Ensaios e Ciência
ISSN 1415-6938 e ISSN 2178-695X

DOI: https://doi.org/10.17921/1415-6938.2026v30n2p184-197

Rhizobacteria with Emphasis on Biological Nitrogen Fixation and Phosphorus Solubilization in Alfalfa Crops

Rizobactérias com Ênfase em Fixação Biológica de Nitrogênio e Solubilização de Fosfóro na Cultura de Alfafa

Erich dos Reis Duarte: Faculdade Anhanguera. PR, Brasil

Aline Vanessa Sauer: Universidade Estadual do Norte do Paraná. PR, Brasil

Eduardo Lopes Cancellier: Universidade Federal de Lavras. MG, Brasil

Rodrigo Foltran: Universidade Estadual Júlio Mesquita Filho. SP, Brasil

Cristiano Reschke Lajus: Universidade Comunitária da Região de Chapecó. SC, Brasil.

Éttore Guilherme Poletto Diel: Universidade Comunitária da Região de Chapecó. SC, Brasil.
ettorepoletto77@gmail.com

Received: December 2025
Accepted: May 2026

Abstract

The alfalfa (*Medicago sativa*) in addition to promoting cultivation diversification and generating jobs in the pioneer north of Paraná-Brazil, stands out in animal feed due to its qualitative and agronomic characteristics. The objective of this study was to evaluate the application of rhizobacteria in post emergence in the management of alfalfa crop irrigated by sprinklers. The rhizobacteria were covered in alfalfa crops. Commercial products based on *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6), *Bacillus megaterium* (isolated BRM 119) + *Bacillus subtilis* (isolated BRM 2084) and *Sinorhizobium meliloti* (isolated SEMIA 134 e SEMIA 135) were applied. The cultivation area was irrigated for two hours before application and four hours after application. Seven cuts of alfalfa were performed every 39 to 41 days. Before each cut, the height of 20 plants per plot was measured on the first leaf below flowering. The leaf area index (LAI) and dry matter (DM) total area considered (m^2) were evaluated. The experimental design used was randomized blocks, with 8 treatments and 3 repetitions, totaling 24 plots. The application of rhizobacteria in coverage showed significant differences in alfalfa crops. Dry matter yield increased 28.15% per cut, above the control. To facilitate the implementation of management for rural producers, the results propose post-emergence applications in alfalfa crops.

Keywords: Forage Legume. Alfalfa Productivity. Microrganisms.

Resumo

A alfafa (*Medicago sativa*) além de promover a diversificação de culturas e gerar empregos no norte pioneiro do Paraná-Brasil, se destaca na alimentação animal devido suas características qualitativas e agronômicas. O objetivo deste trabalho foi avaliar a aplicação de rizobactérias em pós emergência no manejo da cultura de alfafa irrigada por aspersores. As rizobactérias foram aplicadas em cobertura na cultura de alfafa. Foram aplicados os produtos Pseudomonas fluorescens (isolado CCTB03) + *Azospirillum brasilense* (isolado AbV6), *Bacillus megaterium* (isolado BRM 119) + *Bacillus subtilis* (isolado BRM 2084) e *Sinorhizobium meliloti* (isolados SEMIA 134 e SEMIA 135). A área de cultivo foi irrigada por duas horas antes da aplicação e quatro horas após a aplicação. Foram realizados sete cortes da alfafa a cada 39 a 41 dias. Antes de cada corte mensurou-se a altura de 20 plantas por parcela na primeira folha abaixo da floração. Avaliou-se o índice de área foliar (IAF) e matéria seca (MS) área total considerada (m2). O delineamento experimental utilizado foi de blocos casualizados, com 8 tratamentos e 3 repetições, totalizando 24 parcelas. A aplicação das rizobactérias em cobertura demonstraram diferenças significativas na cultura da alfafa. A produtividade de matéria seca aumentou 28,15% por corte, acima do controle. Para facilitar a implantação do manejo para os produtores rurais os resultados propõem as aplicações em pós emergência na cultura de alfafa.

Palavras-chave: Forrageira Leguminosa. Produtividade da Alfafa. Microrganismos.

1 Introduction

Alfalfa (*Medicago sativa*), native of Asia Minor and South Caucasus, is a forage legume that is adaptable to different types of climate and soil, which makes it adaptable to all agricultural regions of the world (Vilela, 2020). When compared with other legumes, it stands out due to the properties of its fibers, high nutritional value (20% to 25% of crude protein in dry matter), and for its ability to produce soft forage that is palatable to animals. (Moreira *et al*., 2017).

Alfalfa cultivation is based on the use of bacteria of species *Sinorhizobium meliloti* and *Azospirillum brasilense* in seed treatment. However, the use only in a single phase of legume cultivation, raises the need for studies on the viability of other bacteria and other forms of application in the culture, such as post-emergence application. In addition to the species of genus *Rizhobium* sp., other growth-promoting bacteria and plants (BPCP) can be favourable, especially under conditions of low soil fertility (Hungria *et al*., 2010)

Soil conditions, especially in terms of chemistry, are among the fundamental factors that interfere with alfalfa cultivation in Brazil. In this regard, research results from other countries reveal that the pH for the crop should be between 6.5 and 7.5. For cultivars more adapted to Brazilian conditions, such as those in the Southeast, soil acidity correction is based on base saturation, for which a level of 80% is expected, with a pH between 6.0 and 6.5 (Santos *et al*., 2006). Limestone should be applied between three and six months before sowing alfalfa in acid soils, always respecting the previous crop for the period and soil analysis obtained (Honda; Honda, 1999).

Alfalfa cultivation under optimal conditions requires high soil fertility, especially regarding adequate levels of organic matter, nitrogen, phosphorus, potassium, calcium, and magnesium. Proper nutrient management is essential to maintain plant persistence, regrowth capacity, dry matter production, and forage quality throughout the production cycles (Bernardi et al., 2007; Sarmento *et al.*, 2001). According to Moreira *et al.* (2007), fertilization management is fundamental for both crop establishment and maintenance. Basal fertilization requires broadcast application and soil incorporation throughout the cultivated area. At sowing, phosphorus (P_2O_5) and potassium (K_2O) rates should be adjusted according to regional recommendations, while topdressing potassium fertilization should be performed according to crop demand and technical recommendations (Rando, 1992).

The amount of nitrogen (N) supply for alfalfa is realized exclusively by the symbiosis between plant and bacterium *Sinorhizobium meliloti*. The biological interactions of *Rhizobium* and *Bradyrhizobium* bacteria in the soil have great economic impacts due to the increase in nodulation and greater growth experienced by forages and legumes in response to the positive interaction between symbiotic and diazotrophic bacteria, especially those belonging to genus *Azospirillum* spp. (Silva, 2020). Nitrogen is considered one of the most important nutrients for alfalfa development because it directly affects chlorophyll synthesis, protein formation, photosynthetic activity, and dry matter accumulation, contributing significantly to plant growth and forage productivity (Taiz; Zeiger, 2015).

Nitrogen for alfalfa culture, as for other legumes, can be supplied through the symbiotic fixation of aerobic bacteria of species Sinorhizobium melilotti (specific for alfalfa). This species is considered native especially in soils capable of nodulating alfalfa, but it is found in low population levels in the soil (Rassini; Freitas, 1998). The bacteria inoculation in the seeds of forage legume occurs with the use of efficient isolates, which have the following attributes: ability to adjust to the soil conditions and climate of the region; aptitude for nodule formation and nitrogen fixation in all alfalfa cultivars adapted to the region; and high ability to survive in soil and compete with other isolates.

Rural producers have sought more technologies to become more competitive. Only rural properties that manage to optimize their costs, improve their scale economies and, combined with increased productivity, become viable in agricultural activities. Thus, improving cultivation techniques, with lower production costs and impact on the environment, is essential (Duarte, 2020; Duarte *et al.*, 2021).

The possibility of replacing agrochemicals with alternative products, such as biological products, to increase productivity, nitrogen fixation, and phosphorus (P) solubilization has been increasingly adopted by alfalfa producers in the northern region of Paraná. Phosphorus is an essential nutrient for alfalfa cultivation because it participates in energy transfer, root development, nodulation, and biological nitrogen fixation. Adequate phosphorus availability contributes to greater plant establishment, dry matter accumulation, and forage productivity (Taiz; Zeiger, 2015).

The use of poultry litter as an organic fertilizer has become the best alternative example. Considered safe and profitable, the waste destination from poultry farming aims to reduce the production cost in substitution of chemical fertilizers (Benedetti *et al.*; 2009). This work aimed to evaluate the application of products based on bacteria of species *Azospirillum brasilense*, *Pseudomonas fluorescens, Bacillus subtilis, B. megaterium* and *Sinorhizobium meliloti* in coverage in the management of alfalfa cv. Crioula.

2 Material and Methods

The experiment was carried out at Sitio São José in the city of Bandeirantes-PR located between coordinates 50º37'63" WO and 23º15'79" S. The area soil is classified as eutroferric red latosol and presents the following chemical and physical characteristics: pH in H_2O, 6,2; pH in $CaCl_2$, 5,78; MO, 21,49 g kg^{-1}; P, 16,94 mg dm^{-3}; K, 1,06 cmolc dm^3; Ca, 9,18 cmolc dm^{-3}; Mg 2,01 cmolc dm^{-3}; Al 0 cmolc dm^{-3} and V, 74.68% and texture (0-20 cm) g kg^{-1} Sand 120, Silt 240, Clay 680 (Pavinato *et al.*, 2017).

Soil analysis was carried out and based on this, organic mineral fertilization and soil correction were carried out according to Moreira *et al*., (2007). In soil preparation, the soil deepest layers were unpacked through harrowing.

Sowing cv. Crioula of alfafa (*M. sativa* L.) was carried out on February 6, 2020, mechanized, in furrows spaced 20 centimeters apart (Rassini; Freitas, 1998). 20 kg ha^{-1} of seeds inoculated with a commercial mixture of Rhizobium meliloti strains (BR 7407, BR 7408 and BR 7409) (commercial product Total Rizho®) recommended by Moreira *et al*. (2007) were used.

Bacteria used in the cover application in management of the alfalfa crop were obtained from commercial products: Pseudomonas fluorescens (isolate CCTB03) + Azospirillum brasilense (isolate AbV6) (commercial product Biofree®), Bacillus megaterium (BRM 119) + Bacillus subtilis (isolate BRM 2084) (commercial product BiomaPhos®), and Sinorhizobium meliloti (isolates SEMIA 134 and SEMIA 135) (commercial product TotalNitro®). The products were purchased from sealed commercial packaging. The products were applied 60 days after sowing with a Jacto manual knapsack sprayer, Magnojet ceramic spray tip (AD110º02). After each treatment application, the sprayer cleaning was carried out to avoid contamination between plots. Irrigation was performed with a LWP 2450 sprinkler at a flow rate of 400 L/hour for 2 hours before applying the treatments. The plant irrigation process was repeated 4 hours after the application of each treatment with commercial products. The experimental design used was randomized blocks with 3 replications, with plots of 9 m² (3 x 3 m) and 20 cm spacing between rows, constituting 08 treatments with 24 plots (Table 1).

Table 1 - Description of bacterial treatments in commercial mixtures, their combinations and respective doses, applied as cover in alfalfa (Medicago sativa) crop at Sitio São José, in the city of Bandeirantes, PR

Treatments	**Lineage**	**Dosage kg/L ha^{-1}**
T01	Witness:	0
T02	*Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135)	0.3
T03	*Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6)	0.3
T04	*Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)	0.3
T05	*Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6)	0.3/0.3
T06	*Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6)	0.3/0.3
T07	*Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolate BRM 2084) / *Sinorhizobium meliloti* (isolates SEMIA 134 and SEMIA 135)	0.3/0.3
T08	*Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) / *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)	0.3/0.3/0.3

Source: Research data.

Seven cuts of the alfalfa crop were performed (Table 2). Before each cut, the heights of 20 plants per plot were measured, considering the plant base to the first leaf below flowering, using a metric ruler. The leaf area index (IAF)/m^{-2} was evaluated by LAI=(AF.NP)/AT where: AF - average leaf area of 2 plants (m^2), NP - number of plants per square meter (plants m^2), AT - total area considered 1 (m^2) (FERRAGINE *et al*., 2004). The stem diameter was measured with the caliper device at the height of the second node on the plant. To collect the material, 0.5 m of borders were discarded and 1 m² was collected in each plot. After each cut, the samples were taken to a forced ventilation oven at 65 °C, until constant weight was obtained, to determine the sample dry weight and calculate the average dry matter yields.

Table 2 - Intervals between cuttings for alfalfa crop (Medicago sativa) and rainfall rates between cuttings at Sítio São José, in the city of Bandeirantes, PR

Alfalfa crop cut-off dates	**alfalfa crop cuts cut number**	**Intervals between cuts (days)**	**Temperature (ºC)**	**Precipitation (mm) between cut intervals**
06/14/2020	1st	-	25.5	-
07/23/2020	2nd	39	23.5	23
08/31/2020	3rd	38	29.5	15
09/11/2020	4th	41	32.5	20
10/21/2020	5th	40	38.5	37
11/26/2020	6th	35	33.5	104
01/02/2020	7th	36	34.5	94

Source: research data.

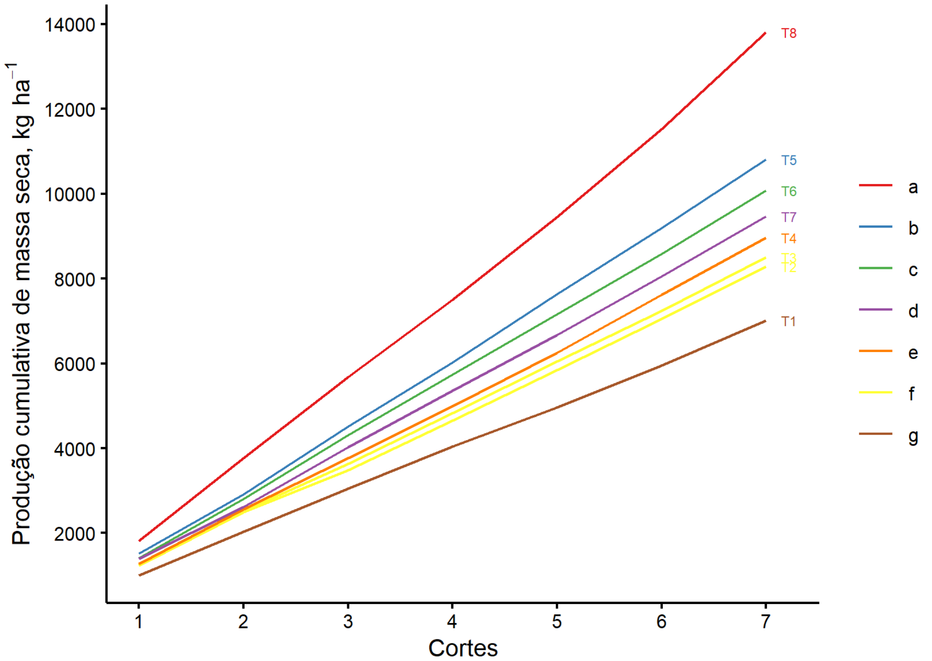
After obtaining the results, the leaf area index (IAF), stem diameter (DC), plant height (AP) and their productivity correlations for each treatment were calculated, based on dry matter weight (PMS) kg ha^{-1} of hay produced.

The data obtained for leaf area index (LAI), stem diameter (SD), plant height (PH), and dry matter production (DM) were organized in spreadsheets and subjected to analysis of variance (ANOVA). When significant differences were observed, the means were compared using Tukey's test at 5% probability ($p \leq 0.05$). Pearson's correlation analysis was also performed between the evaluated variables. Statistical analyses were performed using SISVAR statistical software.

3 Results and Discussion

The results found from the application of Pseudomonas fluorescens (isolated CCTB03) + Azospirillum brasilense (isolated AbV6), Bacillus megaterium (BRM 119) + Bacillus subtilis (isolated BRM 2084), and Sinorhizobium meliloti (isolated SEMIA 134 and SEMIA 135), corresponding to treatment 8 (T08), demonstrated a significant increase in hay dry matter production in the alfalfa crop when compared to the control treatment (Figure 1).

Figure 1 - Accumulated dry matter production (kg ha^{-1}) of alfalfa accumulated over seven cuts of alfalfa (Medicago sativa) crop based on *Sinorhizobium meliloti* applications (isolated SEMIA 134 and SEMIA 135); *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6); *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolate BRM 2084) and their different treatment combinations



[Legend: Accumulated dry matter production, kg ha^{-1} / Crop**]**
Source: Research data.

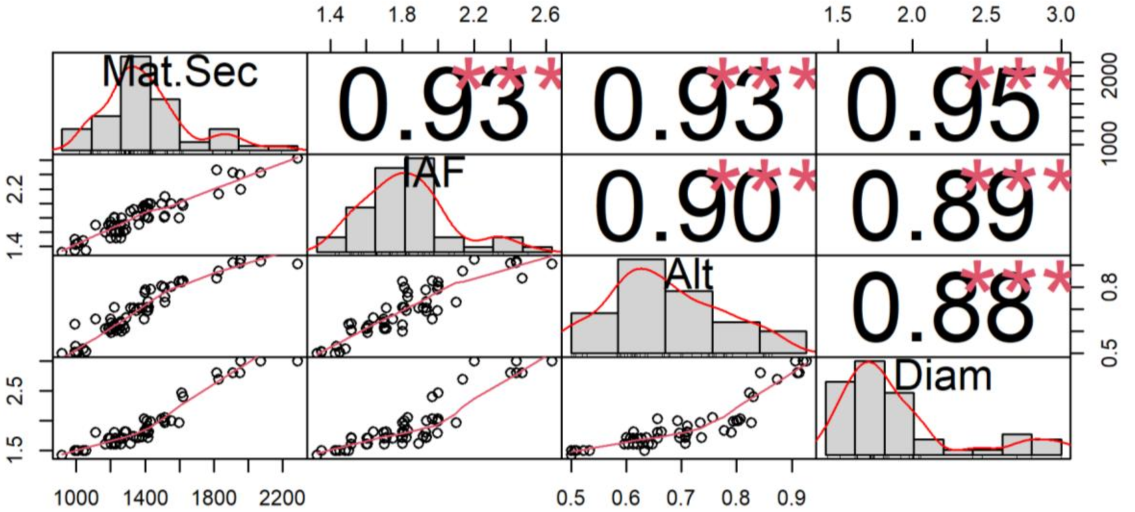
Bacteria *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135), *Pseudomonas fluorescens* (isolate CCTB03) + *Azospirillum brasilense* (isolated AbV6), used in treatments 2 (T02) and 3 (T03), differed from the control with production of 8281 and 8504 kg ha-1, respectively. Significant differences were observed for the use of *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) treatment T06. The results differed from the control, with a production of 10083 kg ha^{-1}. The use of bacteria together in T8 treatment (*Sinorhizobium meliloti* (isolates SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) / *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)), showed the highest result in dry matter accumulation in the 7 analyzed cuts, with a result of 13815 kg ha^{-1}. These results demonstrate to alfalfa producers the best technology to be implemented in management. The level of investment is justified by the respective return in weight of dry matter from the alfalfa crop.

The results showed a significantly greater increase in dry matter (PMS) in treatment 8 (Sinorhizobium meliloti (isolated SEMIA 134 and SEMIA 135) / Pseudomonas fluorescens (isolated CCTB03) + Azospirillum brasilense (isolated AbV6) / Bacillus megaterium (BRM 119) + Bacillus subtilis (BRM 2084 isolated)) at doses of 0.3/0.3/0.3 kg/L ha−1, respectively. The treatment obtained a superior response compared to the others when using the mixture of bacteria obtained from commercial products. Treatments consisting of commercial isolates or a commercial mixture of isolates, when applied individually, showed superior PMS results compared to the control. The bacterial association used in treatment 8 showed the highest dry matter accumulation among the seven evaluated cuts, with a production of 13,815 kg ha−1. This value represented an increase of 28.15% in dry matter production compared to the control treatment, demonstrating the positive effect of bacterial association on alfalfa productivity.

In addition to the increase in PMS, the application of bacteria can provide benefits to the alfalfa crop. The use of bacteria capable of producing indole acetic acid (AIA) and carrying out biological nitrogen fixation (FBN) may increase the total accumulation of nitrogen (N), phosphorus (P), dry mass and increase productivity in plants (Lobo, 2018). Phosphorus is the second fundamental nutrient after nitrogen necessary for plant development. An important element in all living systems and, in general, phosphate solubilizing microorganisms play a fundamental role in phosphorus nutrition, changing availability to plants through the release of inorganic and organic phosphorus from the soil, with solubilization and mineralization occurring (Khan *et al*., 2014).

A significant correlation result was obtained between the variables (Figure 2) of dry matter (DM), leaf area index (LAI), plant height (ALT) and stem diameter (DC). Therefore, when an increase in dry mass weight (PMS) was observed, it also correlated with the other analyzed variables. This demonstrates, for example, that taller plants also have greater leaf area and greater stem diameter. This allows us to conclude that the greater production of dry matter comes from a joint action of the stem diameter and the greater leaf area. In a similar study, a correlation was observed between the data for IAF, DC and ALT with dry matter productivity, which showed the highest dry matter value with more than 8,000 kg/h^{-1} (Duarte, 2020).

Figure 2 - Pearson's correlation between variables analyzed in alfalfa culture (Alt = Plant height, Diam = Stem diameter, LAI = leaf area index and Mat. Sec = Dry matter production.) ***significant at 0.1%

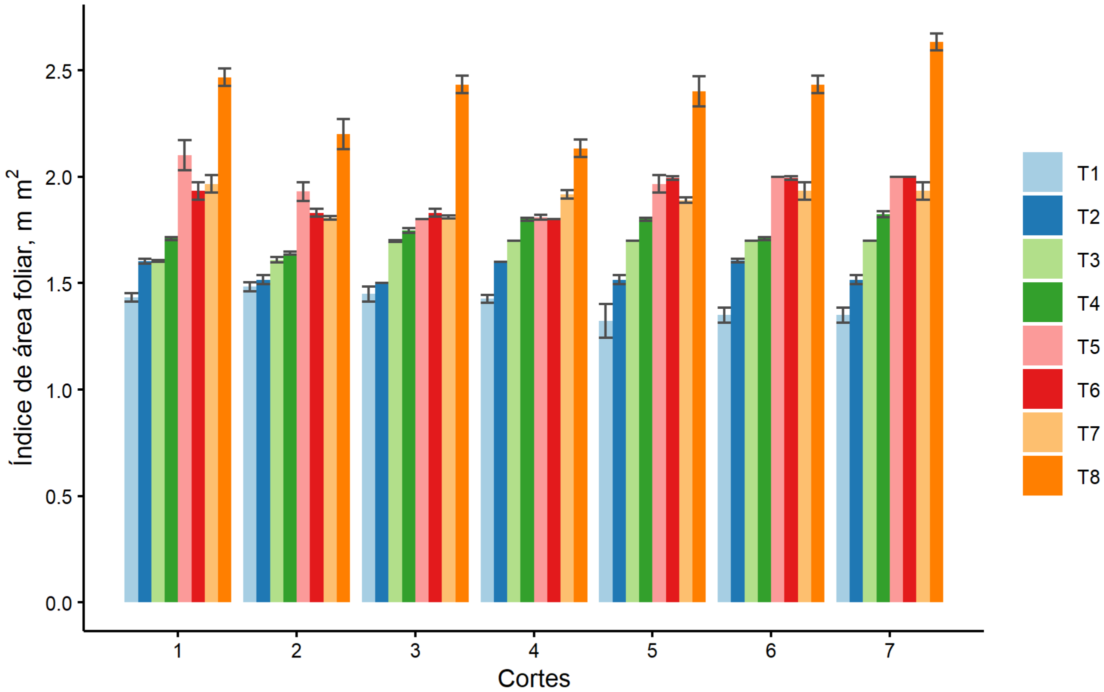


Source: Research data.

At each cut of the alfalfa crop, the yield is based on the dry mass that comes from the stem and leaves, which is why IAF and DC are so important. These indexes must be maintained with good soil fertility, high levels of organic matter, nitrogen, phosphorus and potassium, in addition to calcium and magnesium. Alfalfa crop, to be carried out under ideal conditions, requires maintaining a high level of soil fertility. Nutrients must be applied to the soil through liming and fertilization. Therefore, due to the fact that they play a great role in increasing the productivity of alfalfa crop, bacteria are fundamental inputs for this forage (Bernardi *et al*., 2007; Sarmento *et al*., 2001).

The treatments aim to analyze the application of bacteria in their respective isolated commercial products or in different combinations between products and bacterial species, which were applied post-emergence in a single step. The results per treatment for IAF per cut (Figure 3), demonstrated that the application of bacteria in treatments 8, 7, 6, 5, 4, 3 and 2 significantly increased the levels of leaf area index (IAF). The different treatment combination demonstrated that the use of different bacterial species provided larger plants and larger leaf areas (Figure 4), and the results reflected in the increase in dry matter productivity. Similar results were reported by Silva (2020), who observed greater vegetative development and biomass accumulation in alfalfa plants subjected to bacterial co-inoculation.

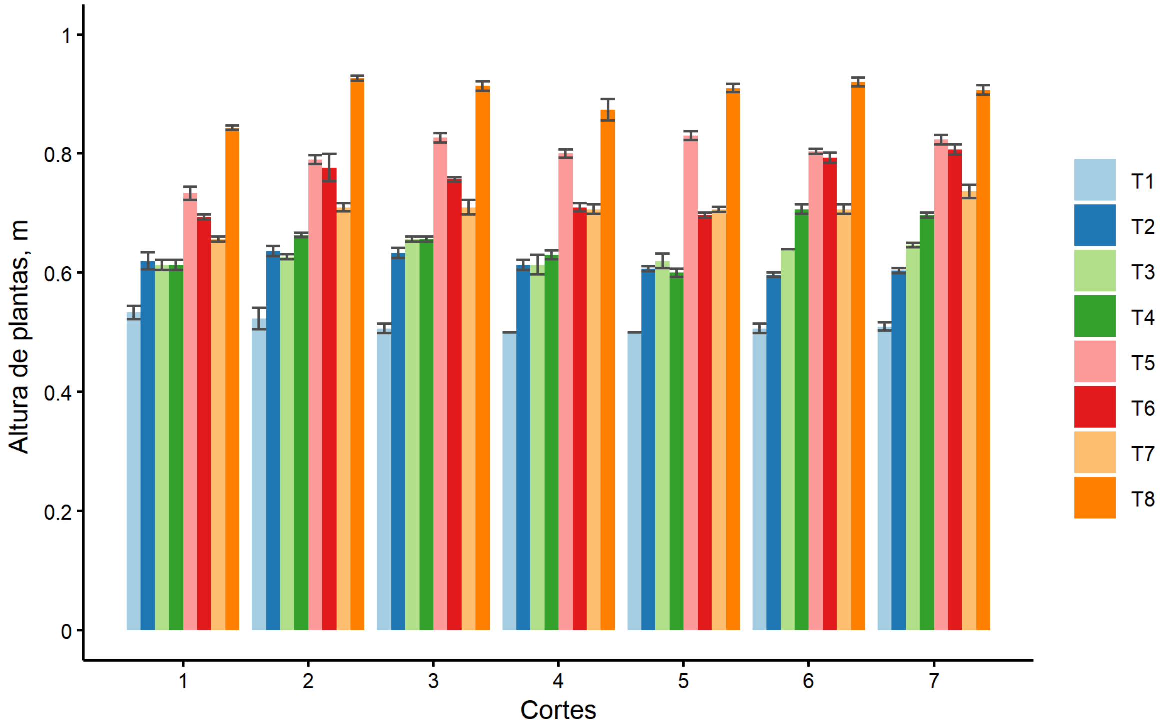
Figure 3 - Leaf area index of alfalfa crop under the influence of different biological managements



[Legend**:** Leaf area index, m^2 / Crop**]** (T1= Control; T2= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); T3= *Pseudomonas fluorescens* (isolated CCTB03) + Azospiril lum brasilense (isolated AbV6); T4 = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084); T5= *Sinorhizobium meliloti* (isolatesd SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolate AbV6); T6= *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolate AbV6); T7 = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); T8= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) / *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)). Error bars represent average standard error (n=3).
Source: research data.

For plant height, the treatment application differed from the control (Figure 4). The obtained results demonstrated that the application with the bacteria was significant in treatments 8, 7, 6, 5, 4, 3 and 2 for the increase in the height of the plants. Bacteria ability to promote plant growth was demonstrated at higher plant heights and reflected in higher yields respectively. Plant height varied on average from 37.9 to 53.3 cm. The lowest averages were observed in Treatment 1, which consisted of the absolute control (no N and no inoculation). Plants inoculated with plant growth-promoting bacteria tend to have a greater stimulus to grow, according to plant height analysis. Similar results were obtained by Silva (2020) when using bacteria, he detected an increase in the height of alfalfa plants in his variance analysis, plant height (cm) was significantly affected by the interaction between treatments and evaluation cuts. Other results indicated that liming application practices and potassium fertilization can contribute decisively to increase alfalfa longevity and height (Bernardi *et al.*, 2007).

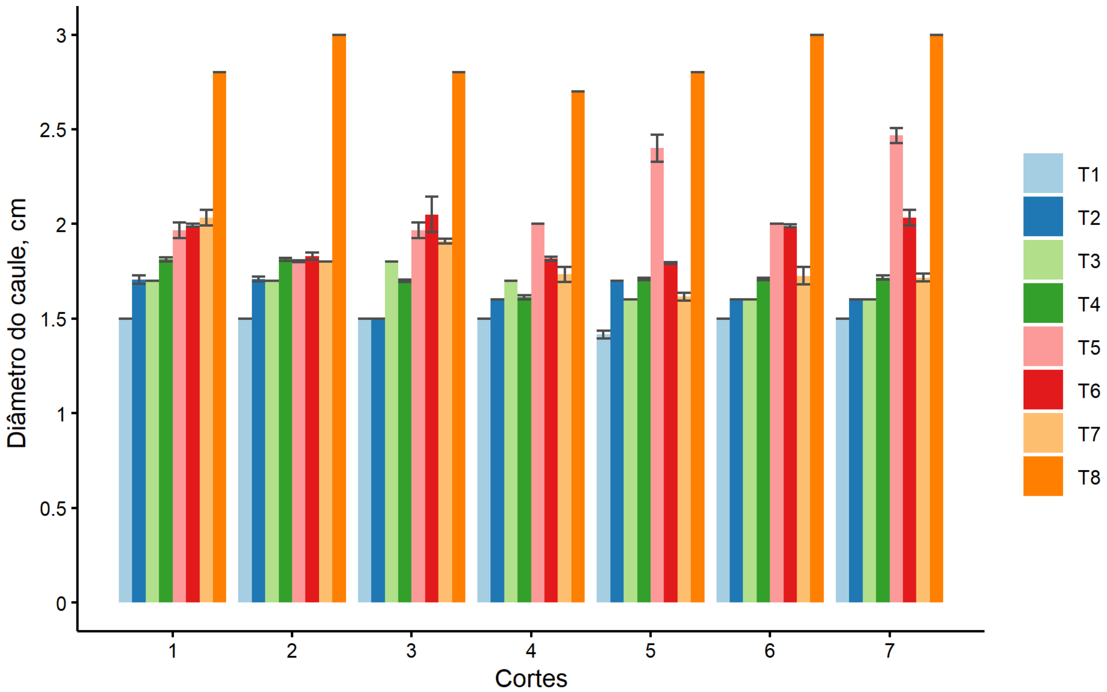
Figure 4 - Height of alfalfa plants under the influence of different biological managements. Error bars represent average standard error (n=3)



[Legend: Height of plants, m / Crop**]** (**T1**= Control; **T2**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); **T3**= *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6); **T4** = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) ; **T5**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6); **T6**= *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Pseudomonas fluorescens* (isolated CCTB03)) + *Azospirillum brasilense* (isolate AbV6); **T7** = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); **T8**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) / *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)). Error bars represent average standard error (n=3).
Source: research data.

The alfalfa crop stem diameter under the influence of different biological managements, showed that the treatments presented different responses in the crop development (Figure 5). Treatments 7, 6, 5, 4, 3 and 2 showed parity between stem diameter results. Treatment 8 differed from the others by showing greater stem thickness, up to 3 cm in diameter. These results indicate that the combination of different bacteria, in addition to helping to increase dry matter and leaf area index, can also promote greater plant stem thickness and provide greater robustness to the alfalfa crop. Similar results were found by Duarte *et al.*, (2021).

Figure 5 - Stem diameter of alfalfa crop under the influence of different biological managements. Error bars represent average standard error (n=3)



[Legend: Stem diameter, cm / Crop**]** (**T1**= Control; **T2**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); **T3**= *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6); **T4** = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) ; **T5**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6); **T6**= *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Pseudomonas fluorescens* (isolated CCTB03)) + *Azospirillum brasilense* (isolate AbV6); **T7** = *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084) / *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135); **T8**= *Sinorhizobium meliloti* (isolated SEMIA 134 and SEMIA 135) / *Pseudomonas fluorescens* (isolated CCTB03) + *Azospirillum brasilense* (isolated AbV6) / *Bacillus megaterium* (BRM 119) + *Bacillus subtilis* (isolated BRM 2084)). Error bars represent average standard error (n=3).
Source: Research data.

The management of alfalfa requires good organic nutrition, since, in addition to improve physical properties of the soil, fertilization improves fertility, especially in terms of micronutrients. The loss of ammonia, due to the mixture of manure and corrective (limestone), is irrelevant, since the N must come from the symbiotic nitrogen fixation (Rassini, 1998).

Honda and Honda (1999) show that potassium is a fundamental fertilizer to increase PMS and the high quality of alfalfa, since, if well nourished, the plants increase their ability to better use the nitrogen fixed by the bacteria and transform it into protein. To reduce losses, potassium fertilization should be carried out at sowing and in cover, in portions after every two cuts to balance the cost/benefit.

The commercial production of organic and organomineral fertilizers from humic substances has a geological origin rich in humified carbon, such as peat, rocks, lignite, mineral or vegetable coal, manure and organic residues. In agriculture, these products have been used to increase and conserve soil microbial life, whether in grain production, which provide increased efficiency in the use of fertilizers and reduce water consumption for irrigation, or in forage production (Schneider, 2020).

The positive effects observed in the present study are consistent with previous reports involving plant growth-promoting bacteria in forage crops. Silva (2020) observed increases in biomass production and vegetative development in alfalfa subjected to bacterial co-inoculation. Similarly, Hungria et al. (2010) reported that Azospirillum brasilense promotes improvements in root development and nutrient uptake, contributing to greater plant growth and productivity. In addition, phosphate-solubilizing bacteria such as Bacillus megaterium may increase phosphorus availability in the soil, favoring plant establishment and dry matter accumulation (Khan et al., 2014).

The assumptions for analysis of variance were verified for dry matter production data using graphical methods. Residual normality was assessed using Q-Q plots of residuals versus expected values, while homogeneity of variances was evaluated through standardized residuals versus fitted values. After confirming these assumptions, analysis of variance (ANOVA) was performed, followed by the Scott-Knott grouping test at 5% probability. For the individual analysis of cuts, a split-plot-in-time structure was adopted in the factorial ANOVA.

For plant height, stem diameter, and leaf area index variables, analysis of variance could not be performed due to the occurrence of repeated numerical observations, which generated mathematical limitations associated with division by zero. Therefore, these variables were discussed descriptively based on standard error bars. All statistical analyses were performed in the R environment (R Core Team, 2019), using the ScottKnott package (Jelihovschi *et al.*, 2014), while graphical analyses were performed using the ggplot2 package (Wickham, 2016).

The positive effects observed in the present study are consistent with previous reports involving plant growth-promoting bacteria in alfalfa cultivation. Silva (2020) observed increases in biomass production and vegetative development in alfalfa subjected to bacterial co-inoculation. Similarly, Hungria et al. (2010) reported that Azospirillum brasilense promotes improvements in root development and nutrient uptake, contributing to greater plant growth and productivity. In addition, phosphate-solubilizing bacteria such as Bacillus megaterium may increase phosphorus availability in the soil, favoring plant establishment and dry matter accumulation (Khan *et al.*, 2014).

4 Conclusion

The results demonstrated that the application of products based on Azospirillum brasilense + Pseudomonas fluorescens, Bacillus subtilis + Bacillus megaterium, and Sinorhizobium meliloti promoted increases in alfalfa productivity. The best responses were observed when bacterial associations were applied together in post-emergence management. The combined application of the evaluated rhizobacteria resulted in the highest dry matter accumulation, with an increase of 28.15%

compared to the control treatment. Therefore, the use of plant growth-promoting bacteria represents a promising and sustainable strategy to improve alfalfa productivity and optimize crop management.

References

BENEDETTI, P.M. *et al.* Fertilization with chicken litter in pasture. In: Brazilian Congress of Animal Science, XIX, 2009, Águas de Lindóia. ZOOTEC Annals: Pirassununga: Faculdade de Zootecnia e Engenharia de Alimentos, Brazilian Association of Zootechnicians. 2009.

BERNARDI, A.C.C.; RASSINI J.B.; FERREIRA. R .P . *Soil potassium content, nutritional status and dry matter production of alfalfa as a function of doses and frequency of potassium fertilization after two years of cultivation.* Electronic data. São Carlos: Embrapa Livestock Southeast, 2012.

CARVALHO, J.G.; ASSIS, R.P.; MOREIRA, A. Liming requirement for alfalfa cultivation. In: BOTREL, M.A. *et al.* Workshop on forage potential of alfalfa (Medicago sativa L.) in the tropics. Juiz de Fora: CNPGL, 1994. p.117-125.

DUARTE, E.R. Fertilization management in alfalfa crop. 1st edition São Paulo: Lux, 2020.

DUARTE, E.R. *et al*. The application of liquid and solid fertilizers in alfalfa crops. Braz. J. Agrotechnol. v.11, p.1-06, 2021.

FERRAGINE, M.C. et al. Seasonal production, leaf area index and light interception of alfalfa cultivars under grazing. Braz. Agricul. Res., v.39, n.10, p.1099-1052, 2004.

HONDA, C.S.; HONDA, A.S. The Alfafa Crop. 2. Cambará: Iara Artes Gráficas, 1999.

HUNGRIA, M. *et al.* ScottKnott: a package for performing the Scott-Knott clustering algorithm in R. Tema, v.15, n.1, p.3-17, 2014.

KHAN, M.S.; ZAID, A.; AHMAD, E. Mechanism of Phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms, in: phosphate solubilizing microorganisms. Springer International Publishing, Cham, 2014, p.31–62. doi: 10.1007/978-3-319-08216-5_2

LOBO, L.L.B. Potential of endophytic bacteria in promoting growth in maize plants Jaboticabal, 2018

MOREIRA, A. et al. Soil fertility and nutritional status of alfalfa cultivated in the tropics. Documents/Embrapa Livestock Southeast, 67. São Carlos: Embrapa Livestock Southeast, 2007.

PAVINATO, P.S. *et al.* Fertilization and liming manual for the State of Paraná. Curitiba: SBCS/NEPAR, 2017.

R CORE TEAM. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2019.

RANDO, A**.** Recommendations for potassium topdressing fertilization in alfalfa crops. 1992**.**

RASSINI, J.B.; FREITAS, A.R. Development of alfalfa (Medicago sativa L.) under different dose of potassium fertilization. Braz. J. Zootec., v.27, n.3, p.487-490, 1998.

SANTOS, H.G. et al. Brazilian system of soil classification. Rio de Janeiro: Embrapa Soils, 2006.

306 p.

SARMENTO, P.; CORSI, M.; CAMPOS, F. P. Alfalfa response to phosphorus sources associated with gypsum and liming. Scie. Agric., v.58, p.81-390, 2001.

SCHNEIDER, F.L. Organic carbon sources on the agronomic performance of the soybean crop and phosphorus and potassium levels in the soil. Pato Branco, 2020. 1 text file (52f): PDF ; 1,4 MB.

SILVA, L. Aurélio. ;Biomass production and bromatological composition of alfalfa subjected to co-inoculation with bacteria. Universidade Estadual Paulista (UNESP) – Dracena – SP – Brasil 52f. 2020.

TAIZ, L.; ZEIGER, E. Plant Physiology and Development. 6.ed. Sunderland: Sinauer Associates, 2015.

VILELA, Duarte. Hay: A promising market in the world and little explored in Brazil. Online magazine https://alavoura.com.br/pesquisa-inovacao/tecnologiaagricola/feno-um-mercado-promissor-no-mundo-e-pouco-explorado-no-brasil/. Access April. 2020.

WICKHAM, H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag, 2016.