

Bioinput treatments enhance germination and vigor of *Mimosa bimucronata* seeds¹

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ABSTRACT

Seed treatment with bioinputs can improve the efficiency of direct seeding by enhancing both its establishment and early growth. This study aimed to evaluate the effects of five commercial bioinputs (Acadian®, Asokop®, AURAS®, Trichodermil® and Stimulate®) on the germination and vigor of maricá [*Mimosa bimucronata* (DC.) Kuntze] seeds. The germination percentage, germination speed index, mean germination time and primary root and shoot lengths were measured. Asokop® and AURAS® promoted a more uniform germination, whereas Asokop®, AURAS® and Acadian® significantly increased the germination speed. AURAS® also promoted the greatest root development.

KEYWORDS: Plant growth promoters, bioregulators, ecological restoration, direct seeding.

RESUMO

Tratamentos com bioinsumos melhoram a germinação e o vigor de sementes de *Mimosa bimucronata*

O tratamento de sementes com bioinsumos pode aprimorar a eficiência da semeadura direta, melhorando tanto o seu estabelecimento quanto o crescimento inicial. Objetivou-se analisar os efeitos de cinco bioinsumos comerciais (Acadian®, Asokop®, AURAS®, Trichodermil® e Stimulate®) na germinação e vigor de sementes de maricá [*Mimosa bimucronata* (DC.) Kuntze]. Avaliaram-se a porcentagem de germinação, índice de velocidade de germinação, tempo médio de germinação e os comprimentos da raiz primária e da parte aérea. Asokop® e AURAS® proporcionaram a germinação mais uniforme, enquanto Asokop®, AURAS® e Acadian® aumentaram significativamente a velocidade de germinação. AURAS® também promoveu o maior desenvolvimento radicular.

PALAVRAS-CHAVE: Promotores de crescimento de plantas, biorreguladores, restauração ecológica, semeadura direta.

INTRODUCTION

Direct seeding has been recognized as a cost-effective alternative for the large-scale restoration of degraded ecosystems (Raupp et al. 2020). Although it often achieves plant densities higher than seedling transplantation (Freitas et al. 2019), it still suffers from low rates of species establishment in the field due to multiple biotic and abiotic stresses (Souza & Engel 2023).

Adapting seed treatment technologies from agriculture to forestry may offer a short-term strategy for scaling up direct seeding and achieving ecological restoration targets by 2030 (Brasil 2017). However, there is still a critical gap in studies evaluating seed

treatments with bioinputs, specifically for native species used in direct seeding for restoration purposes.

Agricultural seed treatment inputs intended for ecological restoration must combine efficacy with minimal environmental impact. Bioinputs - biological products derived from animal, plant or microbial sources - protect seeds and enhance their establishment, growth and eventual yield (Afzal et al. 2020, Souza et al. 2022). In Brazil, over 500 bioinputs are officially registered, covering functional classes such as biological control agents and plant-growth promoters (Oliveira et al. 2023).

Bioinputs may contain bioregulators or other chemical and biological compounds that promote seed and seedling development. For example, *Azospirillum*

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sp. and *Bacillus* sp. strains have shown to enhance germination and growth in several economically important species (Elsayed et al. 2022, Ribeiro et al. 2022), while algal extracts [*Ascophyllum nodosum* (L.) Le Jolis] improve resilience against abiotic stresses (Shukla et al. 2019). Although these products are widely used in large-scale agriculture (Meyer et al. 2022), their application in forestry remains largely limited to yield-focused plantation trials and mycorrhizal inoculation (Russo & Berlyn 2021, Cordero et al. 2022).

Maricá [*Mimosa bimucronata* (DC.) Kuntze] was selected as a model species due to its reliable germination performance under laboratory conditions (Santos et al. 2019, Ramos et al. 2020), enabling a clearer assessment of bioinput effects in controlled settings. The hypothesis is that treating its seeds with bioinputs containing bioregulators enhances germination speed and seedling vigor, thereby improving the success of direct seeding for ecological restoration. By reducing the germination time and standardizing emergence, this approach may better explore brief favorable windows, such as those expected under climate change scenarios (Klupczyńska & Pawłowski 2021).

Therefore, this study aimed to evaluate the potential of bioinputs containing bioregulators to improve germination, vigor and seedling development of Maricá, thus contributing to a more successful direct seeding in ecological restoration projects.

MATERIAL AND METHODS

The experiments were conducted at the Universidade Federal de São Carlos, in Sorocaba, São Paulo state, Brazil, from September to October 2021.

Five bioinputs were selected based on their documented application in crop species and their bioregulatory properties (Chagas et al. 2017, Ghosh et al. 2018, Guerra et al. 2023, Kumari et al. 2023, Solanha et al. 2023), all supplied in liquid formulation. The products included: Azokop® - *Azospirillum brasilense* (strains AbV5 and AbV6), with a minimum concentration of 2×10^8 CFU mL⁻¹ (Koppert, Brazil); AURAS® - *Bacillus aryabhattai* (strain CMAA 1363), at 1×10^8 CFU mL⁻¹ (Embrapa, Brazil); Acadian® - seaweed extract (*Ascophyllum nodosum*) containing 5.3 % of water-soluble potassium (K₂O; 61.46 g L⁻¹), 6.0 % of total organic carbon (69.60 g L⁻¹), pH of 8.0, density

at 20 °C of 1.16 g mL⁻¹ and salt index of 18 % (Koppert, Brazil); Trichodermil® - *Trichoderma harzianum* (strain ESALQ 1306), with 2.0×10^9 viable conidia mL⁻¹ (Koppert, Brazil); Stimulate® - biostimulant containing kinetin (0.09 g L⁻¹), gibberellic acid (0.05 g L⁻¹) and 4-indole-3-butyric acid (0.05 g L⁻¹) (Stoller, Brazil).

Maricá (*Mimosa bimucronata*, Fabaceae) seeds were used as a model due to their high germination rate (> 60 %) (Santos et al. 2019, Ramos et al. 2020, Dutra et al. 2023) and widespread application in ecological restoration through direct seeding (Barbosa et al. 2017, Balestrin et al. 2023, Piotrowski et al. 2023). The seed lot was assembled from nine mother trees distributed across the northwestern region of the São Paulo state, near the Tietê River reservoirs (e.g., Promissão and Ibitinga). Seed quality tests indicated 99.8 % of purity, 1,000-seed weight of 8.6001 g and moisture content of 10.1 % (Brasil 2024).

Six treatments were defined with the following concentrations: control (no bioinput); Azokop® at 10 mL L⁻¹; AURAS® at 10 mL L⁻¹; Acadian® at 8.5 mL L⁻¹; Trichodermil® at 8 mL L⁻¹; and Stimulate® at 10 mL L⁻¹. These concentrations were selected based on the manufacturers' recommendations and supported by the literature (Hungria 2011, Oliveira et al. 2020, Smiderle & Souza 2022). Each solution was applied directly to the seeds by placing them in 7 × 10 cm transparent plastic zipper bags, shaking manually for 30 seconds, and then allowing them to remain in contact with the solution for 30 minutes before removal for germination testing.

Prior to bioinput application, seed dormancy was broken by immersing the seeds in water heated to 80 °C, then removing the heat source and keeping them in the same water for 24 hours, following one of the methods recommended by Brasil (2024). The treated seeds were placed in Petri dishes lined with filter paper moistened with distilled water and incubated in a biochemical oxygen demand (B.O.D.) chamber at constant 30 °C, under a 12-hour photoperiod. The substrate was re-moistened twice weekly by adding 1 mL of distilled water.

The experiment followed a completely randomized design with ten replicates of 30 seeds per treatment, totaling 1,800 seeds. The first count was performed at two days after setup and subsequently at the same interval up to 14 days (Brasil 2024). Germination was defined by the emergence of the

primary root to at least 2 mm in length (Castellani et al. 2009), from which the germination percentage was calculated.

At the end of the test, 20 seedlings per treatment were selected by stratified random sampling across all replicates to ensure representation of within-treatment variation. Primary root and shoot lengths were measured using a millimeter ruler. The germination percentage was used to assess the germination potential, germination speed index (Ranal et al. 2009) and seedling growth parameters, to estimate vigor. The mean germination time (Labouriau 1983) was used to evaluate both the germination vigor and uniformity. All treatments were applied simultaneously at the start of the experiment, to ensure uniform environmental conditions and temporal consistency. The substrate moisture was maintained at optimal levels through periodic watering based on visual inspection and weight monitoring to prevent desiccation or waterlogging.

Data normality was assessed using the Shapiro-Wilk test and inspection of residual plots. Homogeneity of variance was evaluated with the Levene's test prior to the analysis of variance (Anova). The experimental data were then subjected to the Anova, followed by the Scott-Knott multiple comparison test, to prevent overlapping groupings (Jelichovski et al. 2014). Exploratory analyses included boxplots for germination percentage, germination speed index, mean germination time, primary root and shoot length. All statistical analyses were performed in the R software (version 4.0.5) (R Core Team 2024).

RESULTS AND DISCUSSION

No significant differences were observed for final germination among the treatments ($F = 2.063$;

$p = 0.0844$; Table 1); however, Azokop® and AURAS® showed the lowest variability in root emergence, indicating a greater uniformity in the germination percentage, when compared to the other treatments (Figure 1A).

Azokop®, AURAS® and Acadian® yielded the highest germination speed indices (Table 2), significantly differing from the control and the remaining treatments ($F = 2.8$; $p = 0.0255$). Azokop® exhibited the lowest variability (Figure 1B). This bioinput contains the *Azospirillum brasilense* bacterium, known to promote the production of growth regulators and phytohormones, as well as minerals that enhance primary root development and water uptake (Fibach-Paldi et al. 2012). In other Fabaceae species, *A. brasilense* at 1 g cm^{-3} did not enhance seed germination in the absence of dormancy breaking nor seedling growth of *Cassia leptophylla* Vogel and *Mimosa flocculosa* Burkart (Felix et al. 2021). Conversely, the *A. brasilense* inoculation promoted seedling growth in *Peltophorum dubium* (Spreng.) Taub. and *Leucaena leucocephala* (Lam.) de Wit (Rampim et al. 2014), whereas no effects were observed on the development, diameter or survival rate of *Enterolobium contortisiliquum* (Vell.) Morong and *Mimosa caesalpinifolia* Benth. (Silva et al. 2021). These variable responses may be attributed to factors such as the absence of dormancy breaking and differences in bioinput concentration, reinforcing the need to avoid generalizing results across species or phylogenetic groups. Nevertheless, the studies highlight the potential of such inputs.

Acadian® also positively influenced seedling vigor (Table 1). It contains extracts of the *Ascophyllum nodosum* algae, which are rich in bioregulators that play critical roles in germination and early seedling development (Saeger et al. 2020). This may explain the increase in germination speed,

Table 1. Mean and standard deviation ($\pm$) of the germination percentage (%G), germination speed index (GSI), mean germination time (MGT) and length (cm) of primary root and shoot.

Treatment	Seeds			Seedling	
	%G	GSI	MGT	Root	Shoot
Control	59 $\pm$ 11 a*	3.2 $\pm$ 0.8 b	7.3 $\pm$ 1.1 a	4.1 $\pm$ 0.9 b	4.38 $\pm$ 0.8 a
Azokop®	71 $\pm$ 7.8 a	4.2 $\pm$ 0.7 a	7.0 $\pm$ 0.9 a	4.29 $\pm$ 1 b	4.24 $\pm$ 0.7 a
AURAS®	66 $\pm$ 10.3 a	3.9 $\pm$ 0.8 a	6.8 $\pm$ 0.5 a	4.78 $\pm$ 1.2 a	3.95 $\pm$ 1 a
Acadian®	67 $\pm$ 14.7 a	4.0 $\pm$ 0.9 a	6.6 $\pm$ 1 a	4.05 $\pm$ 0.6 b	3.92 $\pm$ 0.7 a
Trichodermil®	60 $\pm$ 10.7 a	3.2 $\pm$ 0.8 b	7.7 $\pm$ 1.3 a	3.77 $\pm$ 1 b	3.87 $\pm$ 0.7 a
Stimulate®	69 $\pm$ 11 a	3.6 $\pm$ 0.7 b	7.5 $\pm$ 0.8 a	4.08 $\pm$ 1 b	3.81 $\pm$ 0.9 a

* Equal letters in the same column do not differ from each other by the Scott-Knott test ($p < 0.05$).

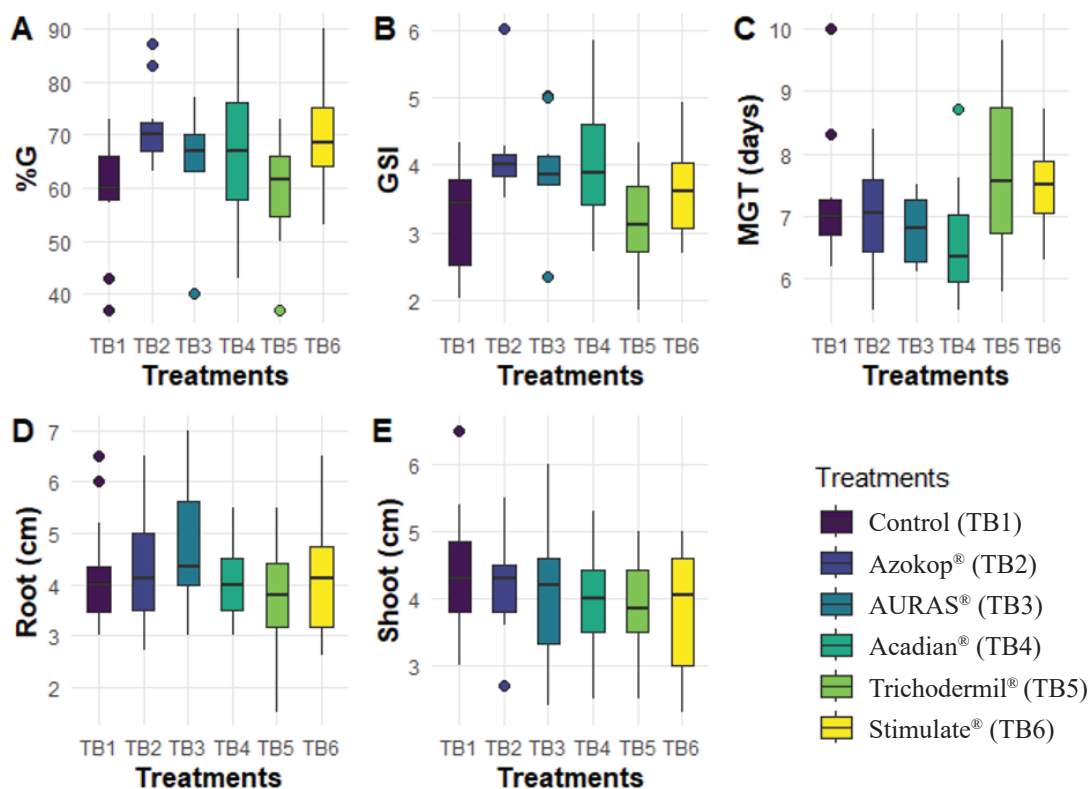


Figure 1. Box plot of germination percentage (%G; A), germination speed index (GSI; B), mean germination time (MGT in days; C), primary root (D; cm) and shoot (E; cm) length of Maricá.

although variability was higher than with Azokop® and AURAS® (Figure 1B).

Among the tested bioinputs, Trichodermil® resulted in the lowest seed vigor. This product contains the *Trichoderma harzianum* fungus, known to stimulate plant growth through mechanisms such as mineral solubilization, root colonization, symbiosis, nutrient uptake, phytohormone production and the secretion of siderophores and enzymes (Benítez et al. 2004, Li et al. 2015). Previous studies have reported improvements in seed vigor and quality in wheat (Anjum et al. 2020) and increases in length and biomass of *Handroanthus serratifolius* (Vahl) S. Grose seedlings (Santos et al. 2020). However, at concentrations above 10 mL L⁻¹, this bioinput can inhibit the emergence of *Hymenaea courbaril* L. seeds (Smiderle & Souza 2022).

Although the mean germination time did not differ significantly among the treatments ($F = 1.924$; $p = 0.105$), Stimulate® promoted the greatest uniformity in the mean germination time values (Figure 1C). This product contains three key bioregulators involved in germination processes: kinetin, gibberellic acid and indole-3-butyric acid (Moterle et al. 2008). Kinetin

helps to mitigate inhibitory effects on germination (Kamran et al. 2021), gibberellic acid can enhance seed quality and vigor (Cornea-Cipcigan et al. 2020) and indole-3-butyric acid promotes root development (Shekhawat & Manokari 2016). Stimulate® has also shown positive results for shoot development in *Lens culinaris* Medik. (Bruno et al. 2021), as well as improved germination and early growth in *Capsicum annuum* L. (Guerra et al. 2023). For native tree species, however, it reduced the germination percentage and germination speed index in *Cedrela fissilis* Vell., despite increasing the seedling length and dry mass (Dourado et al. 2020). When used in conjunction with dormancy-breaking treatments, it improved the emergence of *Hymenaea courbaril*, when compared to other bioinputs (Smiderle & Souza 2022).

Regarding seedling development, the root length was significantly affected only in the AURAS® treatment ($F = 2.421$; $p = 0.0398$; Table 1). This product contains the *Bacillus aryabhattai* bacterium, which produces secondary metabolites associated with plant growth and can function as a biofertilizer (Radhakrishnan et al. 2017). It also promotes

nutrient retention and chlorophyll content (Mun et al. 2024), and contributes to the synthesis of phytohormones (Park et al. 2017) and butanoic acid (Mun et al. 2024), which stimulate the indole-3-butyric acid production - a hormone essential for root development (Shekhawat & Manokari 2016). In addition, *B. aryabhattai* is associated with the transcription of plant growth-related genes (Xu et al. 2022), what may explain the enhanced primary root growth observed for Maricá (*Mimosa bimucronata*).

Among the treatments, only Acadian® promoted uniformity for primary root length (Figure 1D). The shoot development did not differ significantly across the treatments ($F = 1.585$; $p = 0.17$; Table 1) and exhibited considerable variability in the AURAS® and Stimulate® treatments (Figure 1E).

In summary, the bioinputs that enhanced seed vigor (Azokop®, AURAS® and Acadian®) demonstrate potential as seed treatments for direct seeding. This is particularly relevant because the emergence window typically spans from 30 to 180 days after sowing, critically influencing the species assembly that shapes the community structure in subsequent years (Piotrowski et al. 2023).

CONCLUSIONS

1. The application of the bioinputs Azokop®, AURAS® and Acadian® positively influences the vigor of Maricá (*Mimosa bimucronata*) seeds by promoting a more uniform and faster germination, which may enhance seedling establishment in direct seeding practices for ecological restoration;
2. Among the tested products, AURAS® notably improves the primary root growth in Maricá seedlings, indicating its potential to support early seedling development and resource acquisition in the field.

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