

Grain sorghum grown as second crop and inoculated with *Azospirillum brasilense* associated with nitrogen fertilization

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ABSTRACT

Nitrogen fertilization in off-season sorghum may have lower cost-benefit ratios than expected due to the residual effect of previous crops. However, the use of growth-promoting bacteria can be an economical alternative to increase crop yields in Cerrado. The objective was to evaluate the response of grain sorghum to nitrogen fertilization and its inoculation by *Azospirillum brasilense*. This study aims to evaluate grain sorghum responses to nitrogen fertilization and *A. brasilense* inoculation. The experiment was carried out in a fully randomized block design and 5 x 2 factorial scheme, with five replications. Treatments consisted of applying N recommendation (120 kg N-urea ha⁻¹) split as follows: 100% at sowing, 100% as topdressing, 25% at sowing and 75% as topdressing, 50% at sowing and 50% as topdressing, and a control (without application), all these with and without *A. brasilense* seed inoculation. We also assessed the morphological and productivity components of grain sorghum. The application of 100% N at sowing promoted taller plants, while 50% -50% splitting shorter ones. Overall, grain sorghum plants did not respond to *A. brasilense* inoculation. Moreover, yield components of rainfed sorghum grown in succession to soybeans were not affected by *A. brasilense* inoculation and N supplied at once or split.

Keywords: Splitting, Crop succession, *Sorghum bicolor*, PGPB.

Sorgo granífero em segunda safra inoculado com *Azospirillum brasilense* associado a adubação nitrogenada

RESUMO

As atuais recomendações de N para o sorgo segunda safra, em plantio direto, podem ser ineficientes devido a fatores técnicos e edafoclimáticos. Assim, a inoculação por *Azospirillum brasilense* tem sido estudada como uma alternativa econômica para suprir N à cultura no Cerrado. Avaliou-se a resposta do sorgo granífero à adubação nitrogenada associada a inoculação com *A. brasilense*. O delineamento experimental foi em blocos casualizados, em esquema fatorial 5 x 2, com cinco repetições dos seguintes tratamentos: quantidade recomendada de N (120 kg ha⁻¹ de N-ureia) aplicada 100% na semeadura, ou apenas em cobertura, ou parcelada – 25% na semeadura e 75% na cobertura, ou 50% na semeadura e 50% na cobertura, e o controle (sem N); com e sem inoculação via semente do sorgo. Foram avaliados os componentes morfológicos e de produtividade da cultura. A aplicação de 100% do N na semeadura proporcionou plantas com porte maior e o parcelamento 50% -50% proporcionou plantas de menor porte. As plantas não responderam à inoculação por *A. brasilense*. Os componentes de produção do sorgo cultivado em sucessão à soja, em sequeiro, não se alteram devido à inoculação por *A. brasilense* e ao fornecimento ou parcelamento da adubação nitrogenada.

Palavras-chave: Parcelamento, Sucessão de culturas, *Sorghum bicolor*, BPCP.

1. Introduction

Off-season grain sorghum is more efficient than other grain crops such as corn and wheat as for water-to-dry matter conversion (Magalhães et al., 2015), which occurs due to biochemical and morphological mechanisms making plants more drought tolerant. Therefore, including sorghum in crop rotation/succession becomes relevant in no-tillage systems (NTS) in the Brazilian Cerrado. Indeed, NTS consolidation and success is due to the use of straw-producing plants at proper amounts for soil covering (Andreotti et al., 2008), combined with suitable nutritional management and other cultural treatments.

Sorghum responses to nitrogen (N) fertilization depend on previous crops and may range from high and medium to low (Cantarella et al., 1997). Besides identifying its responsiveness, suitable amount and timing for mineral N application are also relevant for decision-making by farmers.

As a rule, succession between legume and non-legume crops has a good effect (Mascarenhas et al., 2011). According to Arif et al. (2011), corn crops perform better when grown with residue from chickpeas if compared to residue from other grains. This is due to the low C/N ratio of legume residues that mineralize quickly and can release N, meeting the demand for subsequent crops (Odhiambo, 2010). In this sense, many farmers do not fertilize crops and only sow on residues from previous crops (usually soybeans). This practice is based on the promotion of atmospheric N fixation by legumes, relatively speeding up N cycling given fast decomposition and mineralization of low C/N ratio residues (Veras et al., 2016). Mineralization is rapid due to the high N rates and low lignin and polyphenol contents in the residue (Abera et al., 2012).

Conversely, the use of nitrogen fertilizers in sorghum crops is deemed to be reduced when compared to demands of crops of great tradition and economic interest in Brazil. Moreover, the high cost of nitrogen fertilizers has driven research into natural N fixation and plant growth-promoting bacteria (PGPB) studies, as these supply all or part of the N demanded by crops or help absorb nutrients at low cost and less environmental impact (Hungria, 2011). In this context, studies on *Azospirillum brasilense* inoculation in grasses have exhibited several benefits to host plants, such as morphological and nutritional changes, increase in crop yield, among others (Longhini et al., 2016; Nakao et al., 2018).

Benefits of *Azospirillum*-host plant interactions have been reported as results from biological nitrogen fixation (BNF) by colonizing bacteria. However, the symbiosis with rhizobacteria can produce plant growth-promoting substances (Bhattacharjee et al., 2008) such as auxins,

cytokinins, and gibberellins (Ona et al., 2003; Cassán et al., 2009). Both mechanisms can occur simultaneously. However, once the efficiency of *A. brasilense* inoculation in plants can be influenced by several environmental, technical, and crop-intrinsic factors, further studies are still needed to evaluate such interactions under different environmental conditions.

In this study, we hypothesized that off-season sorghum under rainfed conditions and after soybean cultivation, grown on a clayey soil of medium fertility, dispense with N fertilization due to residual effects of the legume, and that, under these conditions, seed inoculation with *A. brasilense* improves sorghum development and yield. Therefore, our study aimed to evaluate grain sorghum performance in response to N-fertilization splitting and *A. brasilense* seed inoculation.

2. Material and Methods

The current experiment was conducted at the Teaching, Research and Extension Farm of the College of Engineering of Ilha Solteira. The site is in the city of Selvíria, Mato Grosso do Sul State, Brazil (20° 18' S, 51° 22' W, 370 m above sea level) (Figure 1). The experiment was conducted during the 2016 off-season under rainfed conditions. According to Köppen's classification, the local climate is classified as *Aw*. Figure 2 shows the climate data gathered throughout the experiment.

According to Santos et al. (2018), the soil in the studied area is classified as clayey Red Latosol (Oxisol). Before the experiment, a grain-size analysis was performed (0.0 to 0.20 m depth) by the pipette method (Embrapa, 1997), and the results were as follows: 480, 175, and 345 g kg⁻¹ clay, silt, and sand fractions, respectively. Soil fertility was analyzed in the layers from 0.00 to 0.10 and from 0.10 to 0.20 m, following Raij et al. (2001) (Table 1).

The experimental area had been cultivated under a no-tillage system (NTS) for five years. In the 2016 agricultural year, grain sorghum was grown (autumn/winter) in succession to soybeans (spring/summer). Fifteen days before the experiment, weeds were killed by the application of glyphosate (1.44 kg a.i. ha⁻¹), and plant residues ground using a horizontal mill (Triton).

The experimental design was randomized blocks and a 5 x 2 factorial scheme, with five replications. Treatments comprised 0% N at sowing and 100% as topdressing, 25% at sowing and 75% as topdressing, 50% at sowing and 50% as topdressing, 100% at sowing and 0% as topdressing, and a control (no N application), all with and without sorghum seed inoculation with *A. brasilense*. The fertilization was done manually and followed the recommended dose of 120 kg ha⁻¹ N, using urea as a source.



Figure 1 - Location of the experimental area. Selvíria city (MS), Brazil, 2016. SIRGAS 2000, Cartographic Bases (IBGE, 2020).

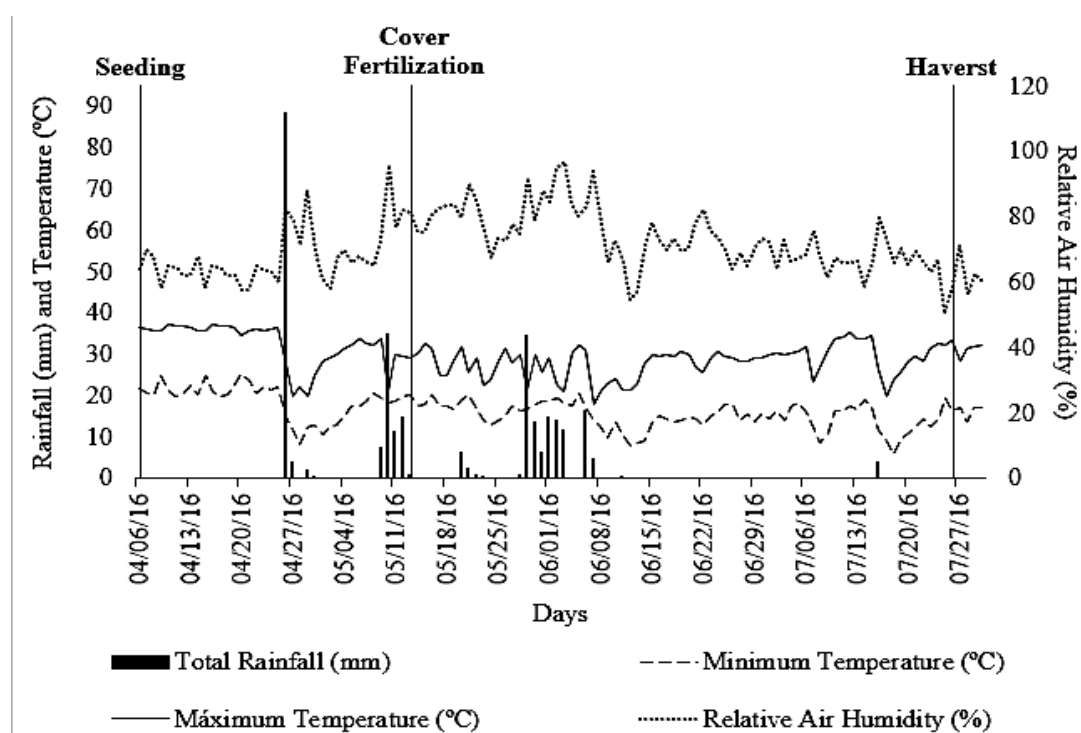


Figure 2 – Daily meteorological data of rainfall (mm), maximum and minimum temperatures (°C), and relative air humidity (%) during the experimental period. Selvíria – MS, Brazil, 2016.

Table 1. Soil chemical properties before sorghum sowing in Selvíria – MS, Brazil, 2016.

Depth	resin P	OM	pH	K	Ca	Mg	H+Al	Al	SB	CEC	V
(m)	(mg dm ⁻³)	(g dm ⁻³)	(CaCl ₂)	(cmol _c dm ⁻³)							(%)
0.00–0.10	27	23.0	5.4	0.4	2.0	1.6	3.6	0.0	4.0	7.5	51
0.10–0.20	18	19.4	5.1	0.3	1.5	1.0	3.9	0.1	2.7	6.6	40

The hybrid sorghum 'Ranchero' was mechanically sown on April 6, 2016, under NTS, and spaced 0.45 m between rows. Sowing fertilization consisted of 90 kg P₂O₅ and 30 kg K₂O, using simple superphosphate and potassium chloride as sources, respectively. Topdressing was done at the early panicle emergence (2-GS2 growth stage) when plants were around 0.30 m tall (May 13, 2016).

Experimental plots comprised seven 6-m-long and 3.15-m-wide sowing rows (22.05 m²). Only the three central rows composed the useful area, disregarding 1.5 m from the ends of each row. The hybrid 'Ranchero' was used since it has an aptitude for grain production. Diazotrophic bacteria were supplied by the AzoTotal™ inoculant, which was developed for corn and wheat crops (under registration number PR-93923-10074-1 by the Brazilian Ministry of Agriculture). It has a liquid physical nature, a density of 1.0 g mL⁻¹, and a dose of 100 mL per 20 kg seeds (containing 2 x 10⁸ colony forming units CFUs per mL of *A. brasilense*, AbV5 and AbV6 strains). Sorghum seeds were inoculated about 30 minutes before sowing under shade.

Nitrogen topdressing was performed manually by applying fertilizer at 10 cm from sorghum plants as a function of each treatment, at about 30 days after emergence (DAE) when plants were about 0.30 m high (13/05/2016) at panicle initiation stage (GS2).

At harvest time (July 26, 2016), the final plant stand (FPS) was determined by counting plants within 3 central rows of each plot, discarding 1.5 m from each end. For evaluations, 10 plants were randomly collected from each useful area, and the following parameters were determined: stem basal diameter (SBD), plant height (PH), panicle insertion height (PIH), panicle length (PL), total plant dry matter (TPDM), panicle dry matter (PaDM), grain mass per panicle (GMP), grain number per panicle (GNP), and rates of stem, leaf, and panicle dry matter in relation to TPDM. These fractions were separated with the aid of pruning shears and then weighed and dried in an oven (65 °C) to determine dry matter and harvest index (ratio between grain dry matter (GDM) and TPDM). One thousand grain mass (TGM) was determined by weighing four subsamples per plot and corrected for 13 % moisture.

All data underwent Shapiro-Wilk's test of normality. Afterwards, data were analyzed by F-test ($p < 0.05$), and the means compared by Tukey's test ($p < 0.05$) using SISVAR 5.7 software (Ferreira, 2019).

3. Results and Discussion

There was no significant interaction between inoculation and nitrogen splitting for the studied parameters ($p > 0.05$). Many studies have reported the benefits of *Azospirillum*-plant interactions (Longhini et

al., 2016; Nakao et al., 2018). However, plant responses to inoculation can be influenced by cropping conditions such as soil pH, temperature, and humidity (Cardoso et al., 2010; Hungary, 2011). These factors proved to be limiting for bacteria due to climatic conditions (Figure 2) during sorghum crop implantation.

Nitrogen splits significantly affected ($p < 0.05$) plant (PH) and panicle insertion (PIH) heights of sorghum plants. Fractional fertilization of 50% at sowing and 50% as topdressing reduces PH and PIH compared to 100% at sowing. The other treatments did not differ from each other (Table 2). Short sorghum plants facilitate mechanized grain harvesting and reduce risks of lodging, increasing final yields (Pereira Filho and Rodrigues, 2015). However, the highest PH (1.4 m) when applying 100 % at sowing does not cause lodging. Molina et al. (2000) observed barely any (0%) lodging losses in sorghum plants between 1.0 to 1.5 m high, which is common in grain sorghum hybrids.

The significant response of sorghum to 100% N fertilization at sowing (Table 2) can be justified both by its larger demand in early growth and intense nutrient uptake between 20 to 30 DAE (7 to 12 fully developed leaves) (Coelho et al., 2002). As regards the clayey soil texture, it contributed to lower N losses from mineral fertilizers (Tahir and Marschner, 2017). Another time of high nutritional demand for sorghum is the grain-forming stage (Coelho et al., 2002), which is when roots are well developed and explore efficiently the soil volume. Therefore, the absence of N topdressing (100 % - 0 %) may have been supplied by organic matter in the soil (23.0 g dm⁻³), contributing greatly to N availability in soil solution by mineralization (Lorenzini et al., 2014).

Averages of panicle dry matter (PaDM), grain dry matter (GDM), and total plant dry matter (TPDM) were not influenced by the interaction between inoculation and splitting forms ($p > 0.05$). According to Macedo et al. (2018), sorghum PH is generally related to PDM, which was not observed in our study (Table 2 and Table 3). Such results corroborate the previously mentioned hypothesis in which sorghum grown in a medium-fertility clayey soil, in succession to soybean cultivation, does not require N fertilization due to legume residual effects. Although it is an off-season crop, grain yield reached suitable values for the season (5.5 to 6.4 t ha⁻¹; Table 3), according to Freitas et al. (2014).

Under favorable climatic conditions, well-managed commercial crops, mainly nutrient supply and disease control, can reach sorghum crop yields between 6 and 8 t grain ha⁻¹ during the off-season (Resende et al., 2009). These yields can also be met even in the Cerrado, where there is less water availability for crops in the off-season (usually after soybeans). This is due to the well-fertilized soils, planting of improved cultivars, and use of technologies for general crop management.

Table 2. Averages of final plant stand (FPS), stem basal diameter (SBD), plant height (PH), panicle insertion height (PIH), and panicle length (PL) of grain sorghum plants under nitrogen fertilization splitting followed by their respective standard errors.

Treatment	FPS	SBD	PH	PIH	PL
<u>Inoculation (I)</u>	(plants ha ⁻¹)	(mm)	(m)	(m)	(cm)
Without	191,605 ± 6,160	17 ± 0.3	1.3 ± 0.01	1.1 ± 0.01	26 ± 0.4
With	175,309 ± 7,473	17 ± 0.3	1.2 ± 0.02	1.0 ± 0.02	27 ± 0.3
<u>N splitting (S)¹</u>					
0 %-100 %	177,778 ± 8,226	17 ± 0.4	1.3 ± 0.02 ab	1.0 ± 0.02 ab	26 ± 0.5
25 %-75 %	173,827 ± 10,018	17 ± 0.3	1.3 ± 0.02 ab	1.0 ± 0.03 ab	27 ± 0.2
50 %-50 %	162,469 ± 7,706	17 ± 0.3	1.2 ± 0.01 b	0.9 ± 0.02 b	27 ± 0.7
100 %-0 %	188,148 ± 12,648	17 ± 0.6	1.4 ± 0.02 a	1.1 ± 0.02 a	26 ± 0.4
Control	194,074 ± 15,472	16 ± 0.7	1.3 ± 0.03 ab	1.0 ± 0.04 ab	26 ± 0.8
	p-values				
I	0.0904 ^{ns}	0.7152 ^{ns}	0.3103 ^{ns}	0.1824 ^{ns}	0.0852 ^{ns}
S	0.2006 ^{ns}	0.7386 ^{ns}	0.0490*	0.0348*	0.2019 ^{ns}
I x S	0.1008 ^{ns}	0.3323 ^{ns}	0.1253 ^{ns}	0.1533 ^{ns}	0.1073 ^{ns}
CV %	12	6	4	4	4

Means followed by different letters differ from each other by Tukey's test at 5 % probability. ¹at sowing and as topdressing, respectively; *: p<0.05; ns: non-significant by Tukey's test (p>0.05); CV %: coefficient of variation

Table 3. Averages of plant dry matter (PDM), panicle dry matter (PaDM), yield dry matter (YDM), grain yield (YP), harvest index (HI) of grain sorghum under N fertilization splitting, followed by their respective standard errors.

Treatment	PDM	PaDM	YDM	GY	HI
<u>Inoculation (I)</u>	(g)		(Mg ha ⁻¹)	(kg ha ⁻¹)	-
Without	64.4 ± 5.35	38 ± 3	13.3 ± 0.66	6,055 ± 251	0.47 ± 0.04
With	57.8 ± 3.01	32 ± 2	12.9 ± 0.39	6,183 ± 186	0.50 ± 0.02
<u>N splitting (S)¹</u>					
0 %-100 %	66.2 ± 8.31	36 ± 4	14.1 ± 1.04	5,994 ± 297	0.44 ± 0.04
25 %-75 %	66.4 ± 7.30	36 ± 5	12.1 ± 0.90	6,385 ± 170	0.49 ± 0.07
50 %-50 %	67.1 ± 8.21	37 ± 4	13.7 ± 0.42	5,974 ± 374	0.46 ± 0.04
100 %-0 %	59.1 ± 3.25	34 ± 3	12.6 ± 0.82	6,334 ± 416	0.51 ± 0.05
Control	58.7 ± 8.14	39 ± 4	12.8 ± 0.32	5,472 ± 253	0.39 ± 0.03
	p-values				
I	0.3525 ^{ns}	0.0564 ^{ns}	0.6587 ^{ns}	0.6869 ^{ns}	0.6204 ^{ns}
S	0.8579 ^{ns}	0.9430 ^{ns}	0.3677 ^{ns}	0.3367 ^{ns}	0.4874 ^{ns}
I x S	0.3512 ^{ns}	0.5513 ^{ns}	0.3535 ^{ns}	0.4865 ^{ns}	0.7441 ^{ns}
C.V. %	24	25	13	12	23

¹At sowing and as topdressing, respectively; CV %: coefficient of variation; ns: non-significant by Tukey's test (p>0.05).

The above-mentioned results reflected in the harvest index (HI), which was similar in all N-splitting forms (Table 3). This factor is adequate, and although the control had a lower HI, we assume that it is not due to treatment (p>0.05). The index measures genotype efficiency in translocating photoassimilates to grains, and might be negatively influenced by environmental adversities such as water and nutrient availability and temperature (Pereira Filho and Rodrigues, 2015). Such conditions were not limiting for sorghum development in our study. Crop productivity components were not significantly influenced by inoculation and N-fertilization splitting interaction

(p>0.05). Percentages of plant and reproductive sorghum components showed no significant differences. These parameters were not dependent on inoculation and fertilizer application or even respective splitting (Table 4).

Notably, even control (without N fertilization) showed no significant differences for the productivity parameters (p> 0.05) compared to fertilizer treatments (Tables 2, 3, and 4). It may be due to soybean residues with a low C:N ratio, providing sorghum high quality (Odhambo, 2010). Indeed, low lignin and polyphenol contents speed up decomposition and nutrient cycling (Carvalho et al., 2010) for crops sown in succession.

Table 4. Averages of grain mass per panicle (GMPa); one thousand grain mass (TGM); number of grains per panicle (NGP); and percentages of stems (S%), leaves (L%), and panicle (P%) for grain sorghum under nitrogen fertilization splitting and their respective standard errors.

Treatment	GMPa	TGM	NGP	S%	L%	P%
<u>Inoculation (I)</u>	(g)		-	(%)		
Without	32 ± 2	24 ± 1	1,387 ± 73	32 ± 1	14 ± 1	54 ± 1
With	29 ± 2	24 ± 1	1,230 ± 74	31 ± 1	14 ± 1	55 ± 1
<u>N splitting (S)¹</u>						
0 %-100 %	28 ± 1	23 ± 1	1,212 ± 69	32 ± 2	13 ± 1	55 ± 2
25 %-75 %	31 ± 5	25 ± 1	1,233 ± 183	31 ± 1	13 ± 1	55 ± 2
50 %-50 %	30 ± 2	23 ± 2	1,314 ± 130	30 ± 2	14 ± 1	56 ± 1
100 %-0 %	32 ± 3	24 ± 1	1,356 ± 143	32 ± 2	14 ± 1	54 ± 2
Control	27 ± 5	24 ± 1	1,141 ± 100	29 ± 1	14 ± 1	57 ± 2
	p-values					
I	0.2162 ^{ns}	0.7377 ^{ns}	0.1446 ^{ns}	0.5535 ^{ns}	0.6185 ^{ns}	0.4856 ^{ns}
S	0.5979 ^{ns}	0.8731 ^{ns}	0.7632 ^{ns}	0.6178 ^{ns}	0.9165 ^{ns}	0.6398 ^{ns}
I x S	0.3537 ^{ns}	0.5891 ^{ns}	0.2110 ^{ns}	0.5784 ^{ns}	0.9585 ^{ns}	0.5714 ^{ns}
CV %	19	12	17	11	13	6

¹At sowing and as topdressing, respectively; C.V. %: Coefficient of Variation; ns: non-significant by Tukey's test (p>0.05).

According to Mateus et al. (2011), no-tillage crop rotation systems, besides permanent soil cover, cycle nutrients in residues and increase soil N supply for the next crop. Crusciol et al. (2011) reported that soil N supply is mostly due to constant use of N fertilizers in crops comprising the rotation/succession systems, besides N mineralization in high-quality and low C: N ratio plant residues.

The foregoing suggests that regardless of N fertilization or its splitting, soybean residues in soil increased sorghum yield components in succession. Accordingly, the lack of sorghum response to N splitting may be related to previous NT crops in the experimental area (Table 1). Another factor would be the low rainfall rates during the experiment (Figure 2), which, although occurred, did not limit sorghum yield potential, reflected in suitable grain yields (Freitas et al., 2014) that was equivalent among all treatments.

It should also be emphasized that N dynamics in the soil-plant-air system is complex. N efficiency depends on several factors interacting simultaneously such as cropping system, N application amount and timing, plant residues from previous crops, and edaphoclimatic factors acting on N volatilization, leaching, mobilization or non-mobilization (Farinelli and Lemos, 2010, Lorensini et al., 2012, Silva et al., 2017, Ajeigbe et al., 2018).

Although soil texture may improve N-fertilizer maintenance without significant leaching losses, climatic conditions and fertilizer nature must have been considered to evaluate our results. This is because, after soil application, urea is hydrolyzed by urease to form ammonium carbonate [$\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \rightarrow (\text{NH}_4)_2\text{CO}_3$], which decomposes rapidly to ammonium, hydroxyl, and bicarbonate [$(\text{NH}_4)_2\text{CO}_3 + \text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{OH}^- + \text{HCO}_3^-$], increasing pH around fertilizer

granules (Rochette et al., 2009). In short, if urea is not incorporated into the soil, as in this study, part of the NH_4^+ can convert to NH_3 and be lost to the atmosphere (Nascimento et al., 2013; Rochette et al., 2013; Degaspari et al., 2020; Soares et al., 2020).

Ammonia volatilization from urea on the soil surface can be low, totaling 1%, or extremely high, above 50% of the applied N (Cantarella et al., 2008; Sanz-Cobena et al., 2012; Tasca et al., 2011; Rochette et al., 2013). When high N doses are applied, losses can be greater (Ma et al., 2010) when soil moisture for infiltration is low and temperatures are high, as well as a greater amount of vegetation cover on the soil surface (Tasca et al., 2011; Nascimento et al., 2013; Liu et al. 2019; Degaspari et al., 2020; Soares et al., 2020), as observed in the present study.

4. Conclusions

The grain sorghum hybrid 'Ranchero', when grown in the off-season and succession to soybeans, and under water restriction, does not respond to *Azospirillum brasilense* inoculation and nitrogen fertilization. Furthermore, nitrogen splitting does not affect sorghum crop morphological components or grain yield. Application of half the recommended nitrogen dose at sowing and the other half as topdressing provides short-stature plants, without reducing dry matter production.

Authors' Contribution

Deyvison de Asevedo Soares contributed to the installation of the experiment, data collection, statistical analysis and writing of the article. Marcelo Andreotti was the first author's advisor, contributing to the statistical

analysis and advising on the writing and correction of the article. Maria Elisa Vicentini contributed to the implementation of the experiment, data collection, correction of the article and creating the Figure 1. Leandro Alves Freitas contributed to the implementation of the experiment, data collection, advice on the writing and correction of the article.

Viviane Cristina Modesto contributed to the installation of the experiment, data collection and translation of the article. Allan Hisashi Nakao contributed to the installation of the experiment, data collection. Lourdes Dickmann contributed to the installation of the experiment and data collection. Marcelo Carvalho Minhoto Teixeira Filho was the first author's co-advisor, advising on the writing and correction of the article.

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