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Nitrogen fertilization and inoculation with diazotrophic bacteria in corn intercropped with xaraés grass

Vanessa Zirondi Longhini[1], Marcelo Andreotti[2], Wésley Carlos Rossini de Souza[2], Nídia Raquel Costa[3], Marcelo Carvalho Minhoto Teixeira Filho[2], Rafael Montanari[2]

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Via de Acesso Prof. Paulo Donato Castelane, s/n, Vila Industrial, CEP 14884-900, Jaboticabal-SP, Brasil. E-mail: ne_longhini@hotmail.com

[2] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Engenharia de Ilha Solteira, Departamento de Fitossanidade, Engenharia Rural e Solos, Avenida Brasil, 56, Centro, CEP 15385-000, Ilha Solteira-SP, Brasil. Caixa Postal 31. E-mail: dreotti@agr.feis.unesp.br; wesley_rossini@hotmail.com; mcmteixeirafilho@agr.feis.unesp.br; montanari@agr.feis.unesp.br

[3] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agronômicas, Rua Doutor José Barbosa de Barros, 1780, Jardim Paraíso, CEP 18610-307, Botucatu-SP, Brasil. E-mail: nidiarcosta@gmail.com

ABSTRACT

The objective of this work was to evaluate the effect of nitrogen (N) fertilization doses with and without inoculation of seeds with *Azospirillum brasilense* in fall corn intercropped with *Urochloa brizantha* cv. Xaraés over the nutrition, the production and yield components of the corn crop, and the dry matter yield and crude protein levels of xaraés grass under irrigation in the Cerrado region. The experiment had a completely randomized block design with four replications in a 2 × 5 factorial scheme. The treatments consisted in inoculating or not the corn seeds with *A. brasilense* and five N doses (0, 30, 60, 90 and 120 kg ha^{-1} N). The inoculation of corn seeds with *A. brasilense* in intercropping with xaraés grass in the fall season increases nutrition, height growth of plants and corn production components, however, without effect on grain yield. Corn plants from seeds inoculated with *A. brasilense* lead to a lower production of dry mass of xaraés grass in intercropping. Nitrogen fertilization in the cover layer linearly increases the crude protein content of the xaraés grass up to the dose of 120 kg ha^{-1} N.

Key words: *Azospirillum brasilense*; crop-livestock integration; *Urochloa brizantha*; *Zea mays*

Adubação nitrogenada e inoculação com bactérias diazotróficas na cultura do milho em consórcio com capim-xaraés

RESUMO

O presente trabalho objetivou avaliar o efeito de doses de nitrogênio (N) em cobertura, com e sem a inoculação das sementes com *Azospirillum brasilense* no milho outonal, consorciado com *Urochloa brizantha* cv. Xaraés sobre a nutrição, os componentes da produção e produtividade da cultura do milho, e sobre a produtividade de massa seca e teores de proteína bruta do capim-xaraés, sob irrigação na região do Cerrado. O delineamento experimental foi em blocos casualizados, com quatro repetições, em esquema fatorial 2 × 5. Os tratamentos foram constituídos pela inoculação ou não das sementes do milho com *A. brasilense* e cinco doses de N em cobertura (0; 30; 60; 90 e 120 kg ha^{-1} N). A inoculação das sementes de milho com *A. brasilense*, em cultivo consorciado com capim-xaraés na época outonal, incrementa a nutrição, o crescimento em altura das plantas e os componentes de produção do milho, entretanto, sem efeito na produtividade de grãos. Plantas de milho advindas de sementes inoculadas com *A. brasilense* condicionam menor produção de massa seca do capim-xaraés em consórcio. A adubação nitrogenada em cobertura incrementa linearmente os teores de proteína bruta do capim-xaraés, até a dose de 120 kg ha^{-1} N.

Palavras-chave: *Azospirillum brasilense*; integração lavoura-pecuária; *Urochloa brizantha*; *Zea mays*

Introduction

Corn is a cereal of great importance for the Brazilian and world agribusiness, being one of the most demanding crops in terms of fertilizers, especially those of nitrogenous nature, and in conditions of intercropping with tropical forages (Costa et al., 2012a). Most mineral fertilizers come from fossil fuels, and consist in more expensive inputs, thus increasing the total costs of crop production.

Input expenditures (formulated fertilizers and herbicides) can account for more than 50% of the effective operating costs for producers, especially when there is grain crops (corn and sorghum) and tropical forage are associated, due to the higher nutrient demand, i.e. amounts of fertilizers, to reach the final yield (Costa et al., 2012b; Costa et al., 2015a).

In this scenario, new technologies must be sought to reduce the use of nonrenewable sources in the production of industrial fertilizers and to obtain the highest corn crop productivity, concurrently with reducing the production costs and the emission of greenhouse gases. The use of diazotrophic bacteria has proved to promote significant improvement in the production of cereals in different production systems, either by seed inoculation or leaf spraying (Sala et al., 2008; Hungria et al., 2010; Araújo et al., 2014; Brum et al., 2016; Fukami et al., 2016).

Several diazotrophic bacteria have been isolated in corn crop, and the most studied species so far belong to the genus *Azospirillum* and *Herbaspirillum* (Araújo et al., 2016; Brusamarello-Santos et al., 2017; Martins et al., 2017). These species perform atmospheric nitrogen fixation and grow associated with the rhizosphere of corn through symbiosis, thus contributing to nitrogen nutrition (Figueiredo et al., 2009) and promoting plant growth through the production of hormones (Novakowiski et al., 2011) such as auxins and gibberellins. Bacteria are capable of increasing root volume, promoting better soil exploration and ion/root contact for increased phosphorus uptake (Steenhoudt & Vanderleyden, 2000; Bashan et al., 2004; Hungria et al., 2010). Hungria et al. (2010) reported an increase in productivity of 30% in corn attributed to the use of *A. brasiliense,* corresponding to 823 kg ha^{-1} in relation to the control without inoculation.

Brachiaria can be hosts of diazotrophic bacteria native to the soil (Hungria et al., 2010). Therefore, the intercropping between corn and forage of the genus *Urochloa* is an important alternative to improve crop nutrition, growth and productivity, aiming at reducing production costs and improving soil quality.

In *Urochloa*/corn intercropping, the forage grass can be used to form straw under no-tillage system during the period before winter crops. Thus, with correct management, the intercropping will increase the amount of straw, resulting in better soil cover for no-till crop and, in some cases, increased productivity in the successor crop (Costa et al., 2012a; Garcia et al., 2013).

The objective of the present study was to evaluate the effect of nitrogen fertilization doses with and without inoculation of *Azospirillum brasilense* in fall corn (harvests 2012 and 2013) intercropped with *Urochloa brizantha* cv. Xaraés on the macronutrient contents of the leaves, the production and yield components of the corn crop, as well as the dry matter yield and crude protein content of xaraés grass under irrigated conditions in the Cerrado.

Material and Methods

The work was carried out from November 2011 to August 2013 at the Teaching, Research and Outreach Farm, Faculty of Engineering of Ilha Solteira (FEIS/UNESP), located in the municipality of Selvíria-MS, between latitudes 20°18’05”S and 20°18’28”S and the longitudes 52°39’02”W and 52°40’28”W, altitude of 370 m.

The area was irrigated by a sprinkler (central pivot) when necessary, adopting the methodology proposed by Doorenbos & Pruitt (1977). The climate Aw type according to Köppen’s classification, characterized as tropical wet, with rainy season in the summer and dry season in the winter. Daily measures of maximum, average and minimum temperatures and rainfall (Figure 1) were collected during the experiment at

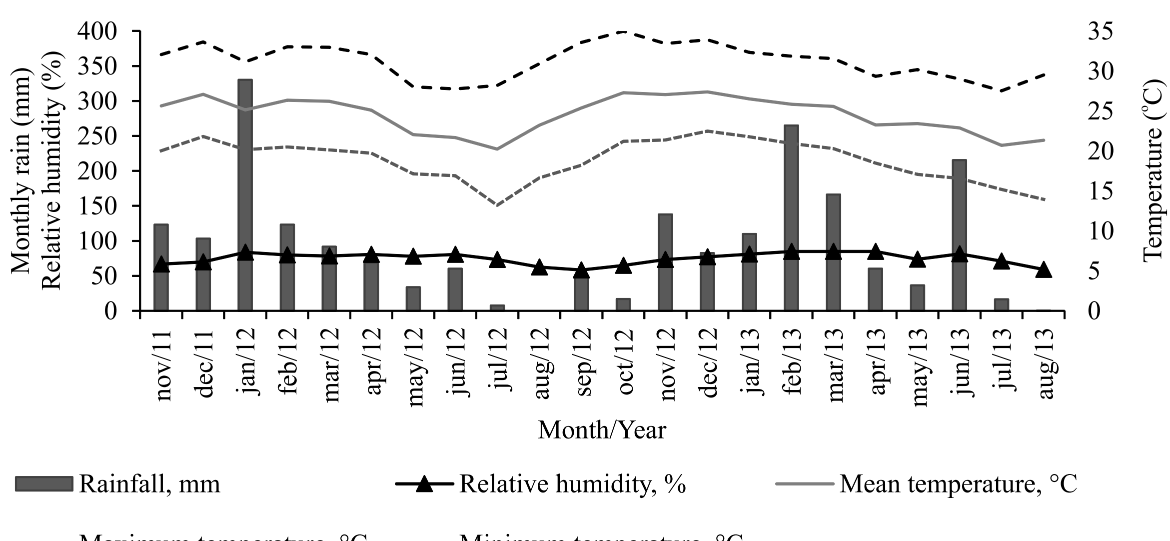


Figure 1. Climatic data of the meteorological station located in the Experimental Farm of FE/UNESP, in the municipality of Selvíria-MS, from November/2011 to August/2013.

the meteorological station located on the farm. The climatic data of the two agricultural years are in agreement with the historical average of the region, with mean annual rainfall of 1,370 mm, mean temperature of 23.5ºC, and relative humidity between 70-80%.

The soil is characterized as dystrophic Red Latosol with clayey texture (Santos et al., 2006), managed in no-tillage system (NTS) for nine years. The methods proposed by Raij et al. (2001) were adopted for determination of soil chemical attributes. For this purpose, 20 soil samples with deformed structure were collected with the aid of an auger from the 0.0-0.20 m layer, for the formation of a composite sample to characterize soil fertility of the experimental area. The samples were collected before the spring/summer crops of 2011/2012 and 2012/2013, which preceded the autumn corn crops in the years 2012 and 2013, respectively (Table 1).

The experiment had a randomized block design with four replications. The treatments were combined in a 2 × 5 factorial scheme, with two treatments of seeds (with inoculation [With Inoc] or without inoculation [Without inoculation] of corn seeds with *Azospirillum brasilense*) and five N fertilization doses (0; 60; 90; and 120 kg ha^{-1}) in the corn crop. Each experimental plot consisted of four rows of corn spaced 0.90 m apart, 6 m long, totaling 21.60 m².

In the two harvests, plants present in the experimental area were desiccated days before sowing of the corn and the xaraés grass in order to form straw for maintenance and continuity of the no-tillage system (NTS). The herbicide glyphosate (1.44 kg ha^{-1} of the active ingredient) was used, with subsequent harvesting of plants using a horizontal vegetable waste crusher (Triton).

The simple hybrid AG 8088 YG was mechanically seeded in the two crops, respectively. A sowing machine was used with a stem-like (machete) mechanism for NTS, depositing the seeds at a depth of 0.05 m, with 5.4 m^{-1} seeds, aiming to reach a final stand near 60,000 plants ha^{-1}. As sowing fertilizer, 300 kg ha^{-1} of the formula 08-28-16 (24 kg ha^{-1} N, 84 kg ha^{-1} P_2O_5 and 48 kg ha^{-1} K_2O) were applied.

Corn sowing was carried out on 11/04 in the two years (2012 and 2013). To treat the corn seeds, 150 g L^{-1} a.i. of imidacloprid + 450 g L^{-1} a.i. of thiodicarb were applied before sowing. In the treatments with inoculation, the inoculant was applied to the seeds with two strains of *A. brasilense* (Ab-V5 and Ab-V6) diazotrophic bacteria using 100 mL of the Azo Total® product for each 25 kg of seeds.

The sowing of *Urochloa brizantha* cv. Xaraés was carried out after sowing the corn, with another sowing machine with a double alternating disc and furrower type mechanism for NTS, with two lines of grass spaced 0.34 m between corn lines (Costa et al. 2012a). The seeds were deposited at an average depth of 0.08 m using approximately 7 kg ha^{-1} of viable pure seeds (CV = 76%). The seeds of the grass were placed in greater depth in relation to corn, following the recommendations of Kluthcouski et al. (2000), with the objective of delaying its emergence and reducing competition between species in the initial period of development of the intercrop.

Cover fertilization with N doses was done manually when the corn had reached the V6 phenological stage (six fully developed leaves). Ammonium sulphate (21% N and 23% S) was used as source, and the fertilizer was applied near the corn lines.

At the corn flour blooming stage (26/06/2012 and 19/06/2013), 20 opposite leaves and below the spikes were collected in the experimental area (two central lines with 4 meters in length), which had their average third separated for leaf analysis. The material was dried at 65°C in a forced air circulation oven for 72 h, ground and sent for analysis for macronutrient contents (Malavolta et al., 1997).

At the moment of corn harvest (12/09/2012 and 16/08/2013), measurements on 10 plants per plot were carried out to determine the production components of the corn crop. The stalk diameter (SD) was determined, with measurements performed in the second visible internode with the aid of a digital caliper. The mean plant height (PH) was evaluated by measuring the height of the plants until the top of the flag leaf sheath, as well as the height of insertion of the main spike (HIMS). For both measurements a ruler graduated in cm was used. The final plant stand ha^{-1} (FPS) and the final number of spikes ha^{-1} (FNS) were also evaluated, counting all the plants and spikes within the useful area of the plot.

Ten spikes per plot were randomly collected to determine length (SL) and diameter (SD). The mass of 100 grains (M100) was determined by the average counting and weighing of 4 samples (13% moisture). In order to determine grain yield (GY), all spikes within the useful area of the plots were collected, corrected to 13% moisture, and extrapolated to kg ha^{-1}.

In order to determine the dry matter yield of the aerial part (DMY) of xaraés grass, three samples per plot were randomly collected with the help of a 1.0 x 1.0 m metallic square. The grass was harvested at approximately 0.05 m from the soil surface on the day of the corn harvest. The samples were weighed and taken to drying at 65°C in a forced air circulation oven for 72 h to determine the dry matter yield. Afterwards, these samples were ground and sent to determine the N and crude protein content (CP = N% × 6.25), according to the methodology suggested by Silva & Queiroz (2002).

The data obtained in the experiments were submitted to analysis of variance to verify the effect of the isolated factors inoculation (I) and nitrogen dose (D) and the interaction between them (I × D). The effect of year was not included in the analysis and was evaluated separately. The Tukey test ($p \leq 0.05$) was used to compare the means of the treatments with and without inoculation. The effect of nitrogen coverage doses

Table 1. Characterization of soil chemical attributes at of 0 - 0.2 m depth. Selvíria, Mato Grosso do Sul. 2011 and 2012.

Year	$P_{(resin)}$ ($mg\ dm^{-3}$)	SOM* ($g\ dm^{-3}$)	pH ($CaCl_2$)	K^+	Ca^{2+}	Mg^{2+}	H+Al	Al^{3+}	BS**	CEC***	$S-SO_4$ ($mg\ dm^{-3}$)	V	m****
				($mmol_c\ dm^{-3}$)								(%)	
2011	68	23	5.8	2.6	34	26	20	0	62.6	82.6	2	76	0
2012	42	22	4.9	3.1	23	19	31	2	45.1	76.1	5	59	4

* SOM - Soil organic matter; ** BS - Base saturation; *** CEC - Cation exchange capacity; **** m - Aluminum saturation.

was evaluated through polynomial regression analysis. All analyses were performed using the statistical software Sisvar® 5.0 (Ferreira, 2011).

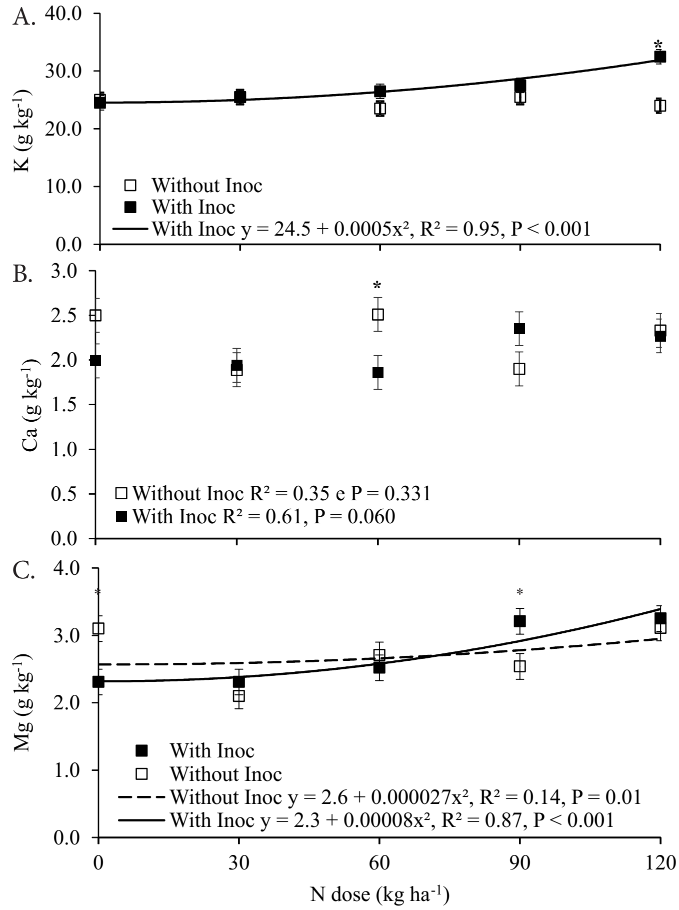
Results and Discussion

In the 2012 harvest, the inoculation of the seeds with *Azospirillum* had the effect of increasing the N, K and S leaf contents (Table 2). As a result of nitrogen coverage doses in 2012, positive linear adjustments were obtained for N and Mg contents (R^2 = 0.86 and 0.71, respectively). In the harvest of 2013, an effect of the inoculation with *Azospirillum* was seen in increasing the N content, with reduction of Ca and Mg contents, while the cover fertilization increased the N and P leaf contents (R^2 = 0.60 and 0.50, respectively). These nutrients are of great importance for the growth and development of corn, especially N, because they are part of the chlorophyll molecule, acting in the formation of substances such as proteins, enzymes and nucleic acids (Gross et al., 2006).

The highest leaf contents of N and of other nutrients confirm the positive nutritional effect of corn seed inoculation with *A. brasilense* (Table 2). These bacteria promote the fixation of atmospheric N_2 and contribute to the production of auxins, which are substances responsible for the stimulation of growth, mainly root growth, improving soil exploration and consequent nutrient absorption and nutrition of plants (Bashan & Holguin, 1997; Steenhoudt & Vanderleyden, 2000). The results obtained corroborate the reports of Hungria et al. (2010), in which the authors affirm that not only the increase in leaf absorption of N is related to seed inoculation with *Azospirillum*, but also of other nutrients such as K and S.

Leaf N contents were close to levels considered adequate by Malavolta et al. (1997), especially in the presence of inoculation with the diazotrophic bacteria (Table 2). As for the contents of the other nutrients, the values are within the appropriate levels regardless of treatment (Malavolta et al., 1997), thus promoting proper corn crop nutrition and development.

There was an interaction between the factors inoculation with *Azospirillum* and N dose (I × D) for K, Ca and Mg contents in the 2012 harvest (Figure 2). N doses and inoculation with *Azospirillum* positively influenced leaf K content, with data adjusted to a linear increasing equation (R^2 = 0.95, and p < 0.001). The interaction provided a higher K content mainly in the case of the treatment with inoculation + 120 kg ha^{-1} N. It is also observed that when the inoculation + 60 kg ha^{-1} N was carried out, there was a greater accumulation of K in corn leaves



* Means differ between each other according to Tukey test (p ≤ 0.05).

Figure 2. Unfolding of the interaction of the macronutrient leaf contents in the flowering corn as a function of seed inoculation (I) with *Azospirillum brasilense* and N coverage doses (D) in the 2012 harvest. A) Potassium content; B) Calcium content; C) Magnesium content.

Table 2. Macronutrient leaf contents in flowering corn as a function of seed inoculation with *Azospirillum brasilense* and N coverage doses. Selvíria - MS, 2012 and 2013 harvests.

Item	Inoculation (I)		N dose, kg ha^{-1} (D)					CV, %	P-value		
	With	Without	0	30	60	90	120		I	D	I × D
Harvest 2012											
N, g kg^{-1}	25.4a	22.6b	22.6(1)	23.6	23.0	24.1	26.5	13.0	<0.001	0.002	0.714
P, g kg^{-1}	3.8	3.3	4.0	3.8	3.5	3.6	2.9	24.5	0.125	0.121	0.957
K, g kg^{-1}	27.3a	24.7b	24.8	25.5	25.0	26.5	28.2	9.6	0.003	0.060	0.009
Ca, g kg^{-1}	2.1	2.2	2.2	1.9	2.2	2.1	2.3	18.3	0.255	0.346	0.058
Mg, g kg^{-1}	2.7	2.7	2.7(2)	2.2	2.6	2.9	3.2	14.7	0.937	0.001	0.015
S, g kg^{-1}	1.6a	1.4b	1.5	1.4	1.4	1.5	1.6	14.7	0.003	0.098	0.231
Harvest 2013											
N, g kg^{-1}	27.7a	26.5b	23.4(3)	26.8	27.9	28.4	29.1	6.2	0.041	<0.001	0.424
P, g kg^{-1}	4.0	3.9	3.7(4)	4.0	3.9	4.1	4.1	4.4	0.182	0.006	0.240
K, g kg^{-1}	23.1	22.7	22.3	21.8	22.5	24.3	23.8	10.1	0.588	0.183	0.498
Ca, g kg^{-1}	2.7b	3.0a	2.6	2.8	3.1	2.8	2.9	10.0	<0.001	0.150	0.562
Mg, g kg^{-1}	2.1b	2.3a	2.1	2.2	2.3	2.3	2.2	10.4	0.008	0.497	0.362
S, g kg^{-1}	0.7	0.8	0.7	0.8	0.8	0.8	0.8	19.7	0.135	0.309	0.585

[1]There was a significant effect of the isolated factors according to the F test (p ≤ 0.05) and of the interaction of the factors according to the Tukey test (p ≤ 0.05).
Means followed by distinct letters in the lines differ from each other. (1) N content = 22.6 + 0.00024x (R^2 = 0.86 and p < 0.001); (2) Mg content = 2.4 + 0.00005x (R^2 = 0.71 and p < 0.001); (3) N content = 25.5 + 0.000293x (R^2 = 0.60 and p < 0.001); (4) P content = 3.9 + 0.0000147x (R^2 = 0.50 and p = 0.01).

when compared without inoculation + 90 kg ha^{-1} N and without inoculation + 120 kg ha^{-1} N, resulting in a possible reduction of 30 to 60 kg ha^{-1} of nitrogen fertilizer, respectively (Figure 2A). As for Ca content, the highest observed value was for the treatment without inoculation + 60 kg ha^{-1} N (Figure 2B). As for Mg, there was a positive linear adjustment for inoculation and N dose ($R^2 = 0.87$, $p < 0.001$), the highest value observed for the treatment with inoculation 90 kg ha^{-1} N and 120 kg ha^{-1} N, with a reduction of 30 kg ha^{-1} of nitrogen fertilizer (Figure 2C).

The inoculation of corn seeds with *A. brasilense* increased the PH in the 2012 crop and AD, PH, FPS and FNS in the 2013 crops, but did not influence grain yield in any crop (Table 3, Figure 3). In the 2013 crop, there was a reduction in the M100 in the treatment with inoculation, however, the higher vegetative growth provided by the higher FPS and FNS resulted in a GY similar to the treatment without inoculation (Table 3). These results corroborate those obtained by Cavallet et al. (2000), who reported that the effects of the bacterium *Azospirillum* spp. in grasses have been observed in relation to the vegetative and physiological aspects, such as the increase in the number of lateral and adventitious roots, and the increase of the activity of photosynthetic enzymes and nitrogen assimilation, which are linked to the growth potential, and the dry mass yield, and grain yield. These authors concluded that the inoculation did not promote a significant increase of GY, as also seen in the present research.

Therefore, the benefits of inoculation with *Azospirillum* may not culminate in grain yield gains. There are many reports of positive responses to the inoculation of diazotrophic bacteria in cereals (Roesch et al.; 2006; Sala et al., 2008; Hungria et al., 2010), although absence of effect of inoculation has also been reported (Ogüt et al., 2005; Godoy et al., 2011). According to Roesch et al. (2006), the potential of atmospheric nitrogen fixation, the promotion of corn crop growth and the productivity in symbiosis with diazotrophic bacteria depend on biotic and abiotic factors such as plant genotype, soil microbiological community and availability of nitrogen in the system. Another important factor is the use of pesticides in the seeds, which have become much frequent; the toxicity caused in the inoculated bacteria in the culture is not well known, but it might likely limit or even inhibit their positive action (Fukami et al., 2016).

Increasing linear regressions were adjusted for the effect of N coverage doses on the variables SL, SD and M100 (R^2 = 0.77, 0.81 and 0.99, respectively), also resulting in a linear increase of corn GY ($R^2 = 0.86$) in 2012, which did not occur in 2013, when only SL, FPS and FNS (R^2 = 0.32, 0.60 and 0.45, respectively) were increased as greater contribution of N in coverage was provided. However, with low regression coefficients for SL and FNS, since the increment of the final plant stand together with the increase of N doses apparently led to greater vegetative growth of the plants and intraspecific competition, which did not result in grain yield gains, as happened in 2012.

Mean GY values for the inoculated treatment were 5,373 and 4,639 kg ha^{-1} and for the non-inoculated were 5,462 to 5,005 kg ha^{-1} (2012 and 2013 harvest, respectively). As a function of N doses, the 2012 and 2013 values were 3,648 and 4,767 kg ha^{-1}, respectively, for the control without application of N and 6.276 and 4.594 kg ha^{-1} for application of 120 kg ha^{-1} N. According to the National Supply Company (Conab, 2017), the national average productivity in the 2015/16 harvest was 4,799 kg ha^{-1} for the first harvest and 3,861 kg ha^{-1} for the second harvest. Thus, the productivity obtained in the present research was very close and even superior, in some treatments, to the national productivity.

According to the results found in the present research, positive effects of the treatments in the production system

Table 3. Stalk diameter (SD), plant height (PH), height of the main stem insertion (HIMS), spike length (SL), spike diameter (SD), final plant stand per hectare (PS), number of spikes per hectare (NS), mass of 100 grains (M100) and corn grain yield (GY) as a function of seed inoculation with *Azospirillum brasilense* and nitrogen coverage doses. Selvíria - MS, 2012 and 2013 harvests.

Item	Inoculation (I)		N dose, kg ha^{-1} (D)					CV%	P-value		
	With	Without	0	30	60	90	120		I	D	I × D
Harvest 2012											
DC, mm	23.9	23.1	22.7	23.1	23.5	23.9	24.2	10.4	0.185	0.124	0.469
ALTP, m	2.39a	2.24b	2.29	2.29	2.30	2.32	2.36	3.1	0.009	0.201	0.755
AIEP, m	1.27	1.16	1.23	1.20	1.19	1.21	1.24	6.0	0.071	0.251	0.762
SL, cm	15.1	14.9	12.9(1)	14.6	15.6	16.0	15.8	5.4	0.568	0.006	0.627
SD, mm	44	44	42(2)	43	44	45	45	2.4	0.522	0.003	0.852
FPS, number ha^{-1}	58,111	60,333	58,905	59,798	59,956	59,381	58,071	6.3	0.063	0.968	0.674
FNS, number ha^{-1}	59,611b	62,778a	60,615	60,845	61,135	61,484	61,892	5.9	0.015	0.132	0.389
M100, g	26.7	25.8	23.7(3)	25.0	26.3	27.6	28.9	6.7	0.929	0.040	0.741
GY, kg ha^{-1}	5,373	5,462	3,648(4)	4,989	5,874	6,303	6,276	20.1	0.141	<0.001	0.805
Harvest 2013											
DC, mm	17.8a	16.1b	16.2	17.1	17.5	17.3	16.5	10.7	0.006	0.604	0.954
ALTP, m	2.26a	2.17b	2.20	2.21	2.20	2.27	2.19	3.9	0.003	0.424	0.833
AIEP, m	1.33	1.29	1.30	1.31	1.32	1.30	1.33	7.8	0.222	0.969	0.820
SL, cm	11.6	11.7	10.6(5)	11.3	12.1	12.3	11.8	8.3	0.706	0.011	0.810
SD, mm	37b	39a	37	37	38	38	38	5.0	0.025	0.151	0.983
FPS, number ha^{-1}	63,333a	59,833b	59,722(6)	58,056	63,056	63,472	63,611	6.1	0.006	0.015	0.083
FNS, number ha^{-1}	54,278a	49,889b	47,639(7)	49,028	55,139	54,722	53,889	10.6	0.017	0.026	0.218
M100, g	17.6b	21.8a	22.8	18.6	20.3	17.5	19.2	28.2	0.024	0.388	0.544
GY, kg ha^{-1}	4,639	5,005	4,767	4,509	5,388	4,854	4,594	23.5	0.196	0.308	0.640

[1]There was a significant effect of the isolated factors according to the F test ($p \leq 0.05$) and of the interaction of the factors according to the Tukey test ($p \leq 0.05$). Means followed by distinct letters in the lines differ from each other.

(1) SL = 12.9 + 0.0712x (R^2 = 0.77 and p < 0.001); (2) SD = 42 + 0.0708x (R^2 = 0.81 and p < 0.001); (3) M100 = 22.4+ 0.0130x (R^2 = 0.99 and p < 0.001); (4) GY = 3447 + 35.7x (R^2 = 0.86 and p < 0.001); (5) SL = 11.3 + 0.000065x (R^2 = 0.32 and p = 0.032); (6) FPS = 59.797 + 0.03308x (R^2 = 0.60 and p = 0.006); (7) FNS = 49.952 + 0.03947x (R^2 = 0.45 and p = 0.022).

evaluated in tropical regions, both in the nutrition and in the production of the cultures over time, were evident (Table 3). It is likely that previous fertilizations during the cultivations (residual effect) provided nutrient requirements for the corn crop, reducing the effect of the inoculation and the nitrogen fertilization, thus influencing the absence of interaction between the I × D factors (Figure 3).

It is also worth noting the benefits of straw in the production system, since this is an agricultural area with a history of nine-year NTS. The decomposition and mineralization processes of plant residues make it possible the occurrence of cycling and nutrient release in successive crops (Pariz et al., 2011b; Costa et al., 2014, 2015b).

Although there was no significant effect of the interaction between inoculation with *Azospirillum* and N dose for GY (Figure 3B), a higher GY for the inoculation + 60 kg ha^{-1} N was seen when the means were separately observed during the inoculation treatment in the 2013 harvest, lowering around 30 to 60 kg ha^{-1} of nitrogen fertilization when compared with the inoculation + 90 kg ha^{-1} N and inoculation + 120 kg ha^{-1} N (Fig. 3B).

In general, the second crop of the agricultural year can also benefit crop nutrition. This is mainly due to the improvement of the production system by the adoption of conservation practices such as NTS, crop rotation and intercropping, which aim to improve soil quality throughout crops. Crop rotation systems produce higher amounts of dry matter in the off-season, releasing more nutrients later, through the decomposition of straw (Rosolem et al., 2006; Calonego and Rosolem, 2010; Raphael et al., 2016; Tiritan et al., 2016).

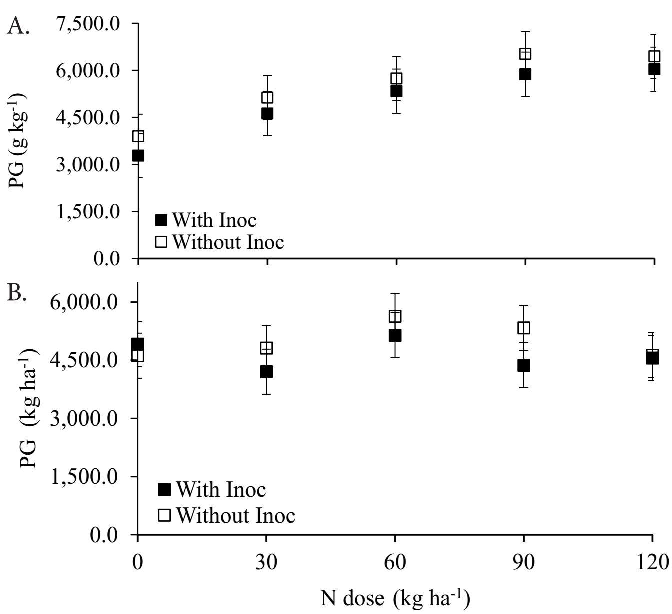


Figure 3. Unfolding of corn grain yield (GY) interaction as a function of seed inoculation (I) with *Azospirillum brasilense* and N coverage doses (D). A) 2012 harvest; B) 2013 harvest.

In order to achieve success in corn grain yield in a intercropping with forage grasses, several factors must be taken into account, mainly because competition between the two species in the same area may happen. In the present work, the 0.90 m spacing for corn and 0.34 m for grass did not result in competition among the intercropped species. On the other hand, narrower spacing of 0.45 m between rows for corn, only one row of grass is recommended in the inter-row of the grain crop. Thus, the knowledge of species behavior in the search for water, light and nutrients becomes of great importance (Borghi & Crusciol, 2007; Pariz et al., 2011a).

Inoculation of corn seeds with *Azospirillum* promoted lower DMY of the grass for both crops (Table 4). Such an outcome may have occurred due to competition in the corn intercrop. As a result of the inoculation, the corn plants presented higher vegetative growth in SD and PH, which reflected in lower growth of the intercropped xaraés grass. Thus, more intense shading by corn may cause the dry mass accumulation rate of the grass to decrease, leading to dispense the application of herbicide sub-doses to restrict the growth of intercropped grass and contributing to lower the cost of production of corn in this system.

Nitrogen coverage fertilization in corn did not change the DMY of the Xaraés grass (Table 4). However, the amount of plant material produced by xaraés grass demonstrates the great potential of this production system, which can guarantee forage feed in the off-season. After harvesting the grains, grazing is already practically established and, after grazing, desiccation makes it possible to obtain straw for NTS, contributing to soil cover and nutrient cycling (Costa et al., 2012a; 2014).

No change in CP content of the Xaraés grass as a function of the inoculation of corn seeds with *Azospirillum* was seen in any harvest (Table 4). Positive linear regression adjustment was obtained for CP content of the grass ($R^2 = 0.64$) as a function of the N coverage doses, indicating a greater accumulation of CP following the increase of the N supply. This fact can provide enhanced digestibility due to the increase of cell content (amino acids and proteins) and cell wall dilution, thus reflecting the nutritional utilization by the animals, when used as forage (Van Soest, 1994).

The CP contents of the xaraés grass at the time of corn harvest were well above the 7% recommended by Van Soest (1994), which is considered the minimum acceptable for the

Table 4. Dry mass yield (DMY) and crude protein (CP) content of xaraés grass (*Urochloa brizantha*) intercropped with corn in fall crop as a function of inoculation of corn with *Azospirillum brasilense* and N coverage doses. Selvíria - MS, 2012 and 2013 harvests.

Item	Inoculation (I)		N dose (kg ha^{-1})					CV%	P-value		
	With	Without	0	30	60	90	120		I	D	I × D
					Harvest 2012						
DMY, kg ha^{-1}	3,280b	5,080a	4,325	4,050	4,275	3,750	4,500	30.1	<0.001	0.792	0.184
CP, %	14.1	14.4	9.3(1)	13.2	15.5	16.6	17.0	8.1	0.518	<0.001	0.240
					Harvest 2013						
DMY, kg ha^{-1}	2,755b	4,267a	3,633	3,402	3,591	3,150	3,780	30.1	<0.001	0.792	0.187
CP, %	12.0	12.2	7.9(2)	11.3	13.1	13.8	14.4	8.1	0.964	<0.001	0.289

[1]There was a significant effect of the isolated factors according to the F test ($p \leq 0.05$) and of their interaction according to the Tukey test ($p \leq 0.05$). Means followed by distinct letters in the lines differ from each other. (1) CP content = 12.0 + 0.00042x ($R^2 = 0.64$ and $p < 0.001$); (2) CB content = 10.2 + 0.000358x ($R^2 = 0.64$ and $p < 0.001$).

proper functioning of the rumen microbiota. Thus, the results obtained in the present research demonstrate the efficiency and relevance of nitrogen fertilization, both via mineral and via biological fixation of atmospheric N by diazotrophic bacteria, both for the adequate nutrition and development of corn intercropped with Xaraés grass.

According to Hungria et al. (2010), one of the main factors to be considered in relation to the benefits of diazotrophic bacteria is that grasses, such as brachiaria, in successive crops may be hosts of these native soil bacteria. Therefore, more studies on intercropping are needed, considering the importance of more sustainable systems in the world agricultural scenario, aiming to take advantage of the secondary species in the development of the main crops and to the improvement of soil quality.

Conclusions

The inoculation of corn seeds with *A. brasilense* in intercropping with xaraés grass in the fall season increases nutrition, height growth of plants and components of corn production, however, without effect on grain yield.

Corn plants from seeds inoculated with *A. brasilense* lead to lower production of dry mass of xaraés grass in intercropping.

Nitrogen coverage fertilization linearly increases the crude protein content of the Xaraés grass, up to the dose of 120 kg ha^{-1} N.

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