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Dual-purpose wheat forage productivity and grain yield under defoliations and different types of inoculation with *Azospirillum brasilense*

Produtividade de forragem e grãos de trigo duplo-propósito submetido a desfolhas e diferentes tipos de inoculação com *Azospirillum brasilense*

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Abstract

The use of inoculants containing plant growth-promoting bacteria is an alternative to increase crop yields. This study aimed to verify the effects of different types of inoculations on forage and grain yield of wheat dual-purpose BRS Umbu cultivar under cutting management. A randomized complete block design was used and treatments consisted of a combination of types of inoculation and number of cuts, with four replications. Cumulative forage productivity, tiller population density, grain yield, number of spikelets per spike, grains per spike and one thousand grain weight were measured. Regardless of number of cuts, the inoculant application by leaf spraying provided greater cumulative forage yield (up to 58 % increase), demonstrating shoot growth stimulation. The use of inoculants in different types of application did not affect yield components or grain yield.

Additional keywords: defoliation, diazotrophic bacteria, grain yield, *Triticum aestivum* L.

Resumo

O uso de inoculantes contendo bactérias promotoras de crescimento de plantas é uma alternativa para aumentar a produtividade das culturas. Este trabalho teve como objetivo verificar os efeitos de diferentes tipos de inoculações na produtividade de forragem e no rendimento de grãos de trigo da cultivar BRS Umbu sob manejo de corte. Foi utilizado delineamento de blocos completos casualizados e os tratamentos consistiram na combinação dos tipos de inoculação e número de cortes, com quatro repetições. Produtividade acumulada de forragem, densidade populacional de perfilhos, rendimento de grãos, número de espiguetas por espiga, grãos por espiga e peso de mil grãos foram medidos. Independentemente do número de cortes, a aplicação de inoculante por pulverização foliar proporcionou maior produtividade acumulada de forragem (aumento de até 58%), demonstrando estímulo ao crescimento da parte aérea. O uso de inoculantes em diferentes tipos de aplicação não afetou os componentes de rendimento nem o rendimento de grãos de trigo.

Palavras-chave adicionais: bactérias diazotróficas; desfolhação; produção de grãos; Triticum aestivum L.

Introduction

Wheat (*Triticum aestivum* L.) is the most cultivated cereal in the world, after maize, supporting 40% of the global population as their staple food and contributes about 20 % of total protein and daily calories of human diet (Ghahremaninejad et al., 2021, Saddiq et al., 2021). This cereal is extensively cultivated around the world because of its high demand and cultivars that are adapted to different environmental conditions and that can be used for both human consumption and animal feed (Ghasemi-Mobtaker et al., 2020, Gaweda & Haliniarz, 2021).

Wheat is an important cereal crop with a high demand for nitrogen fertilizer for growth, yield and quality products such as bread, pasta and baked goods (Zörb et al., 2018). Wheat conducted in a dual-purpose system provides forage for animal feed and grain production in the same crop (Mondal et al., 2020). With source-sink changes caused by defoliations, adequate supply of nutrients, especially nitrogen, is fundamental and should be performed through replacement fertilization to maintain production and grain quality. Therefore, one needs to search solutions, particularly sustainable and biological ones, to enable increased grain yield.

Plant growth-promoting rhizobacteria (PGPR) are microorganisms that produces phytohormones and have been made available to increase the availability of nutrients to growing plants and crop yields, especially nitrogen-fixing bacteria, such as Azospirillum brasilense Tarrand et al. (previously classified as Roseomonas fauriae Rihs et al.) (Helsel et al. 2006, Silva & Pires, 2017, Fukami et al., 2018). In this sense, application of PGPR is an important strategy in cereal cultivation, i.e., it is an alternative to increase the efficiency of nitrogen fertilizers (Quatrin et al., 2019).

Previous studies reported results of nitrogen fertilization associated with inoculation. According to Galindo et al. (2016), increases in the efficiency of N fertilization, combined with *A. brasilense* inoculation, were reported for corn grain yield in the Cerrado region. According to Dobbelaere et al. (2003), positive responses to inoculation with *A. brasilense* are found even when the crops are grown in soils with a high N content, which indicates that plant responses do not occur only because of biological nitrogen fixation, but also because of production of phytohormones such as cytokinin, gibberellin, and auxin (indoleacetic acid). Lemos et al. (2013) evaluated five wheat cultivars, with and without inoculation with *A. brasilense*, associated with N fertilization and found that the response to inoculation was satisfactory when associated with N topdressing.

Inoculation with *A. brasilense* is commonly applied to seeds, but the contact between bacteria and chemicals (such as insecticides and fungicides) during treatments may compromise inoculation efficiency (Fukami et al., 2016, Munareto et al., 2018). In this way, alternative methods, for example, inoculant leaf application, have been studied in wheat with this bacterium (Pereira et al. 2017, Ribeiro et al. 2018, Correia et al., 2020).

A better understanding of alternative management practices with inoculation is necessary, mainly for wheat conducted in a dual-purpose system, in which forage and grain yield studies are scarce. Based on these facts, this work aimed to verify the effects of different types of inoculations on forage productivity and grain yield of wheat dual-purpose BRS Umbu cultivar under cutting management.

Material and Methods

Experiments were conducted in Lages, Santa Catarina state, southern Brazil, in 2014 and 2015 with wheat BRS Umbu cultivar. Experimental area soil was classified as loamy Haplumbrept (Santos et al., 2013), and according to analysis, (0 to 20 cm layer) soil pH (water), organic matter, Mehlich-1 P, Mehlich-1 K, Ca, Mg, Al and H+Al were, respectively, 5.6, 33 g kg-1, 8.3 mg dm-3, 0.47 cmolc dm-3, 4.5 cmolc dm-3, 2.4 cmolc dm-3, 0.7 cmolc dm-3 and 9.9 cmolc dm-3.

In 2013, the area was made up of rangeland. Preparation was made with application of 7 tons of dolomitic limestone followed by plowing and harrowing. In 2014 and 2015, wheat was sown under no-tillage in common bean and soybean straw, respectively. Sowing was done on May 19 in the first year and May 7 in the second year. Mineral fertilizer N-P2O5-K2O in a 5-20-10(%) formulation was used at a rate of 400 kg ha-1, per crop season. Nitrogen topdressing from urea was applied with 50 kg ha-1 N, at tillering stage (GS 21), at first visible node (GS 31), and after each cut as a way to replace the nitrogen eliminated as a result defoliation. When replacement fertilization coincided with fertilization of GS 31 stage, N fertilization was the same, that is, performed only once. The phenologic stage of plants undergoing each treatment, was evaluated using the Zadoks scale (Zadoks et al., 1974).

Seeds were treated with fungicide and insecticide suitable for the culture: carbendazim (Methyl benzimidazol-2-ylcarbamate), imidacloprid [1-(6-chloro-3-pyridylmethyl) -N-nitroimidazolidin-2-ylideneamine] and, tiodicarb (3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetraazapentadeca-3,12-diene-6,10dione). Moments before sowing, part of the seeds were treated with liquid inoculant Azototal® containing strains AbV5 and AbV6 of A. brasilense with a concentration of 2 x 108 CFU mL-1. The inoculant was applied at a dose of 125 mL per 50 kg of seed, as recommended by the manufacturer. Sowings were made with a plot seeder; each plot was made up of five rows spaced 20 cm and seeds were deposited at 2-5 cm depth. Seeding density was 350 seeds per square meter (Fontaneli et al., 2012).

The treatments consisted in seed inoculation (SI), leaf inoculation spraying (LI) or no inoculation to seeds or leaves (Control) and number of cuts (1, 2 or 3), resulting in treatments: SI/1, SI/2, SI/3, LI/1, LI/2, LI/3, Control/1, Control/2, Control/3. A randomized complete block design was used, with four replications. Size of each plot was 6 m2 and the experiment was composed of 36 plots. For insecticide and fungicide applications, the same recommendations for traditional grain production were followed.

Wheat plant height before defoliation was the criterion for cuts; thus, 30 cm canopy was adopted (Fontaneli et al., 2009, Hastenpflug et al., 2011, Martin et al., 2013, Meinerz et al., 2012). Canopy height was monitored weekly, using a graduated sward stick at 30 random points per plot. For defoliation intensity, 50% of canopy height was used, based on intensities used by Mezzalira et al. (2014) for black oat (Avena strigosa Schreb.), resulting in 15 cm residual height. As done by Carletto et al. (2015) and Meinerz et al. (2012), three successive cuts were made at the plants' regrowth.

When plants achieved 30 cm, before each cut, tiller population density (TPD) was evaluated by number of tillers contained within a 50 cm x 50 cm frame placed in three central lines of plot. After that, plants were cut using scissors and a 0.5 m edge was discarded from each end of plot. All shoot material from three central lines was cut and collected. Then, shoot material was dried in forced air oven at 60 ºC to constant weight, and after that forage production was calculated.

After each, cut nitrogen fertilization was performed, as well as sprinkling of inoculant via leaf for plants allocated to this treatment. The leaf inoculation process used a dose of 250 mL of the commercial product Azototal® per hectare. The inoculant was diluted in water and applied to the leaves through a spray bar system with CO2 pressurization, regulated to a flow of 200 L ha-1.

With plant regrowth and grain production, before harvest, spikes were collected within 1 linear meter of the central line of each plot and, the number of spikelets per spike (NSS) and the number of grains per spike (NGS) were later estimated. Grains were harvested when plants reached harvest maturity, with a combined plot (Wintersteiger), on December 2, 2014 and December 12, 2015. Thousand-grain weight (TGW) was determined by counting one thousand grains from each experimental plot, with an automatic grain counter (Sanick model ESC 2011) and subsequent weighing. Grain yield (GY) was determined on the basis of production in the useful area of the plots, corrected at 13% standard moisture.

For statistical analysis, due to season significant effect, crop seasons were not considered as factors and were used for data reliability; therefore no comparisons could be made for cropping period. Also due to the significant effect of the cuts, it was decided to analyze each cut separately. Thus, data underwent analysis of variance (ANOVA) and when a significant F-test was found, means were compared by Tukey's test at 5% error probability using the statistical SAS® program version 9.0.

Results and Discussion

Regarding forage yield, inoculation can change the morphology of plant roots through the production of growth regulating substances and increase the number of lateral roots and root hairs. This results in greater uptake of water and nutrients, which is the main factor in increasing plant growth (Brum et al., 2021). For the variable cumulative forage productivity, the treatment that showed the greatest results was LI, regardless of number of cuts and crop season (Table 1). These results corroborate the statement by Freitas et al. (2019) that inoculation of forage grasses with *A. brasilense* increases forage yield, especially when performed together with nitrogen fertilization. For cumulative forage productivity, there was an increase of 79; 50 and 46%, respectively for one, two and three cuts, in the LI treatment compared to Control in the 2014 crop season. In the 2015 crop season, the superiority of LI over control had lower results, of 30; 27 and 19.5%, respectively for one, two and three cuts. The higher values found for plants that had one cut may be related to the fact that they are less subjected to defoliations. This result for the LI treatment demonstrates the stimulus of plant growth from inoculation may help a faster forage regrowth after defoliations, contributing for efficiency of forage production (Hungria et al., 2021).

Working with dual-purpose wheat BRS Tarumã cultivar whose seeds were inoculated with *A. brasilense*, using three cuts and four nitrogen doses, Quatrin et al. (2019) found for the average number of cuts that the inoculation promoted an increase of forage mass by approximately 25% for all N doses. For another grass species, Picazevicz et al. (2020) found for *Panicum maximum* BRS Zuri cultivar, whose seeds were inoculated with *A. brasilense*, with 50 kg N. ha^{-1} application, that inoculation resulted in increased aerial dry matter. Hungria et al. (2016), when inoculating *Urochloa brizantha* with *A. brasilense*, found a 24.7% increase in biomass production when it was performed together with N fertilization (40 kg N.ha^{-1}).The present work differs from the others mentioned above, as they all worked with seed inoculation, while this study focused on other inoculation forms and the possible results depend on the number of defoliations employed.

In general, with respect to tiller population density, regardless of number of cuts and crop season, there was a tendency for plants under the SI treatment to have lower TPD than the others. Comparing the SI treatment with the LI treatment, the latter showed greater plant tillering, demonstrating that spray inoculation can activate plant growth hormones. However, the results of this variable for the LI and control treatments did not show any significant difference ($p > 0.05$) (Table 1). Therefore, despite the LI treatment presenting better results, it does not justify the inoculant application to generate new tillers. Similarly, working with *Brachiaria brizantha* cv. Marandu with inoculant foliar spray application, Pedreira et al., (2017) observed the inoculant did not influence the tillers population dynamics. Unlike the result of this present work, in the previously mentioned study by Quatrin et al. (2019), number of tillers per m² was positively influenced by N rates and inoculation, since N availability to plants is fundamental for new tillers to emerge.

Table 1 - Cumulative forage productivity, tiller population density, grain yield, number of spikelets per spike, number of grains per spike and weight of one thousand grain of dual-purpose wheat BRS Umbu cultivar, submitted to seed inoculation (SI), leaf inoculation spraying (LI) or without inoculation to seeds or leaves (Control) and number of cuts, in two crop seasons.

Treatments	Season 2014			Season 2015		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
	------------- Cumulative forage productivity (kg ha^{-1}) -----------------					
SI	400.0 c	433.3 c	533.3 c	1386,7 c	1275.0 c	1612.5 c
LI	1695.8 a	1579.2 a	2016.7 a	3112.5 a	3116.7 a	2858.3 a
Control	945.8 b	1050.0 b	1383.3 b	2391,7 b	2454.2 b	2391.7 b
CV (%)	19.6	19.7	8.7	12.1	14.5	9.4
p>F	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	------------------Tiller population density (tillers m^{-2})-------------------					
SI	816	682 b	681 b	803 b	694 b	691 b
LI	947	963 a	832 a	966 a	935 a	839 a
Control	979	886 a	778 a	968 a	843 a	806 a
CV (%)	13.4	9.5	6.4	6.6	7.4	4.1
p>F	0.19 ns	<0.01	<0.01	<0.01	<0.01	<0.01
	-----------------------------Grain yield (kg ha^{-1}) --------------------					
SI	2712.2 b	2708.9 b	2289.9 b	185.6	231.7	351.7
LI	4363.8 a	4160.6 a	3840.0 a	310.0	369.2	173.0
Control	4075.2 a	3763.6 a	3811.0 a	338.2	346.2	288.4
CV (%)	11.5	8.4	12.8	20.3	22.1	20.6
p>F	<0.01	<0.01	<0.01	0.07 ns	0.06 ns	0.12 ns
	-----------------------------Number of spikelets per spike-----------------------------					
SI	10.8 b	12.8	12.8	12.5 b	12.2 b	14.8
LI	13.5 a	14.0	12.0	15.0 a	15.0 a	13.2
Control	13.5 a	11.8	13.5	16.0 a	16.0 a	14.5
CV (%)	10.2	9.5	6.3	6.9	7.6	7.6
p>F	<0.05	0.08 ns	0.07 ns	<0.01	<0.01	0.16 ns
	-----------------------------Number of grains per spike-----------------------------					
SI	17.4 b	17.4 b	13.0 b	2.6	2.4	2.4
LI	27.2 a	30.1 a	27.8 a	2.2	2.5	2.9
Control	25.2 a	25.3 a	22.5 a	2.4	2.5	2.3
CV (%)	16.8	14.2	16.2	21.4	27.2	13.3
p>F	<0.05	<0.05	<0.01	0.60 ns	0.99 ns	0.07 ns
	-----------------------------Weight of one thousand grain (g)------------------------------					
SI	27.4 b	27.5 b	27.7 b	22.1	20.7	20.9
LI	30.2 a	30.4 a	30.3 a	19.9	23.6	21.7
Control	30.8 a	28.8 a	29.1 a	20.2	22.1	24.4
CV (%)	3.5	3.0	4.4	9.3	6.3	8.4
p>F	<0.01	<0.01	<0.05	0.27 ns	0.06ns	0.10 ns

Means followed by different lowercase letter within the column, differ by Tukey's test at 5% error probability. CV: coefficient of variation; ns: not significant.

When evaluating grain yield in 2014, it is clear that regardless of the number of cuts made, the results for this variable referring to the SI treatment were significantly lower. Grain yield rates referring to LI and control treatments were higher, but statistically equal, which does not justify the costs of inoculant application (Table 1). Thus, it can be inferred that the use of inoculants via both seeds and leaves did not contribute to an increase in grain yield. In the 2015 crop season, there were no significant results for this variable according to the treatments being used. As with this work, other authors have found no effects of inoculant use on increasing wheat grain yield. When testing the inoculant application via leaves, Boleta et al. (2020), Santos et al. (2020) and Galindo et al. (2015) reported that the use of *A. brasilense* inoculant did not increase crop yield. Using the inoculant application via seeds and leaves, and only seeds, Ribeiro et al. (2018) and Quatrin et al. (2019), respectively, found the same result as the authors mentioned above. Silva & Pires (2017), using treatments with inoculant application in three stages: in seed, in sowing and via leaves, also did not find effects on grain yield in condition of absence of water and nutritional deficiencies.

In the other hand, in other works, results can be found with increased grain yield. Galindo et al. (2017) found that the seeds inoculated with *A. brasilense*, associated with 140 kg. ha^{-1} N application, provided the highest wheat grain yields. When evaluating the agronomic efficiency of association of different N doses and types of *A. brasilense* application (via seeds, leaves and furrow), Pereira et al. (2017) found that the application via seed treatment and with the N total dose in topdressing fertilization provided the greatest increase in grain yield.

Corroborating with the results discussed so far, Ferreira et al. (2014) cited that few studies have demonstrated the benefits from symbiotic interaction in grass leaves that are capable of providing the action of diazotrophic bacteria on entering the plant tissue, thus causing increases in productivity. The lack of productivity increase in wheat yield with inoculation was also attributed by Rodrigues et al. (2014) due to competition for space and nutrients among various soil microorganisms, where even the action by diazotrophic bacteria present in the soil was not enough to increases gain yield. In a review on plant growth promoting rhizobacteria, Tabassum et al. (2017) and Ferreira et al. (2019) highlighted that inoculation with *A. brasilense* does not always increase yield. The authors argued that inoculation responses are frequently expressed in plants in adverse situations such as water deficit, pathogen attack, competition by light and water, and nutrient deficiencies.

Regarding yield components variables number of spikelets per spike, number of grains per spike and thousand-grain weight, the trend of the results was the same for grain yield, that is, in general, the results for the treatments applied did not show significant differences or do not justify the costs with inoculant application (Table 1). Regardless of application method, in seeds, in sowing furrow or via leaves, no significant results were found in inoculant use containing *A. brasilense* on the same yield components studied in the present work for wheat (Galindo et al. 2015. Galindo et al. 2017. Silva & Pires 2017, Ribeiro et al. 2018; Correia et al. 2020).

With these findings, the lack of positive results for wheat grain yield and their components owing to the use of inoculants, it can be inferred that biological nitrogen fixation by the bacteria and phytohormone production were not effective at promoting increments in grain yield when conducted in an environment without adverse situations that can promote biotic and abiotic stresses. For all evaluated traits, it can be observed the SI treatment was worse than LI treatment, emphasizing what has already been mentioned by Fukami et al., (2016) and Munareto et al., (2018), that the bacteria's contact with pesticides used in seed may compromise the inoculation technique.

However, it was possible to observe LI treatment superiority compared to SI for all variables and regardless of the number of cuts performed. These results can be attributed to the inoculant application after defoliation, where leaf tissues are exposed and more susceptible to infection and colonization by bacteria (Pedreira et al., 2017). Although the LI treatment proved to be superior, it was significantly equal to the control for the variables analyzed, and this makes the inoculant application not recommended. Therefore, further studies are needed to assess whether the inoculant via foliar application can be effective in providing higher yields, through tests with the association of the inoculant with N rates, in the dual-purpose wheat production system.

Conclusion

It was found in general that regardless of the number of cuts performed, the results observed for all analyzed variables showed the same tendency. For cumulative forage productivity, the treatment carried out with only leaf inoculation by spraying had greater results, justifying the use of the inoculant for the vegetative phase of plant production. For tillering, grain yield and yield components, the inoculant application in seeds did not promote increases, and although foliar application has better results, it is not recommended for wheat dual-purpose BRS Umbu cultivar.

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