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Article

Biostimulants in initial Growth of Discovery™ Bermudagrass

Uso de bioestimulantes no crescimento inicial da grama Discovery™

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Abstract: The use of bacteria and seaweed extracts as biostimulants to enhance plant growth holds promise for sustainable turfgrass management. This study aimed to investigate the effects of soil application of *Azospirillum brasilense* (bacterium) and *Ascophyllum nodosum* (seaweed) extract on the initial growth of Discovery™ bermudagrass. The study was conducted using a completely randomized design with plots measuring 0.25 m², each with a 0.5 m border. Two separate experiments were conducted, each involving four doses of biostimulants and five repetitions. The biostimulant treatments consisted of 0, 2, 4, and 6 mL L^1 *A. brasilense* inoculant and 0, 5, 10, and 15 mL L^1 *A. nodosum* seaweed extract. These treatments were uniformly applied to the soil at a rate of 100 mL m², with applications at 0, 30, and 60 days after the start of the experiment. After 90 days, the parameters green color index, green cover rate, turfgrass height, and vegetation index (normalized difference), were evaluated. The results indicated that both biostimulants significantly promoted the initial growth of Discovery™ bermudagrass. As the doses of the biostimulants increased, there was a corresponding increase in biomass and improved development of the turfgrass. The most pronounced responses were observed with a dose of 6 mL L^1 of the bacteria inoculant and 15 mL L^1 of the seaweed extract. These biostimulants fostered better turf coverage, making it challenging for weeds to establish, and potentially accelerating the production of sod grass.
Keywords: *Azospirillum brasilense*, *Ascophyllum nodosum*, *Cynodon dactylon*, growth-promoting bacteria, seaweed extract.

Resumo: O uso de extratos de bactérias e algas marinhas como bioestimulantes para promover o crescimento das plantas apresenta potencial para o manejo sustentável em áreas de produção de grama. Este estudo teve como objetivo investigar os efeitos da aplicação no solo do *Azospirillum brasilense* (bactéria) e do extrato de *Ascophyllum nodosum* (alga marinha) no crescimento inicial do capim bermuda Discovery™. O estudo foi conduzido usando um desenho completamente aleatório com parcelas de 0,25 m², com bordas de 0,5 m. Foram realizados dois experimentos separados, cada um envolvendo quatro doses de bioestimulantes e cinco repetições. Os tratamentos com bioestimulantes consistiram em 0, 2, 4 e 6 mL L^1 de inoculante de *A. brasilense* e 0, 5, 10 e 15 mL L^1 de extrato de alga marinha *A. nodosum*. Os tratamentos foram aplicados uniformemente no solo (100 mL m²), com aplicações feitas 0, 30 e 60 dias após o início do experimento. Após 90 dias, foram avaliados os parâmetros índice de cor verde, taxa de cobertura verde, altura do gramado e índice de vegetação (diferença normalizada). Os resultados mostraram que os bioestimulantes promoveram o crescimento inicial da grama Discovery™. Houve aumento da biomassa e melhor desenvolvimento do gramado, à medida que aumentavam as doses dos bioestimulantes. As respostas mais significativas foram observadas com a dose de 6 mL L^{-1} do inoculante bacteriano e 15 mL L^{-1} do extrato de algas marinhas. Os bioestimulantes promoveram melhor fechamento do gramado, dificultando o aparecimento de ervas daninhas e possivelmente acelerando a produção de grama.
Palavras-chave: *Azospirillum brasilense*, *Ascophyllum nodosum*, *Cynodon dactylon*, bactérias promotoras de crescimento, extrato de algas marinhas.

Introduction

The studies on ornamental turfgrass management have gained significant recognition due to their functionality and aesthetic value (Santos and Castilho, 2018). Within this field, the production of turfgrass for landscape and sporting purposes holds particular importance, especially in regions like United States and Europe (Beard and Gibbs, 2017). In Brazil, the estimated area dedicated to sod grass production is 32,500 hectares, with the southeast region accounting for approximately 49.7% of the national production (Associação Nacional Grama Legal, 2023). The Discovery™ turfgrass (*Cynodon dactylon*) is a bermudagrass variety developed in Australia and the USA. It is highly regarded for its applications in residential and commercial areas, mainly due to its desirable characteristics such as soft leaves, uniform growth pattern, and slow growth rate (Nascimento et al., 2020; Prates et al., 2020; Silvério et al., 2020).

For the proper development of turfgrass, it is essential to implement effective nutritional management that aligns with its specific uses and requirements (Oliveira et al., 2018; Santos et al., 2022). Nitrogen (N) plays a crucial role as the primary nutrient involved in promoting quality, growth, and aesthetics of grass. It contributes to enhancing the green color of turfgrass and reducing recovery time from mechanical injuries, particularly those caused by trampling (Gazola et al., 2016; Santos et al., 2019). Higher doses of nitrogen enhance the lawn's visual appearance. However, from an economic perspective, it is more advisable to apply 30 to 40 g N m^{-2} only once during the winter for the growth of DiscoveryTM bermudagrass (Prates et al., 2020). Nitrogen can also be used in combination with low doses of glyphosate as a plant growth regulator to control turfgrass growth (Begueline et al., 2021). This, combined with the maintenance costs involved, highlights the need to explore alternative approaches that can achieve a balance between production and sustainability.

Biostimulants derived from seaweed and microorganisms, such as *Azospirillum* spp. bacteria, are recognized as highly effective promoters of plant growth, by production of growth phytohormones like auxins, gibberellins, and cytokinins (Numan et al., 2018). The primary reported advantage attributed to *Azospirillum* is its ability to fix atmospheric nitrogen (N_2), leading to enhanced plant growth (Fukami et al., 2018). Seaweed extracts, such as from *Ascophyllum nodosum*, are also valuable biostimulants as they enhance various physiological processes in plants,

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including nutrient absorption and photosynthesis, and having on their composition macro and micronutrients (Santos et al., 2019). A seaweed-based fertilizer is produced using marine resources and is rich in nitrogen (N), phosphorus (P), potassium (K), as well as a variety of essential medium and trace elements. The active constituents are converted into small-molecule compounds, enabling quick absorption and utilization by crops (Qiqin et al., 2023).

The use of *Ascophyllum nodosum* extract has gained considerable attention in recent years, particularly in agricultural crop studies (Galindo et al., 2019; Santos et al., 2019). Photosynthetic organisms, such as eukaryotic microseaweed and certain cyanobacteria, present a sustainable alternative for turfgrass production areas (Li et al., 2017). Therefore, this study aims to investigate the effects of soil application of *Azospirillum brasilense* (bacterium) and *Ascophyllum nodosum* (seaweed) extract on the initial growth of DiscoveryTM bermudagrass, promoting greener and more sustainable agriculture.

Material and Methods

The study was conducted in experimental area covered with bermudagrass Discovery™, during the winter of 2019, with an average temperature of 18.9 ºC, relative humidity of 62.59%, and accumulated precipitation of 161 mm. The climate in the region is classified as Cfa, which corresponds to a humid subtropical climate with abundant and evenly distributed precipitation throughout the year, according to the Köppen climate classification. The soil type is characterized as a Red Dystrophic Latosol-LVd (Table 1).

Table 1. Result of chemical analysis of the soil in the experimental area.

pH	O.M.	P_{resina}	H+Al	K	Ca	Mg	SB	CEC	V%
($CaCl_2$)	(g dm^{-3})	(mg dm^{-3})	---------------------- ($mmol_c$ dm^{-3}) ----------------------						
5.6	20	108	14	2.9	26	8	37	50	73

The soil was corrected to achieve a target base saturation of 70% (Godoy et al., 2012) based on the BS (base saturation) (%) of the 10-20 cm soil layer, using a 72% TNRP (Total Neutralization Relative Power) dolomitic limestone. This amendment was evenly distributed across the entire lawn 20 days before the experiment was installed. The experiment was irrigated every two days, with the amount of water provided calculated to replace the evapotranspiration that occurred over the preceding two days. The sprinklers used were of the rotor type (5000 Plus model from Rain Bird®), connected to an electronic controller.

The experiment was initiated on July 3rd, 2019. Turfgrass mats were removed using a specific harvester, allowing for new regrowth and growth of the turfgrass. Ten days later, the experimental plots were marked and the treatments were applied. Two experiments were conducted simultaneously, evaluating different doses of *A. brasilense* growth-promoting bacteria and *A. nodosum* extract (seaweed). Each experimental plot had a size of 0.25 m^2 (0.50 x 0.50 m) and was arranged in a completely randomized design. Each experiment consisted of 5 repetitions. The doses used were 0, 2, 4, and 6 mL L^{-1} of *A. brasilense* inoculant and 0, 5, 10, and 15 mL L^{-1} of *A. nodosum* extract. The treatments were uniformly sprayed on the soil at a rate of 100 mL m^{-2}, applied at 0, 30, and 60 days after the start of the experiment.

The evaluations were conducted 90 days after the experiment was initiated and included the following parameters:

- Green Color Index (GCI): Measured using the FieldScout CM 1000 Chlorophyll Meter, which utilizes light reflectance. Samples were obtained parallel to the turfgrass surface at a height of 1.0 m.

- Green Coverage Rate (GCR): Assessed through digital image analysis using a 12 Mp camera fixed in a “light box” structure, similar to Peterson et al. (2011). The structure is a fully sealed box with lamps, ensured to standardized brightness and area images across all treatments. The Canopeo® software was used to analyze the images and determine the turfgrass coverage rate.

- Turfgrass Height: Measured using the “Grass Height Prism Gauge,” a dedicated instrument for turfgrass height measurement.

- Normalized Difference Vegetation Index (NDVI): Determined using the portable GreenSeeker device, which estimates turfgrass vigor and density.

The collected data were subjected to analysis of variance (ANOVA), and regression analysys was performed at a significance level of 5%. The statistical analysis was carried out using the “Statistix 10” software.

Results and Discussion

The results from the experiment with *A. brasilense* inoculation (Fig.1) demonstrated statistically significant differences for all the parameters, indicating that higher doses resulted in greater turfgrass development. Specifically, the concentration 6 mL L^{-1} showed the best results across all variables and significantly differed from the other treatments.

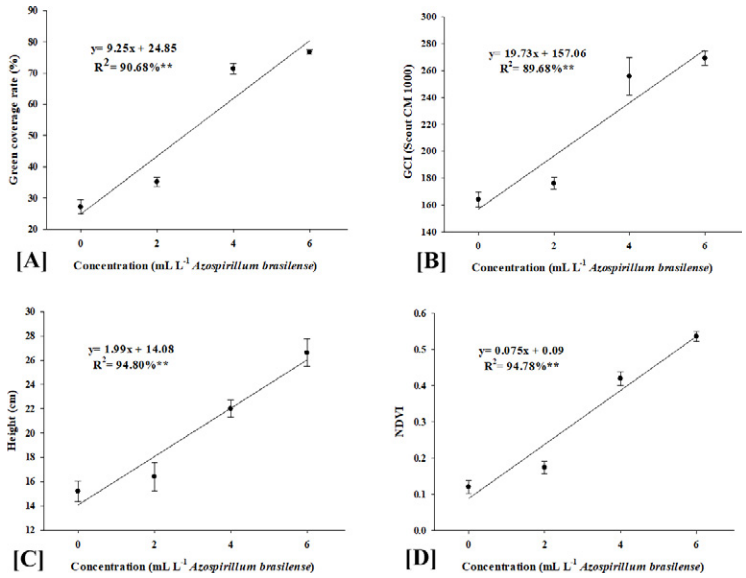

Fig. 1. Regression analysis of: a) Green cover rate (GCR); b) Green Color Index (GCI); c) Height and d) Normalized Difference Vegetation Index (NDVI) after 90 days of application of *A. brasilense.*

The rise in values for CGR, GCI, height, and NDVI can be attributed to the qualities of *A. brasilense* bacteria as a symbiotic biofertilizer, playing a role in biological nitrogen fixation (BNF) (Peng et al., 2020). This, in turn, results in a substantial nitrogen supply in the soil, which is vital for the growth of turfgrass (Gazola et al., 2019). Our experiment suggests that, when applying a dosage of 6 mL L^{-1} every 30 days, there may be no need for additional nitrogen supplementation for a duration of up to 90 days. Therefore, the use of bacteria from the genus *Azospirillum* spp. presents an alternative for turfgrass nutrition and has shown promising results in other agronomically important grasses (Afzal et al., 2019).

There is a well-established correlation between nitrogen fertilization and turfgrass leaf color (Oliveira et al., 2018; Gazola et al., 2016). Consequently, GCI indirectly reflects the chlorophyll content and turfgrass nutrition since higher GCI values are associated with increased concentrations of nitrogen and magnesium in plants (Oliveira et al., 2018; Santos et al., 2019). Higher values of CGR indicate a shorter period required for turfgrass coverage, which is crucial for preventing weed invasion. A shorter coverage period would lead to an earlier sod harvest, as observed in DiscoveryTM bermudagrass treated with and composted sewage sludge (Rezende et al., 2020).

The increase in plant biomass, promoted by *A. brasilense*, was clearly observed in the NDVI, which serves as an excellent indicator for evaluating turfgrass conditions (values around 1 indicate greater biomass and denser turfgrass). Notably, the dose of 6 mL L^{-1} yielded the best result with an NDVI value of 0.53. Moreover, there exists a positive correlation between green color and nitrogen levels in turfgrass leaves, as estimated by NDVI in bermudagrass hybrids (Caturegli et al., 2019). Marín et al. (2020) also recommend the use of NDVI to assess turfgrass quality. The visual aspect of the turfgrass (Fig.2) aligns with our data (Fig.1), with higher doses corresponding to increased Crop Growth Rate (CGR) and plant biomass. Turfgrass height is also influenced by higher nitrogen levels and the GCI, as increased chlorophyll concentrations contribute to more efficient photosynthetic processes, resulting in enhanced growth and adaptability (Santos et al., 2019). Even though, nitrogen is a primary nutrient required by turfgrass and is commonly used extensively in production areas, the results of our study suggest that *A. brasilense* has the potential to partially replace the nitrogen required by turfgrass, thereby reducing production costs.

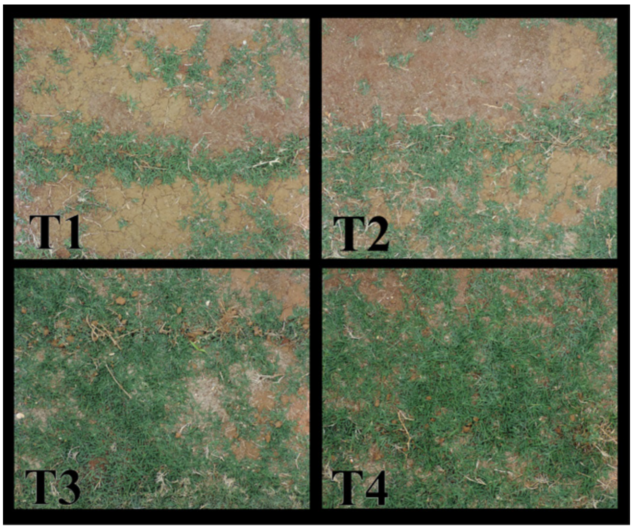

Fig. 2. Visual aspect of Discovery^{TM} bermudagrass 90 days after the application of *A. brasilense*. T1 - 0 mL L^{-1}, T2 - 5 mL L^{-1}, T3 - 10 mL L^{-1}, T4 - 15 mL L^{-1}.

The application of 15 mL L^{-1} of *A. nodosum* extract at 90 days produced the most favorable responses for CGR, GCI, height, and NDVI. The seaweed extract contains bioactive compounds and essential enzymes involved in nutrient assimilation, especially nitrogen (Van Oosten et al., 2017), which likely contributed to the enhanced development of the turfgrass, resulting in increased CGR and, consequently, GCI. Previous studies have demonstrated that the application of seaweed extract leads to an increase in chlorophyll concentration (Zhang et al., 2010) and enhances photosynthetic rates and growth in *Paspalum vaginatum* turfgrass (Elansary et al., 2017). Seaweed extracts also contain phytohormones, including cytokinins (Ali et al., 2021). Foliar application of seaweed (*Ascophyllum nodosum* Jol.) extract-based cytokinins has been shown to elevate leaf cytokinin content and delay senescence in bentgrass (Zhang et al., 2010). Therefore, they directly contribute to plant development and growth, while also providing protection against abiotic stresses in turfgrass (Bonomelli et al., 2018; Di Stasio et al., 2018).

The increase in biomass with the application of seaweed extract can be attributed to the nutritive substances present in the seaweed, which promote cell division and elongation. The higher doses of seaweed extract resulted in increased turfgrass biomass (Fig.3), which in turn influenced the reflectance area in the spectrum evaluated by NDVI. Consequently, seaweed extract can enhance turfgrass production and accelerate sod harvest (Neumann et al., 2017). Our results suggest that the dose of 15 mL L^{-1} of seaweed extract favored turfgrass development, potentially leading to an earlier harvest. While there are no specific reports on the effects of *A. nodosum* on DiscoveryTM bermudagrass, the use of seaweed-based biostimulants has proven to be an important tool in promoting the initial growth and closure of turfgrass (Fig. 4). However, it should be noted that many biostimulants also contain macro and micronutrients, raising the question of whether the positive effects observed are solely due to improved nutrition (Santos et al., 2019).

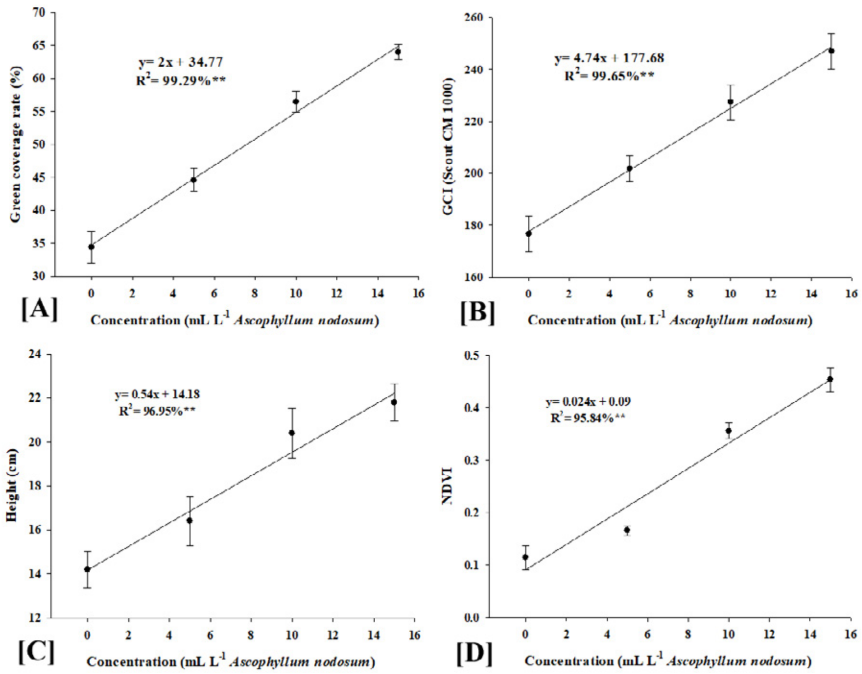

Fig. 3. Regression analysis of: a) Green cover rate (GCR); b) Green Color Index (GCI); c) Height and d) Normalized Difference Vegetation Index (NDVI) after 90 days of application of *A. nodosum*.

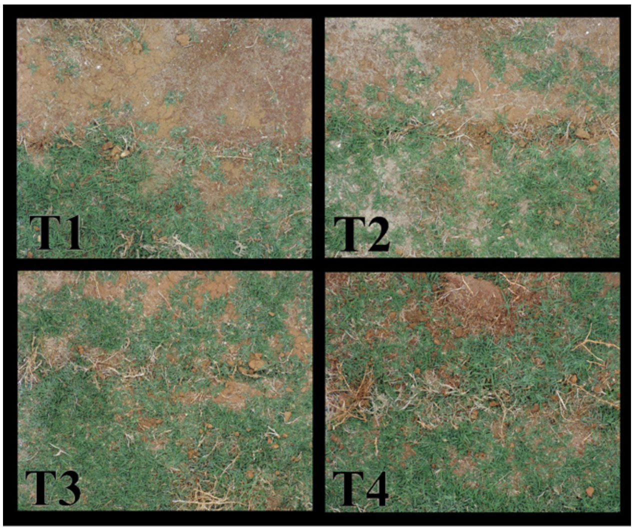

Fig. 4. Visual aspect of Discovery™ bermudagrass 90 days after the application of *A. nodosum* extract. T1 - 0 mL L^{-1}, T2 - 2 mL L^{-1}, T3 - 4 mL L^{-1}, T4 - 6 mL L^{-1}.

Conclusions

The application of 6 mL L^{-1} of *A. brasilense* and 15 mL L^{-1} of *A. nodosum* enhance the initial growth of Discovery™ bermudagrass. These doses resulted in a reduced closing period of the turfgrass, which is crucial for preventing the growth of invasive plants and potentially shortening the time to sod harvest.

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Author Contribution

PLFS: conceptualization, validation, investigation, data curation, writing. **ARZ**: investigation, data curation, writing. **PSTS**: investigation, data curation, writing. **MVLN**: investigation, data curation, writing. **LJGG**: data curation, writing. **ART**: data curation, writing. **RLVB**: conceptualization, validation, resources, data curation, writing, supervision.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on request.

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