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PASTURE AND FORAGE UTILIZATION

Forage yield of Coastcross-1 pastures inoculated with *Azospirillum brasilense*

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ABSTRACT. The high cost of nitrogen fertilizers increases the expenses in pasture-based animal production. The inoculation with plant growth-promoting bacteria is an alternative to reduce the costs. This study evaluated the forage yield of Coastcross-1 pastures inoculated with *Azospirillum brasilense,* fertilized with different levels of nitrogen and subjected to cuts. The experiment was a factorial randomized block design with three replications. The factors were the inoculation (without inoculation, inoculated only at planting and reinoculated in the second year), levels of nitrogen (0, 100 and 200 kg ha^{-1} $year^{-1}$ N) and seasons (spring, summer, fall and winter) when cuts were made. Forage yield, forage accumulation rate, botanical and structural pasture composition were evaluated. Forage yield without nitrogen fertilizer in pastures was in the first year 9.1, 11.7 and 11.7 t ha^{-1} DM, and in the second year 8.6, 11.2 and 11.5 t ha^{-1} DM, for the factor inoculation, respectively. The forage yield rises with inoculation at pasture planting, without nitrogen fertilization. Reinoculation is not necessary.

Keywords: botanical composition, *Cynodon dactylon*, diazotrophic bacteria.

Produção de forragem em pastos de Coastcross-1 inoculados com *Azospirillum brasilense*

RESUMO. O elevado preço dos fertilizantes nitrogenados onera a produção animal baseada no pasto. A inoculação com bactérias promotoras de crescimento vegetal pode ser uma alternativa para redução destes gastos. Assim, objetivou-se avaliar a produção de forragem de pastos de Coastcross-1, inoculados com *Azospirillum brasilense*, fertilizados com diferentes doses de N e submetidos ao regime de cortes. O delineamento experimental foi o de blocos ao acaso, com três repetições, em esquema fatorial. Os fatores foram o uso da inoculação (não inoculado, inoculado somente no plantio e reinoculado no 2º ano), doses de nitrogênio (0, 100 e 200 kg ha^{-1} ano^{-1} de N), e as estações do ano (primavera, verão, outono e inverno) quando foram realizados os cortes. Avaliaram-se a produção de forragem, a taxa de acúmulo de forragem e as composições botânica e estrutural do pasto. As produções de forragem, nos pastos sem nitrogênio, no primeiro ano, foram de 9,1; 11,7 e 11,7 t ha^{-1} de MS, e no segundo ano de 8,6; 11,2 e 11,5 t ha^{-1} de MS, para o fator inoculação, respectivamente. Há aumento na produção de forragem com a inoculação na implantação dos pastos, quando não há aplicação de nitrogênio, sendo que a reinoculação não é necessária.

Palavras-chave: composição botânica, *Cynodon dactylon*, bactérias diazotróficas.

Introduction

Grasses of the genus *Cynodon*, such as Coastcross-1, have been widely used in tropical and subtropical regions to feed ruminants and horses, both as pasture form and as conserved forage, especially hay (Branco et al., 2012). Due to their response to fertilization, these grasses are usually grown using high amounts of chemical fertilizers, especially nitrogen fertilizers (Corrêa et al., 2007).

Nitrogen (N) is one of the nutrients most required for the intensification of forage grass yield, being the main responsible for the formation of tissues. However, the high cost of nitrogen fertilizers and the growing concern about the development of a more sustainable and less polluting agriculture, lead to the search for alternatives to reduce the environmental impact of chemical fertilizers (Costa, Quirino, Naves, Santos, & Rocha, 2015), without causing losses in productivity.

In this context, the inoculation of grasses with associative diazotrophic bacteria, such as *Azospirillum brasilense*, represents an alternative for reducing the use of nitrogen fertilizers, taking into account that the bacteria can contribute with part of the N necessary for the development of the plant through biological nitrogen fixation. And, on a larger scale, for the production of phytohormones, responsible for the increase of the surface area of the roots of grasses and, consequently, larger area explored in the soil with increase in the absorption of minerals, including the N, and water, resulting in a more productive and resistant plant (Moreira, Silva, Nóbrega, & Carvalho, 2010; Hungria, Campo, Souza & Pedrosa, 2010; Costa et al., 2015).

There is a wide array of researches carried out with inoculation of *A. brasilense* on annual crops, such as corn and wheat, but research of this nature with perennial grasses is rare, especially with the genus *Cynodon*.

The goal of this research was to evaluate the effect of inoculation with *A. brasilense* on forage yield of Coastcross-1 grasses fertilized with different levels of N.

Material and methods

The research was conducted at the Dairy Livestock Sector, Department of Animal Science of the Federal University of Santa Maria (UFSM), located in the Central Depression of the State of Rio Grande do Sul, during two crop years, from August 2014 to May 2016. The climate of the region is humid subtropical (Cfa), according to Köppen classification; the climatological normals (Figure 1) of temperature is 19.2ºC and rainfall is 140.5 mm month^{-1}. For the experimental period, the values of monthly average temperature and rainfall were 20.5ºC and 170.9 mm month^{-1} (Instituto Nacional de Meteorologia [INMET], 2016). During this period, 16 frosts were recorded at the UFSM Weather Station, located approximately 700 m from the experimental area, six in August 2014, seven in June 2015, one in July 2015 and two in September 2015.

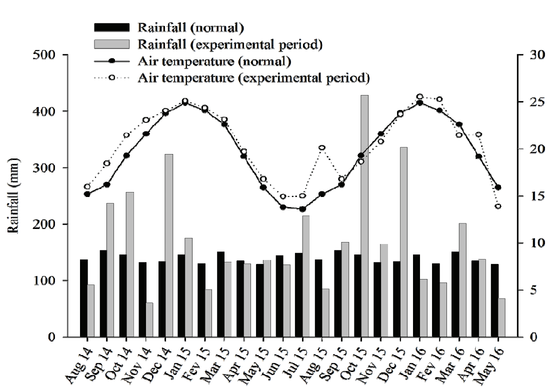

Figure 1. Climatological normals and values recorded in the experimental period for average temperature and monthly cumulative rainfall. Santa Maria, State of Rio Grande do Sul, 2014-2016.

The soil is classified as Sandy Dystrophic Red Argisol (Streck et al., 2008), and, according to the results of soil analysis (0-20 cm) before the implementation of the experiment, the following average values were obtained: pH-H_2O = 5.3; SMP index = 5.7; Clay = 280 g kg^{-1}; P-Mehlich = 7.6 mg dm^{-3}; K = 116 mg dm^{-3}; OM = 32 g kg^{-1}; Al = 0.2 $cmol_c$ dm^{-3}; Ca = 8.2 $cmol_c$ dm^{-3}; Mg = 3.4 $cmol_c$ dm^{-3}; base saturation = 65.7% and Al saturation = 1.7%.

For the evaluations, an area of approximately 700 m^2 was divided into 27 plots with dimensions of 5 m in length and 3 m in width, and corridors of 1 m wide between them. The experiment consisted of a complete factorial arrangement, in which factor A was the use or not of inoculation (Coastcross-1 not inoculated, Coastcross-1 inoculated only at planting, and Coastcross-1 inoculated at planting and in the second year) and factor B was the different fertilization rates (0, 100 and 200 kg ha^{-1} year^{-1} N). In this way, the following treatments were constituted: Coastcross-1 not inoculated; Coastcross-1 not inoculated + 100 kg ha^{-1} year^{-1} N; Coastcross-1 not inoculated + 200 kg ha^{-1} year^{-1} N; Coastcross-1, seedlings inoculated at planting; Coastcross-1, seedlings inoculated at planting + 100 kg ha^{-1} year^{-1} N; Coastcross-1, seedlings inoculated at planting + 200 kg ha^{-1} year^{-1} N; Coastcross-1, seedlings inoculated at planting + reinoculation in the aerial part of the plants in the second year of use; Coastcross-1, seedlings inoculated at planting + reinoculation in the aerial part of the plants in the second year of use + 100 kg ha^{-1} year^{-1} N; Coastcross-1, seedlings inoculated at planting + reinoculation in the aerial part of the plants in the second year of use + 200 kg ha^{-1} year^{-1} N.

Approximately 45 days before planting Coastcross-1, the soil acidity was corrected with dolomitic limestone by incorporation to the soil with the aid of a harrowing disk. The basal fertilization of the first crop year was carried out in the week prior to planting of the seedlings, applying 100 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O, followed by light harrowing for incorporation; in the following year, 80 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O were applied on the surface. For phosphate and potassium fertilization, as well as acidity correction, the recommendations of the Comissão de Química e Fertilidade do Solo [RS/SC] (2004) were followed for warm-season perennial grasses. For N, the total amount of urea was divided into five applications per year. In the first crop year, the first application was made 20 days after seedling planting and the others after the 1st, 2nd, 3rd and 4th cutting of the pasture; for the second year, the applications were performed after the 1st, 2nd, 3rd and 4th and 5th pasture cutting.

In September 2014, the grass was planted using seedlings of 'Bermuda grass' (*Cynodon dactylon* L. Pers.), cv. Coastcross-1, mature and with roots; in pits of approximately 10 cm of depth, 0.5 m spaced apart. For the inoculation of the seedlings, the commercial product AzoTotal® (liquid inoculant composed of pure culture of *Azospirillum brasilense* bacteria, strains Ab-V5 and Ab-V6, with a concentration of 2.0 x 10^8 CFU mL^{-1}) was used in the respective treatments; the product was diluted in the proportion of 0.5 L inoculant to 199.5 L water. The application was carried out using a backpack sprayer, directly on the seedling in the pit, before covering it with soil, using a proportion of 200 L ha^{-1}. For the treatments with inoculation in the second crop year, we used the same dilution and proportion of inoculant of the first year, applied to the aerial part of the plants at the end of September 2015.

Pastures were managed under a cutting regime, which were made when they reached a canopy height of approximately 25 cm (Ziech et al., 2015). In each plot, the material was cut 7 cm above the ground (Ziech et al., 2015), at random, using a square frame of 0.25 m^2, characterizing the mass of forage available. Then, at the same site, the cut was performed close to the soil surface for determination of residual forage mass. Afterwards, samples were weighed, homogenized, and a sub-sample was separated to determine the botanical composition of the pasture and the structural composition of Coastcross-1 (leaf blade and stem + sheath). After separation, components were dried in a forced air oven at 55ºC to constant weight to determine the percentage of pre-dried matter. After collecting the samples, the remaining material was cut (7 cm from the ground), standardizing the plots. The forage cut was taken from the experimental plots.

Forage yield was calculated by adding the yields of each cut performed in the season. For the other variables is presents by the mean data of the cuts within the same season. The daily forage accumulation rate was obtained by subtracting from the total forage mass of the cut in question the residual forage mass of the previous cut, dividing by the number of days of the interval between the cuts. The ratio of leaf blade: stem + sheath was obtained by the ratio of the structural components of Coastcross-1.

The experiment was a randomized block design in a factorial arrangement (inoculation x fertilization levels x seasons) with three replications (plots). The results were tested by analysis of variance, using the MIXED procedure. The effect of the factors and their interactions were subjected to Student's t-test for the comparison of means, at a level of 5% error probability. The covariance matrices used were selected by the lowest AIC (Akaike's Information Criteria) value. Data on forage accumulation rate were subjected to polynomial regression analysis, according to the seasons of the year. The errors were tested for normality. Pearson's correlation coefficient estimated the association between the variables. The analyses were run using the statistical package Statistical Analysis System Institute [SAS] (2016).

The following statistical model was used:

$$Y_{ijkl} = m + T_i + D_j + E_k + T_iD_j + T_iE_k + D_jE_k + \\ + T_iD_jE_k + B_l + B_l(T_iD_j) + \varepsilon_{ijkl}$$

where:

Y_{ijkl} represents the dependent variables;

m is the mean of all observations;

T_i is the effect of inoculation (i = 3);

D_j is the effect of levels of N (j = 3);

E_k is the effect of the seasons (k = 7);

T_iD_j is the interaction between inoculation and N levels;

T_iE_k is the interaction between inoculation and seasons;

D_jE_k is the interaction between N levels and seasons;

$T_iD_jE_k$ is the interaction between inoculation, N levels and seasons;

B_l is the effect of blocks (l = 3);

$B_l(T_iD_j)$ is the effect of blocks within the interaction inoculation x N levels (error a);

ε_{ijkl} is the residual effect (error b).

The effect related to the crop years is not included in the statistical model, because the first year is the implementation of the pasture, then the discussion was between the seasons.

Results and discussion

There were 618 days from seedling planting until the last cut, with a total of 14 pasture cuttings distributed as follows: one in the spring of 2014, three in the summer of 2014/2015, two in the fall of 2015, one in the winter of 2015 (first crop year); two in the spring of 2015, three in the summer of 2015/2016 and two in the fall of 2016 (second crop year). It is worth noting the winter cut of the first year of evaluation, due to the high temperatures, especially in August, with an average of 4.9°C above the climatological normal (Figure 1), and there was no frost in this month, favoring the development of Coastcross-1.

The intervals between cuts in the different seasons, in the first year of evaluation, were 68, 35, 48 and 60 days, for spring, summer, fall and winter, respectively, totalizing a mean interval of cuts of 47 days. In the second year of evaluation, were 64, 29 and 37 days, for spring, summer and fall, respectively, with an average of 41 days. The mean cut interval considering the entire experimental period was 44 days. Pereira et al. (2011), on Coastcross-1 under different regrowth ages (28 and 42 days) and increasing levels of N (0 to 400 kg ha^{-1} N) concluded that, during the season of higher growth, intervals close to 28 days are more suitable for pastures receiving higher levels of N. Pastures without nitrogen fertilization, or with low supply levels, can be managed at longer intervals. The mean intervals obtained in the summer, especially in the second year, when the pasture was established, are close to those recommended by the authors for higher N levels, which may have favored the development of pastures, especiallly those inoculated.

For forage yield in the first crop year (Table 1), no differences were detected between the different seasons, comparing the same levels of N. However, for the total forage yield of the crop year, there was a difference ($p \leq 0.05$) between pastures that did not receive nitrogen fertilization, with higher values for pastures subjected to inoculation with *A. brasilense.*

In the second crop year, for pastures that did not receive nitrogen fertilization, there was superiority ($p \leq 0.05$) in yield, in the spring season and in the total forage yield, for the inoculated pastures; in the fall, however, only in the pasture subjected to reinoculation there was recorded higher production of forage in relation to the non-inoculated one. These results confirm that there was an effect of inoculation, when not associated with nitrogen fertilization, on forage yield, with mean increases of 28.6 and 32.0% for the first and second crop years, respectively.

Moreover, in the inoculated pastures (independent from reinoculation), when fertilized with 100 kg ha^{-1} $year^{-1}$ N, the forage yield in the seasons and also the total yield, in the two crop years, was similar to that observed in the pastures not inoculated receiving 200 kg ha^{-1} $year^{-1}$ N, evidencing the contribution of inoculation also in this level of nitrogen fertilization, indicating a possible synergistic effect (presence of bacteria x nitrogen fertilization) in this level of Lana, Dartora, Marini and Hann (2012) working with corn, recorded gains in the dry biomass of the corn shoot with inoculation, when they did not provide N as topdressing, in the order of 6.5%, but with the application of 100 kg ha^{-1} N as topdressing, these authors found a negative response, with biomass reduction, in the order of 10%, when inoculated. Positive responses from inoculation of *A. brasilense* in grasses, when not associated with topdressing nitrogen fertilization are reported by several authors (Hungria et al., 2010; Lana et al., 2012), but the synergism between the application of N levels as topdressing and the inoculation is still unclear. Hungria, Nogueira and Araújo (2016), evaluated pastures of *Brachiaria* spp. (*B. brizanta* and *B. ruziziensis*) for two years and obtained an average increase of 5.4 and 22.1% of the biomass production, when the pastures were fertilized with 40 kg N ha^{-1}, and when the fertilization was associated (40 kg ha^{-1} N) with inoculation with *A. brasilense*, respectively, compared to production in pastures without nitrogen fertilization and not inoculated.

In the comparison between the pastures inoculated only in the first year and those inoculated in the first and second years, there was no difference between them considering the same levels of N. This indicates that the bacteria remained in the system, without the need to reapply inoculation in the second crop year.

Table 1. Forage yield in Coastcross-1 pastures (t ha-1 DM) inoculated with Azospirillum brasilense and subjected to nitrogen fertilization. Santa Maria, State of Rio Grande do Sul, 2014-2016.

N level (kg)	Seasons (1st crop year)				Total	Seasons (2nd crop year)			Total	CV (%)
					No inoculation					
0	1.8^{Bcd}	4.4^{Ca}	2.0^{Bbc}	0.9^{Ce}	9.1^{D}	2.8^{Cb}	4.6^{Da}	1.2^{Cde}	8.6^{D}	9.5
100	2.4^{ABc}	6.0^{ABa}	1.9^{Bc}	1.2^{BCd}	11.4^{C}	3.6^{Bb}	5.6^{BCDa}	2.0^{ABc}	11.3^{C}	7.4
200	2.7^{ABc}	7.1^{Aa}	2.2^{ABcd}	1.7^{ABd}	13.8^{AB}	4.3^{ABb}	6.9^{ABa}	2.3^{Acd}	13.4^{AB}	6.2
					Inoculated in the first year					
0	2.4^{ABb}	5.7^{BCa}	2.3^{ABbc}	1.3^{ABCc}	11.7^{C}	4.7^{Aa}	5.0^{CDa}	1.5^{BCbc}	11.2^{C}	7.3
100	2.7^{ABcd}	7.0^{ABa}	2.2^{ABd}	1.2^{BCe}	13.1^{BC}	4.0^{ABbc}	5.8^{BCa}	2.2^{ABd}	12.0^{BC}	6.7
200	3.0^{Ac}	7.2^{Aa}	2.4^{ABc}	1.6^{ABCd}	14.3^{AB}	4.5^{Ab}	7.2^{Aa}	2.5^{Ac}	14.2^{A}	5.9
					Inoculated in the first and second years					
0	2.5^{ABc}	5.5^{BCa}	2.3^{ABc}	1.4^{ABCd}	11.7^{C}	4.1^{ABb}	5.2^{CDab}	2.2^{ABc}	11.5^{C}	7.3
100	2.6^{ABc}	6.7^{ABa}	2.1^{ABc}	1.2^{BCd}	12.6^{BC}	4.0^{ABb}	5.7^{BCDab}	2.2^{ABc}	11.9^{BC}	6.9
200	3.1^{Ac}	7.1^{Aa}	2.8^{Acd}	2.0^{Ad}	15.0^{A}	4.5^{Ab}	8.1^{Aa}	2.2^{ABcd}	14.9^{A}	5.6
CV(%)	7.9	3.2	8.9	14.4	8.9	5.0	3.3	9.8	8.6	

Means followed by different letters, upper case in the same column and lower case in the same row, are significantly different by Student's t-test ($p \leq 0.05$). CV = coefficient of variation; Spr = spring; Sum = summer; Fal = fall; Win = winter.

Considering the different N levels, it was observed that there were differences ($p \leq 0.05$) in forage yield, more evident in non-inoculated pastures. In the total forage yield, the variations in non-inoculated pastures were 51.6 and 55.8%, in the first and second crop years, respectively. On the other hand, the average variation in inoculated pastures was 25.2 and 28.2% in the first and second crop years, respectively, which shows the effect of inoculation, when not associated with nitrogen fertilization.

As for the distribution of forage yield throughout the year (Table 1), in general, it was in accordance with the production cycle of Coastcross-1 in subtropical regions, with higher yields in the summer, and lower in the spring and fall (Barbero et al., 2009; Aguirre et al., 2014; Anjos et al., 2016). Although, in the first year of evaluation, the climate had contributed to a cut in the winter, due to the milder temperatures, the production in this season was inferior to the others.

As for the data on the daily forage accumulation rate (Figure 2), grouped by seasons, they present a typical behavior, with a cubic effect with an upward start, from spring to summer, and later drop in fall and also in winter.

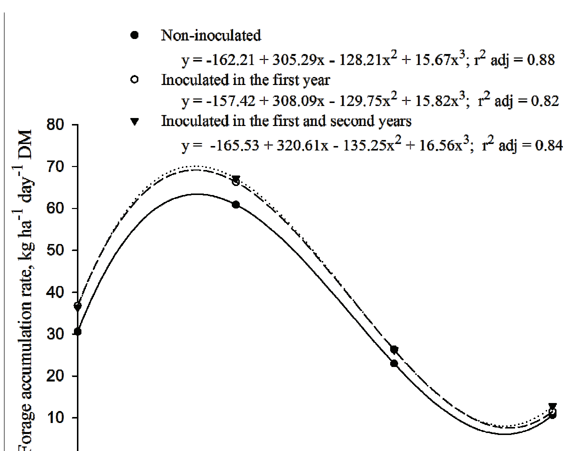

Figure 2. Regression equations for daily forage accumulation rate of the interaction between seasons of the year and inoculation (1 = spring, 2 = summer, 3 = fall and 4 = winter). Santa Maria, State of Rio Grande do Sul, 2014-2016.

Observing the plot of the curves representing the pastures under inoculation, it was observed that there is similarity, demonstrating, therefore, that there is no need to reinoculate the pasture. The daily accumulation rate recorded in the summer for inoculated pastures, of 66.8 kg ha^{-1} DM, is similar to that obtained by Anjos et al. (2016) in the same season, of 67.8 kg ha^{-1} DM, evaluating Coastcross-1, fertilized with 200 kg ha^{-1} $year^{-1}$ N and grazed by dairy cattle.

For the leaf blade: stem + sheath ratio of Coastcross-1 (Table 2), considering the same N levels, only in winter of the first year there was a difference ($p \leq 0.05$) in pastures that received no nitrogen fertilization, with greater value when not inoculated. This behavior, although isolated, may be related to a possible greater development of the roots of inoculated plants, stimulated by the production of phytohormones, with consequently greater soil exploration, greater absorption of the N available (Moreira et al., 2010; Hungria et al., 2010) and, thus, greater shoot development, and proportionally of stems, as observed when N was applied. In general, it was observed that N levels had a greater influence on the structural composition of Coastcross-1, with greater participation of leaves where there was no application of N and decline as applied and increase the level of N.

The mean value of the leaf blade: stem + sheath of Coastcross-1, of 1.28, obtained for pasture without nitrogen fertilization is similar to that obtained by Ziech et al. (2015), of 1.34, evaluating Coastcross-1, fertilized with 80 kg ha^{-1} $year^{-1}$ N, using forage harvesting methodology similar to that used in the present study. High leaf participation values are desirable, as they usually present higher crude protein and lower neutral detergent fiber contents than stems (Branco et al., 2012).

For the botanical composition (Table 3), considering pastures without nitrogen fertilization, differences ($p \leq 0.05$) in Coastcross-1 participation were found in five out of the seven evaluations, and in at least one of the inoculated pastures, Coastcross-1 participation was superior than the non-inoculated. At the level of 200 kg ha^{-1} $year^{-1}$ N, a difference was verified only in the spring of the first crop year, with a higher value in the pasture inoculated only in that year when compared to the non-inoculated pasture. The differences observed, as well as the means of participation, demonstrate the favoring of both the initial development and the maintenance of the participation of the grass under study when inoculated with *A. brasilense*, especially when not fertilized with N. This response may be associated with the increase in the surface area of the roots of grasses, resulting in a more productive and resistant plant (Moreira et al., 2010; Hungria et al., 2010).

Table 2. Leaf blade: stem + sheath ratio of Coastcross-1⋆ inoculated with *Azospirillum brasilense* and subjected to nitrogen fertilization. Santa Maria, State of Rio Grande do Sul, 2014-2016.

N level (kg)	Seasons (1^{st} crop year)				Seasons (2^{nd} crop year)			Mean	CV (%)
	Spr	Sum	Fal	Win	Spr	Sum	Fal		
No inoculation									
0	1.67^{Aa}	1.35^{ABab}	1.35^{Aab}	1.40^{Aab}	1.18^{b}	1.30^{Aab}	1.11^{ABb}	1.34	5.6
100	1.57^{ABa}	1.17^{CDEb}	1.17^{BCb}	1.03^{Bb}	1.12^{b}	1.11^{BCb}	0.90^{Cb}	1.15	6.5
200	1.10^{ABab}	1.02^{DEab}	1.07^{Cab}	0.96^{Bb}	1.25^{a}	1.18^{ABCab}	0.83^{Cb}	1.06	7.0
Inoculated in the first year									
0	1.35^{ABa}	1.45^{Aa}	1.28^{ABab}	1.02^{Bb}	1.18^{ab}	1.30^{Aab}	1.18^{Aab}	1.25	5.9
100	1.03^{Bab}	1.21^{BCDab}	1.24^{ABab}	1.06^{Bab}	1.27^{a}	1.28^{Aa}	0.94^{BCb}	1.15	6.5
200	1.20^{ABa}	1.05^{DEab}	1.05^{Cab}	0.86^{Bb}	1.18^{a}	1.18^{ABCa}	0.82^{Cb}	1.05	7.1
Inoculated in the first and second years									
0	1.36^{ABab}	1.39^{ABa}	1.19^{ABCb}	1.10^{Bb}	1.27^{ab}	1.24^{ABab}	1.27^{Aab}	1.26	5.9
100	1.40^{ABa}	1.10^{DEb}	1.21^{ABCab}	1.03^{Bbc}	1.29^{ab}	1.26^{Aab}	0.72^{Cc}	1.14	6.5
200	1.11^{ABab}	0.98^{Ebc}	1.15^{BCab}	0.89^{Bc}	1.20^{a}	1.08^{Cab}	0.80^{Cc}	1.03	7.2
CV(%)	10.5	3.5	2.9	5.0	4.8	2.1	4.4		

Means followed by different letters, upper case in the same column and lower case in the same row, are significantly different by Student's t-test ($p \leq 0.05$). ⋆Collection of samples 7 cm from the ground. CV = coefficient of variation; Spr = spring; Sum = summer; Fal = fall; Win = winter.

Table 3. Participation of Coastcross-1, other plants and dead material in pastures inoculated with *Azospirillum brasilense* and subjected to nitrogen fertilization. Santa Maria, State of Rio Grande do Sul, 2014-2016.

N level (kg)	Seasons (1st crop year)				Seasons (2nd crop year)			Mean	CV (%)
	Spr	Sum	Fal	Win	Spr	Sum	Fal		
Coastcross-1 (%)⋆									
No inoculation									
0	28.9^{Bc}	37.8^{Bbc}	50.8^{Bab}	56.7^{Ba}	58.1^{a}	55.3^{Ba}	47.6^{Bab}	47.9	6.7
100	31.7^{Bc}	51.3^{Ab}	59.1^{ABab}	73.5^{Aa}	54.2^{b}	54.6^{Bb}	45.2^{Bbc}	52.8	5.1
200	33.8^{Bc}	51.0^{Ab}	69.0^{Aab}	74.9^{Aa}	66.8^{ab}	66.7^{ABab}	53.7^{ABb}	59.4	4.8
Inoculated in the first year									
0	49.6^{Ab}	53.7^{Aab}	64.3^{Aa}	67.8^{ABa}	58.7^{ab}	55.8^{Bab}	59.7^{ABab}	58.5	5.8
100	38.4^{Bc}	50.5^{Abc}	68.7^{Aab}	76.2^{Aa}	59.1^{abc}	60.9^{ABab}	50.5^{Bbc}	57.8	6.7
200	54.6^{A}	56.0^{A}	67.6^{A}	70.2^{AB}	66.3	59.0^{AB}	58.1^{AB}	61.7	5.3
Inoculated in the first and second years									
0	40.2^{ABb}	58.6^{Aa}	68.0^{Aa}	68.0^{ABa}	66.4^{a}	71.0^{Aa}	66.4^{Aa}	62.7	3.2
100	35.1^{Bb}	49.5^{ABab}	67.8^{Aa}	67.2^{ABa}	59.5^{a}	64.0^{ABa}	51.9^{ABa}	56.4	4.9
200	42.2^{ABb}	55.9^{Aab}	65.9^{Aa}	63.6^{ABa}	61.3^{a}	65.9^{ABa}	56.0^{ABab}	58.6	7.9
CV(%)	13.1	6.1	6.1	3.5	6.3	4.6	7.2		
Other plants (%)⋆									
No inoculation									
0	47.1^{Aab}	55.6^{Aa}	25.1^{Ac}	34.3^{Abc}	33.6^{bc}	37.7^{Abc}	39.8^{Abc}	39.0	11.8
100	44.6^{Aa}	44.0^{ABa}	22.9^{ABb}	19.9^{Bb}	37.4^{ab}	37.7^{Aab}	41.4^{Aa}	35.4	12.4
200	42.5^{ABab}	42.8^{ABa}	12.5^{BCc}	21.4^{Bbc}	26.6^{abc}	24.8^{ABabc}	33.0^{ABab}	29.1	13.6
Inoculated in the first year									
0	33.3^{ABa}	40.1^{ABa}	14.8^{BCb}	27.1^{ABab}	32.4^{ab}	37.5^{Aa}	26.4^{Bab}	30.2	12.8
100	42.2^{ABa}	43.5^{ABa}	10.2^{Cc}	18.8^{Bbc}	32.4^{ab}	30.5^{ABab}	36.8^{ABab}	30.6	13.2
200	30.4^{Ba}	36.2^{Ba}	11.0^{Cb}	21.9^{Bab}	26.2^{a}	34.0^{ABa}	28.1^{ABa}	26.8	13.6
Inoculated in the first and second years									
0	38.4^{ABa}	32.9^{Ba}	8.4^{Cb}	27.6^{ABa}	24.3^{ab}	20.4^{Bab}	22.6^{Bab}	24.9	14.3
100	40.6^{ABa}	43.2^{ABa}	10.7^{Cb}	26.4^{ABab}	31.5^{a}	29.6^{ABa}	31.3^{ABa}	30.5	12.9
200	36.7^{ABa}	36.8^{Ba}	15.4^{BCb}	27.9^{ABa}	26.5^{ab}	27.2^{ABab}	30.9^{ABa}	28.8	13.1
CV(%)	5.7	5.6	8.4	6.6	6.3	6.2	6.2		
Dead material (%)⋆									
No inoculation									
0	24.0^{Aa}	6.6^{ABb}	24.1^{a}	9.0^{Ab}	8.3^{ABb}	7.0^{b}	12.6^{Bb}	13.1	11.8
100	23.8^{Aa}	4.8^{Bc}	18.0^{a}	6.6^{ABc}	8.4^{ABbc}	7.7^{bc}	13.4^{ABab}	11.8	13.1
200	23.7^{Aa}	6.3^{ABd}	18.5^{ab}	3.7^{Bd}	6.6^{Bd}	8.5^{dc}	13.4^{ABbc}	11.5	13.4
Inoculated in the first year									
0	17.1^{ABab}	6.2^{ABd}	20.9^{a}	5.1^{ABd}	9.0^{ABcd}	6.7^{d}	13.9^{ABbc}	11.2	13.7
100	19.5^{ABa}	6.0^{ABc}	21.1^{a}	4.9^{ABc}	8.5^{ABbc}	8.6^{bc}	12.8^{Bab}	11.6	13.3
200	15.0^{Bb}	7.8^{Ad}	21.4^{a}	8.0^{Acd}	7.5^{Bd}	6.9^{d}	13.8^{ABbc}	11.5	13.4
Inoculated in the first and second years									
0	21.4^{ABa}	8.5^{Ab}	23.6^{a}	4.4^{Bb}	9.3^{ABb}	8.6^{b}	11.0^{Bb}	12.4	12.5
100	24.4^{Aa}	7.3^{ABc}	21.5^{ab}	6.4^{ABc}	9.0^{ABc}	6.4^{c}	16.8^{Ab}	13.1	11.8
200	21.1^{ABa}	7.3^{ABc}	18.7^{ab}	8.5^{Ac}	12.2^{Abc}	6.9^{c}	13.1^{ABbc}	12.5	12.3
CV(%)	8.9	10.2	11.3	15.4	10.6	8.6	7.0		

Means followed by different letters, upper case in the same column and lower case in the same row, are significantly different by Student's t-test ($p \leq 0.05$). ⋆Collection of samples 7 cm from the ground. CV = coefficient of variation; Spr = spring; Sum = summer; Fal = fall; Win = winter.

In the comparison of the participation of Coastcross-1 between the seasons, no differences were detected ($p \geq 0.05$) only at the level of 200 kg ha^{-1} $year^{-1}$ N, when inoculated only in the first year, demonstrating the regularity in the participation of Coastcross-1 in this pasture, averaging 61.7%. For the pastures that were inoculated in the two years, at all levels of N, there were generally lower participation of Coastcross-1 in the season of its implantation, with subsequent increase and stabilization of the grass presence. The greatest variability occurred in non-inoculated pastures.

The presence of other plants (Table 3) is negatively correlated (-0.91510; $p < 0.0001$) with the participation of Coastcross-1, possibly due to competition for nutrients, water and light. Thus, in pastures without nitrogen fertilization, the differences ($p \leq 0.05$) observed in the participation of other plants, in the summer and fall of the two crop years, always had higher values recorded in the pasture without inoculation, in relation to at least one of the inoculated pastures. For the level of 100 kg ha^{-1} $year^{-1}$ N, in the fall of the first year, there was greater participation of other species in the pasture without inoculation. The observed differences demonstrate that the greater development of Coastcross-1, possibly due to the greater development of the plant roots (Moreira et al., 2010) promoted by the inoculation, had a positive result considering the control of the development of invasive plants.

During the evaluations, the presence of other plants was higher in the first two seasons evaluated. This occurred because Coastcross-1 was still being established, thus allowing, in the spring of the first year, to have a significant participation of natural ryegrass, with 23% of the forage mass available. In the following year, in the same season, the participation of ryegrass was only 10%. In the summer of the first year, there was development of spontaneous growth species such as setaria grass (*Setaria* spp.), *Paspalum conjugatum*, common bermuda grass (*Cynodon dactylon*), vaseygrass (*Paspalum urvillei* Steud.) and Uruguayan ricegrass (*Piptochaetium montevidense*), which occurred less intensely the following year, because Coastcross-1 was already established. Aguirre et al. (2014) researching established pasture of Coastcross-1, in same local, registered in spring participation of natural ryegrass in order of 44.7%, demonstrating the tendency of grass participation at the local.

Regarding the presence of dead material (Table 3), there was an irregularity in the observed responses, not clearly related to inoculation or N levels. During the two years of evaluation, the highest values were recorded in the spring and fall of the first crop year. This behavior can be explained, in the spring of the first crop year, by the high participation of ryegrass, which when reached the end of its vegetative period, collaborated to increase the participation of dead material in the pastures and, in the fall of the first crop year, by the senescence of spontaneous growth species of the summer cycle, which had high participation in pasture in this season.

Conclusion

The inoculation with *Azospirillum brasilense*, strains Ab-V5 and Ab-V6, at Coastcross-1 planting, leads to a better establishment of the grass, especially when not associated with nitrogen fertilization.

The inoculation provides an increase in forage yield of pastures without nitrogen fertilization, and reinoculation in the second crop year is not necessary.

Gain on inoculation decreases as the applied N level increases.

References

Aguirre, P. F., Olivo, C. J., Simonetti, G. D., Nunes, J. S., Silva, J. O., Santos, M. S., ... Anjos, A. N. A. (2014). Produtividade de pastagens de Coastcross-1 em consórcio com diferentes leguminosas de ciclo hibernal. *Ciência Rural, 44*(12), 2265-2272.

Anjos, A. N. A., Olivo, C. J., Sauter, C. P., Silva, A. R., Santos, F. T., & Seibt, D. C. (2016). Forage yield in pastures with bermuda grass mixed with different legumes. *Acta Scientiarum. Animal Sciences*, 38(3), 261-266.

Barbero, L. M., Cecato, U., Lugão, S. M. B., Gomes, J. A. N., Limão, V. A., & Basso, K. C. (2009). Produção de forragem e componentes morfológicos em pastagem de coastcross consorciada com amendoim forrageiro. *Revista Brasileira de Zootecnia, 38*(5), 788-795.

Branco, A. F., Viana, K. B., Castañeda, R. D., Prohmann, P. E., Coneglian, S. M., & Mouro, G. F. (2012). Chemical composition and crude protein fractions of Coastcross grass under grazing on winter, spring and summer in Southern Brazil. *Acta Scientiarum. Animal Sciences, 34*(2), 183-187.

Comissão de Química e Fertilidade do Solo [RS/SC]. (2004). *Manual de adubação e calagem para os Estados do Rio Grande do Sul e de Santa Catarina* (10a ed.). Porto Alegre: SBCS-CQFS.

Corrêa, L. A., Cantarella, H., Primavesi, A. C., Primavesi, O., Freitas, A. R., & Silva, A. G. (2007). Efeito de fontes e doses de nitrogênio na produção e qualidade da forragem de capim-coastcross. *Revista Brasileira de Zootecnia, 36*(4), 763-772.

Costa, R. R. G. F., Quirino, G. S. F., Naves, D. C. F., Santos, C. B., & Rocha, A. F. S. (2015). Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize. *Pesquisa Agropecuária Tropical, 45*(3), 304-311.

Hungria, M., Nogueira, M. A., & Araújo, R. S. (2016). Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. *Agriculture, Ecosystems and Environment, 221*(1), 125-131.

Hungria, M., Campo, R. J., Souza, E. M., & Pedrosa, F. O. (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil, 331*(1), 413-425.

Instituto Nacional de Meteorologia [INMET]. (2016). *Banco de dados meteorológicos para ensino e pesquisa* (Dados mensais – Cód. A803, 2014-2016). Santa Maria, RS: Estação Meteorológica de Santa Maria.

Lana, M. C., Dartora, J., Marini, D., & Hann, J. E. (2012). Inoculation with Azospirillum, associated with nitrogen fertilization in maize. *Revista Ceres, 59*(3), 399-405.

Moreira, F. M. S., Silva, K., Nóbrega, R. S. A., & Carvalho, F. (2010). Bactérias diazotróficas associativas: diversidade, ecologia e potencial de aplicações. *Comunicata Scientiae, 1*(2), 74-99.

Pereira, O. G., Oliveira, M. A., Pinto, J. C., Santos, M. E. R., Ribeiro, K. G., & Cecon, P. R. (2011). Análise de crescimento do capim Coastcross-1 sob adubação nitrogenada em duas idades de rebrotação. *Revista Brasileira de Zootecnia, 40*(10), 2121-2128.

Statistical Analysis System [SAS]. (2016). SAS, *Studio user's guide version 3.5*. Cary, NC: SAS Institute.

Streck, E. V., Kämpf, N., Dalmolin, R. S. D., Klamt, E., Nascimento, P. C., Schneider, P., ... Pinto L. F. S. (2008). *Solos do Rio Grande do Sul*. (2a ed.) Porto Alegre: EMATER/RS.

Ziech, M. F., Olivo, C. J., Ziech. A. R. D., Paris, W., Agnolin, C. A., & Meinerz, G. R. (2015). Nutritive value of pastures of *Cynodon* mixed with forage peanut in southwestern Paraná State. *Acta Scientiarum. Animal Sciences, 37*(3), 243-249.

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