantity of seed. Even then, the CNT1-NI stand had only 44,000 plants, which was 10% less than the desired number. Therefore, the fact that CNT1-I had the largest stand in this system (CNT1) may also be related to the ability of *A. brasilense* to relieve abiotic stressors such as low nutrient levels (Ferreira *et al.*, 2013).

In addition to having the largest stand, CNT1-I showed a greater number of grains per row and higher grain weight compared to ONT1-NI. Kappes *et al.* (2013) also reported an increase in the values of grain weight, yield, and ear length of inoculated plants.

The increased vegetative growth of corn, and greater accumulation of nutrients in response to inoculation in the CNT1 system, resulted in an increase in yield (Table 9). The CNT1 system showed an average of 4079 kg ha^{-1}, due to inoculation with *A. brasilense*. This represents 74% of the average Brazilian productivity of corn, which was 5522 kg ha^{-1} in the 2017 harvest (Conab, 2017). This reinforces the use of microbial inoculants as an ecological, alternative technology to reduce the application rate of fertilizers and to increase the efficiency of nutrient absorption (Ejaz *et al.*, 2020). Significant increases in corn productivity were also found by Martins *et al.* (2017), who reported an average increase of 1000 kg ha^{-1} of grains resulting from inoculation with *A. brasilense*.

The organic systems were not affected by inoculation. This could be related to the high fertility of the soils in these systems. Soils in the organic systems showed the highest average contents of organic matter, a percentage base saturation of approximately 70%, as well as higher levels of phosphorus. Therefore, the results indicate that soil fertility may affect the response to inoculation with *A. brasilense*.

Organic fertilization of corn using an organic compost at a rate of 40 m^3 ha^{-1} is able to increase soil fertility and to provide satisfactory development of the corn culture (Maia and Cantarutti, 2004). As observed in our organic systems, most notably in the ONT3 system, the consortium of corn with *C. ensiformis* showed a productivity greater than 6 Mg ha^{-1}, whereas that of the conventional system was approximately 4 Mg ha^{-1}. In the conventional systems, the repeated application of mineral fertilizers is associated with a very low quantity of plant biomass, leading to negative impacts on soil organic matter, which may in the long-term, compromise the productive capacity of agroecosystems (Sammauria *et al.*, 2020).

Conclusions

Inoculation with *A. brasilense* is a promising practice for increasing corn production, especially under stress, and leads to increased production of corn grains in soils with low fertility. Therefore, it is an alternative means of increasing crop productivity in marginal land areas with reduced fertilizer application, and/or a low technological input level. Inoculation does not show a significant effect on corn productivity in stabilized organic systems with highly fertile soils.

https://doi.org/10.1017/S1742170520000241 Published online by Cambridge University Press

Acknowledgements. The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq), for their support of this research study, and the Total Biotechnology Company for their donation of the inoculant.

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