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UREA RATES AND *Azospirillum brasilense* APPLICATION STRATEGIES ON CANOLA YIELD AND PARTIAL GROSS MARGIN IN SÃO BORJA, RS

DOSES DE UREIA E ESTRATÉGIAS DE APLICAÇÃO DE *Azospirillum brasilense* NA PRODUTIVIDADE E MARGEM BRUTA PARCIAL DA CANOLA EM SÃO BORJA, RS

DOSIS DE UREA Y ESTRATEGIAS DE APLICACIÓN DE *Azospirillum brasilense* EN LA PRODUCTIVIDAD Y EL MARGEN BRUTO PARCIAL DE LA CANOLA EN SÃO BORJA, RS

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Pages: 1-21

Abstract

The objective of this study was to evaluate the growth, grain yield, and partial gross margin of canola subjected to urea rates and *Azospirillum brasilense* application strategies under field conditions in São Borja, Rio Grande do Sul, Brazil. Two independent field experiments were conducted in 2024 and 2025, each arranged in a randomized block design with three replications and a 3 × 3 factorial arrangement consisting of three urea rates (0, 75, and 150 kg ha^{-1}) and three *A. brasilense* application strategies, which differed between experiments. In Experiment 1 (2024), shoot green biomass, leaf area index, and grain yield were evaluated, whereas only grain yield was evaluated in Experiment 2 (2025). In 2024, urea rates and *A. brasilense* application strategies did not result in statistically detectable differences in the variables evaluated. In 2025, grain yield increased linearly with increasing urea rates, from 1,420 to 1,807 kg ha^{-1} between 0 and 150 kg ha^{-1} of urea, whereas no statistically detectable effect was observed for *A. brasilense* or the interaction between factors. Partial gross margin showed different numerical responses between the experiments: increasing urea rates reduced the margin in 2024, whereas the yield increase associated with urea application resulted in a numerical increase in 2025. The results indicate that canola response to urea differed between the two independent experiments and that economic outcomes depend on the relationship between yield response, input costs, and the environmental and nutritional conditions of each production environment.
Keywords: *Brassica napus*; nitrogen; inoculation; plant growth.

Resumo

O objetivo deste estudo foi avaliar o crescimento, a produtividade de grãos e a margem bruta parcial da canola submetida a diferentes doses de ureia e estratégias de aplicação de *Azospirillum brasilense* em condições de campo em São Borja, Rio Grande do Sul, Brasil. Dois experimentos independentes foram conduzidos em 2024 e 2025, cada um em delineamento de blocos casualizados com três repetições e arranjo fatorial 3 × 3, consistindo em três doses de ureia (0, 75 e 150 kg ha^{-1}) e três estratégias de aplicação de *A. brasilense*, que diferiram entre os experimentos. No Experimento 1 (2024), foram avaliadas a biomassa verde da parte aérea, o índice de área foliar e a produtividade de grãos, enquanto no Experimento 2 (2025) foi avaliada apenas a produtividade de grãos. Em 2024, as doses de ureia e as estratégias de aplicação de *A. brasilense* não resultaram em diferenças estatisticamente significativas nas variáveis avaliadas. Em 2025, a produtividade de grãos aumentou linearmente com o aumento das doses de ureia, de 1.420 para 1.807 kg ha^{-1} entre 0 e 150 kg ha^{-1} de ureia, enquanto nenhum efeito estatisticamente significativo foi observado para *A. brasilense* ou para a interação entre os fatores. A margem bruta parcial apresentou respostas numéricas diferentes entre os experimentos: o aumento das doses de ureia reduziu a margem em 2024, enquanto o aumento de produtividade associado à aplicação de ureia resultou em um aumento numérico em 2025. Os resultados indicam que a resposta da canola à ureia diferiu entre os dois experimentos independentes e que os resultados econômicos dependem da relação entre a resposta da produtividade, os custos de insumos e as condições ambientais e nutricionais de cada ambiente de produção.
Palavras-chave: *Brassica napus*; nitrogênio; inoculação; crescimento vegetal.

Resumen

El objetivo de este estudio fue evaluar el crecimiento, el rendimiento de granos y el margen bruto parcial de canola sometida a dosis de urea y estrategias de aplicación de *Azospirillum brasilense* en condiciones de campo en São Borja, Rio Grande do Sul, Brasil. Se realizaron dos experimentos independientes en 2024 y 2025, cada uno bajo un diseño de bloques al azar, con tres repeticiones y un arreglo factorial 3 × 3, compuesto por tres dosis de urea (0, 75 y 150 kg ha^{-1}) y tres estrategias de aplicación de *A. brasilense*, que difirieron entre los experimentos. En el Experimento 1 (2024), se evaluaron la masa verde de la parte aérea, el índice de área foliar y el rendimiento de granos, mientras que en el Experimento 2 (2025) se evaluó únicamente el rendimiento de granos. En 2024, las dosis de urea y las estrategias de aplicación de *A. brasilense* no produjeron diferencias estadísticamente

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Pages: 1-21

detectables en las variables evaluadas. En 2025, el rendimiento de granos aumentó linealmente con el incremento de las dosis de urea, de 1.420 a 1.807 kg ha^{-1} entre 0 y 150 kg ha^{-1} de urea, mientras que no se detectó un efecto estadísticamente significativo de *A. brasilense* ni de la interacción entre los factores. El margen bruto parcial mostró diferentes respuestas numéricas entre los experimentos: el aumento de las dosis de urea redujo el margen en 2024, mientras que el incremento del rendimiento asociado con la aplicación de urea resultó en un aumento numérico en 2025. Los resultados indican que la respuesta de la canola a la aplicación de urea difirió entre los dos experimentos independientes y que los resultados económicos dependen de la relación entre la respuesta productiva, los costos de los insumos y las condiciones ambientales y nutricionales de cada ambiente de producción.
Palabras clave: *Brassica napus*; nitrógeno; inoculación; crecimiento de las plantas.

1. Introduction

Canola (*Brassica napus* L. var. oleifera) stands out in the Brazilian agricultural landscape as a significant alternative for winter cropping, contributing to the diversification of production systems and crop rotation (Guimarães et al., 2022). Beyond its agronomic importance, the crop holds economic significance due to the high oil content of its seeds, which are used for food and energy purposes. However, achieving high yield potential depends on adequate nutrient supply. Nitrogen (N) stands out among the most important nutrients for the growth, productivity, and quality of canola grains (Mohammadi and Mosleh Ghahfarokhi, 2023).

Nitrogen fertilization plays a fundamental role in the development of canola and influences vegetative growth, leaf area development, dry matter accumulation, and therefore seed yield (Wen et al., 2023). Urea is one of the most important sources of nitrogen in agriculture. However, its application can lead to nutrient losses, particularly through ammonia volatilization, which reduces fertilizer efficiency. Against this backdrop, strategies to optimize nitrogen use efficiency and ensure economic viability are crucial for the sustainability of rapeseed cultivation (Liyanage et al., 2022; Ma et al., 2026).

As a complementary alternative to mineral fertilization, the use of plant growth-promoting bacteria (PGPB) has received increasing attention in agriculture. In canola, recent studies show that growth-promoting bacteria can improve nutrient acquisition, plant development, and yield characteristics, although the responses vary according to the microorganism and cultivation conditions (Jalal-Ud-Din et al., 2024). Among the PGPB, *Azospirillum brasilense* has multiple mechanisms associated with the promotion of plant growth, including biological N fixation,

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phytohormone production, and modulation of root architecture, in addition to favoring nutrient acquisition by plants (Pelagio-Flores et al., 2025). Recent studies show interaction between *A. brasilense* and root exudates in *Brassica napus*, while experiments under salt stress indicate the potential of the bacterium to modify physiological and nutritional responses of plants (Farhangi-Abriz et al., 2020; Fourneau et al., 2026).

In this context, growth-promoting bacteria may complement nitrogen fertilization management by increasing nitrogen use efficiency and helping reduce the need for mineral fertilizers without compromising crop performance (Dobrzyński et al., 2026). However, the response to inoculation with *A. brasilense* may vary according to inoculant dosage, application strategy or method, N supply level, and soil-climatic conditions. Recent literature on the association between *A. brasilense* and nitrogen fertilization management remains limited, particularly under field conditions, which makes it important to assess its agronomic and economic applicability.

The objective of this work was to evaluate the growth, grain yield, and estimated economic return of the canola hybrid Diamond subjected to urea rates and different *Azospirillum brasilense* application strategies under field conditions in the municipality of São Borja, Rio Grande do Sul, during the 2024 and 2025 growing seasons, addressing this gap under local conditions.

2. Methodology

Characterization and conduct of the experiments

Two experiments were conducted under different conditions during the 2024 and 2025 canola growing seasons at the same experimental site located in the municipality of São Borja, Rio Grande do Sul, Brazil (28°54'57.8“ S; 55°30'58.9” W). Both experiments were conducted on land classified as Red Latosol (Oxisol), according to the soil classification system adopted for the region, as defined by the Brazilian Soil Classification System (Santos et al., 2025). Pre-sowing soil chemical analysis data were not available for the experimental area.

In both growing seasons, soybeans were the preceding crop. The Diamond

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Pages: 1-21

hybrid was used, and the experiments were organized in a completely randomized block design with three replications. Each experimental unit consisted of five rows, 5 m long, spaced 0.45 m apart, totaling 11.25 m².

Experiment 1 was conducted during the 2024 growing season, with canola seeding on June 8, 2024, and harvest on October 18, 2024. At seeding, 200 kg ha^{-1} of the 05-10-10 organomineral fertilizer was applied directly into the seed row. The average established plant population was 40.7 plants m^{-2}, corresponding to approximately 406,667 plants ha^{-1}.

The treatments were arranged in a 3 × 3 factorial design, combining three levels of urea application rates and three *A. brasilense* application strategies, for a total of nine treatments. The application strategies with *A. brasilense* consisted of: no application; application of 2 L ha^{-1} in the seed furrow; and application of 2 L ha^{-1} in the seed furrow combined with 2 L ha^{-1} via foliar spraying at the V4 growth stage. Urea was applied at rates of 0, 75, and 150 kg ha^{-1}, corresponding to 0, 34.5, and 69 kg N ha^{-1}, respectively, considering a nitrogen concentration of 46% in urea.

Experiment 2 was conducted during the 2025 growing season; sowing occurred on June 14, 2025, and harvesting on October 24, 2025, with basal fertilization consisting of 100 kg ha^{-1} of the 05-20-20 formulated fertilizer and 100 kg ha^{-1} of ammonium sulfate. The average established plant population was 42.4 plants m^{-2} in the 2025 season, corresponding to approximately 424,444 plants ha^{-1}.

The treatments were arranged in a 3 × 3 factorial design, combining three levels of urea application rates and three *A. brasilense* application strategies, for a total of nine treatments. The application strategies with *A. brasilense* consisted of: no application; application of 4 L ha^{-1} via foliar spraying at the V4 growth stage; and application of 8 L ha^{-1} via foliar spraying at the V4 growth stage. Urea was applied at rates of 0, 75, and 150 kg ha^{-1}, corresponding to 0, 34.5, and 69 kg N ha^{-1}, respectively, considering a nitrogen concentration of 46% in urea.

The N supplied by basal fertilization was kept constant across all treatments within each experiment and therefore was not considered part of the experimental N-rate factor. The N rates evaluated in the experiment corresponded exclusively to the additional N supplied by urea applied as topdressing. Thus, the 0 kg ha^{-1} urea

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treatment represented the absence of additional N fertilization through urea topdressing, rather than the absence of N fertilization throughout the crop cycle.

The inoculant containing *A. brasilense*, composed of strains Ab-V5 and Ab-V6, had a concentration of 1×10^8 CFU mL^{-1}. Urea application was performed manually at the V4 growth stage. Applications were carried out before rainfall events, with recorded precipitation volumes of approximately 10 to 15 mm in the hours following the applications in both crop cycles.

In experiment 1, the *A. brasilense* in-furrow application was carried out at a spray volume of 40 L ha^{-1}. Foliar applications in both experiments 1 and 2 were performed using an electric backpack sprayer at a spray volume of 90 L ha^{-1}.

Meteorological conditions

Meteorological data regarding precipitation and air temperature during the experimental trials were obtained from a weather station located near the experimental site. Records corresponding to the period of each growing season were evaluated, spanning from the pre-sowing period to harvest.

For graphical representation, data were organized into weekly intervals relative to the sowing date, with week 0 considered the pre-sowing period and week 1 as the week in which sowing occurred, followed by subsequent weekly intervals until harvest.

Precipitation was expressed as accumulated rainfall per weekly interval, whereas air temperature was expressed as the mean temperature for the corresponding period, allowing for a comparison of meteorological conditions between growing seasons throughout crop development.

Growth and yield evaluations

In experiment 1 in the 2024 growing season, growth evaluations were performed at the flower bud phenological stage. To determine shoot green biomass (SGB), three plants per plot were collected from three previously defined points within the usable plot area, avoiding plants located at row borders. Plants were weighed individually, and the mean green mass per plant was subsequently calculated.

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Leaf area (LA) was determined using the same three plants collected for green mass evaluation. The width of each leaf was measured at the widest section of the leaf blade, perpendicular to the midrib. The leaf area of each leaf was estimated using equation (1), proposed by Tartaglia et al. (2016):

$$LA = (0.88735 \times L^2) + (0.93503 \times L) \quad (1)$$

where LA corresponds to the estimated leaf area and L to the leaf blade width. The total leaf area per plant was obtained by summing the estimated areas of all evaluated leaves. Leaf area values were converted to m^2 $plant^{-1}$.

The leaf area index (LAI) was calculated as the ratio between the crop leaf area and the ground area occupied by the plants. To estimate the LAI in the 2024 growing season, an average plant population of 40.7 plants m^{-2} was considered, equivalent to approximately 406,667 plants ha^{-1}, sown with a 0.45 m spacing between rows, according to equation (2):

LAI = leaf area per plant X plant population per unit area (2)

Growth evaluations (SGB, LA, and LAI) were evaluated only in Experiment 1 (2024). In Experiment 2 (2025), only grain yield was evaluated. For these variables, the plants collected within each plot were used to obtain a representative mean for the experimental unit, which was then used in the statistical analyses.

Grain yield was determined in both growing seasons when the plants reached the harvesting stage, according to the recommended criteria for the crop. Harvesting was performed manually in the three central rows of each plot, using two linear meters per row, disregarding the border rows. This totaled six linear meters and a sampled area of 2.70 m^2 per plot.

After harvesting, the material was subjected to mechanical threshing to obtain the grains. Yield was determined based on the grain mass obtained from the sampled area and subsequently converted to kg ha^{-1}, with the mass adjusted to a 10% moisture content.

Partial gross margin

The partial gross margin was calculated for each treatment based on grain yield and the costs of the inputs used. To estimate the margin, the commercialization

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price of canola was considered as BRL 130.00 per 60 kg bag. For the inputs, values of BRL 2.50 kg^{-1} for urea and BRL 5.50 L^{-1} for the product containing *A. brasilense* were used. Prices were obtained from commercial price quotes collected during the experimental period.

The partial gross margin was calculated as the difference between the estimated gross revenue from the commercialization of the production and the cost of the products used in each treatment, according to equation (3):

PGM = (GY X P) - (CU + CA) (3)

Where PGM corresponds to the partial gross margin (BRL ha^{-1}), GY to grain yield (bags ha^{-1}), P to the price of canola (BRL 130.00 bag^{-1}), CU to the cost of the urea applied (BRL ha^{-1}), and CA to the cost of the product containing *A. brasilense* (BRL ha^{-1}).

The partial gross margin results are dependent on the prices of canola grain, urea, and *A. brasilense* inoculant adopted in the present analysis and may vary with changes in market prices for agricultural commodities and inputs. Costs related to labor, machinery, product application, and other production operations were not included in the analysis. Therefore, the partial gross margin considered only the costs directly associated with urea and *A. brasilense* inoculant, together with the gross revenue obtained from grain yield.

Because this is a partial analysis, the indicator does not represent the net profit of the activity, but only the estimated return associated with the revenue generated from yield and the evaluated input costs.

Statistical analysis

Experiments 1 and 2 were conducted and statistically analyzed independently, with the results subsequently interpreted together regarding the observed agronomic responses across both experiments.

Before the analysis of variance, residual diagnostics were performed to assess the assumptions of the statistical models and identify potential outliers. Residual normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test, both at a 5% significance level. Residuals were

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also visually inspected using diagnostic plots. No observations were identified as potential outliers requiring exclusion or transformation, and therefore, all observations were retained in the analyses. These diagnostic procedures were performed using R software (version 4.6.1), with the RStudio interface.

The data were subjected to analysis of variance (ANOVA) considering a randomized complete block design and a 3 × 3 factorial arrangement, consisting of three urea rates and three *A. brasilense* application strategies. The block effect was retained in the statistical model for all variables evaluated. The significance of main effects and factor interaction was evaluated using the F-test at a 5% probability level.

In the presence of a significant interaction between factors, the interaction was partitioned and analyzed considering the levels of the corresponding factor. In the absence of interaction, main effects were evaluated separately. For the quantitative factor (urea rate), linear and quadratic regression models were fitted using the urea rates of 0, 75, and 150 kg ha^{-1} as the independent variable. Model significance was evaluated based on the regression analysis, and the coefficient of determination (R^2) was used to assess the fit of the selected model. Model selection was based on coefficient significance and goodness of fit to the data, with preference given to the lowest-degree statistically significant model.

When qualitative effects or factor slicings were significant, means were compared using the Scott-Knott test at a 5% probability level.

Statistical analyses of variance and mean comparisons were performed using SISVAR software (version 5.8).

3. Results and Discussion

Weather conditions (Figure 1) differed between the growing seasons, particularly regarding the precipitation regime. Accumulated precipitation during the cycle was 444.8 mm in the 2024 season and 1,029.4 mm in the 2025 season, representing a volume approximately 2.3 times higher in the second season. Average air temperature was also lower in 2025 (16.2 ºC) compared to 2024 (17.8 ºC), indicating that the two experiments were conducted under different environmental conditions.

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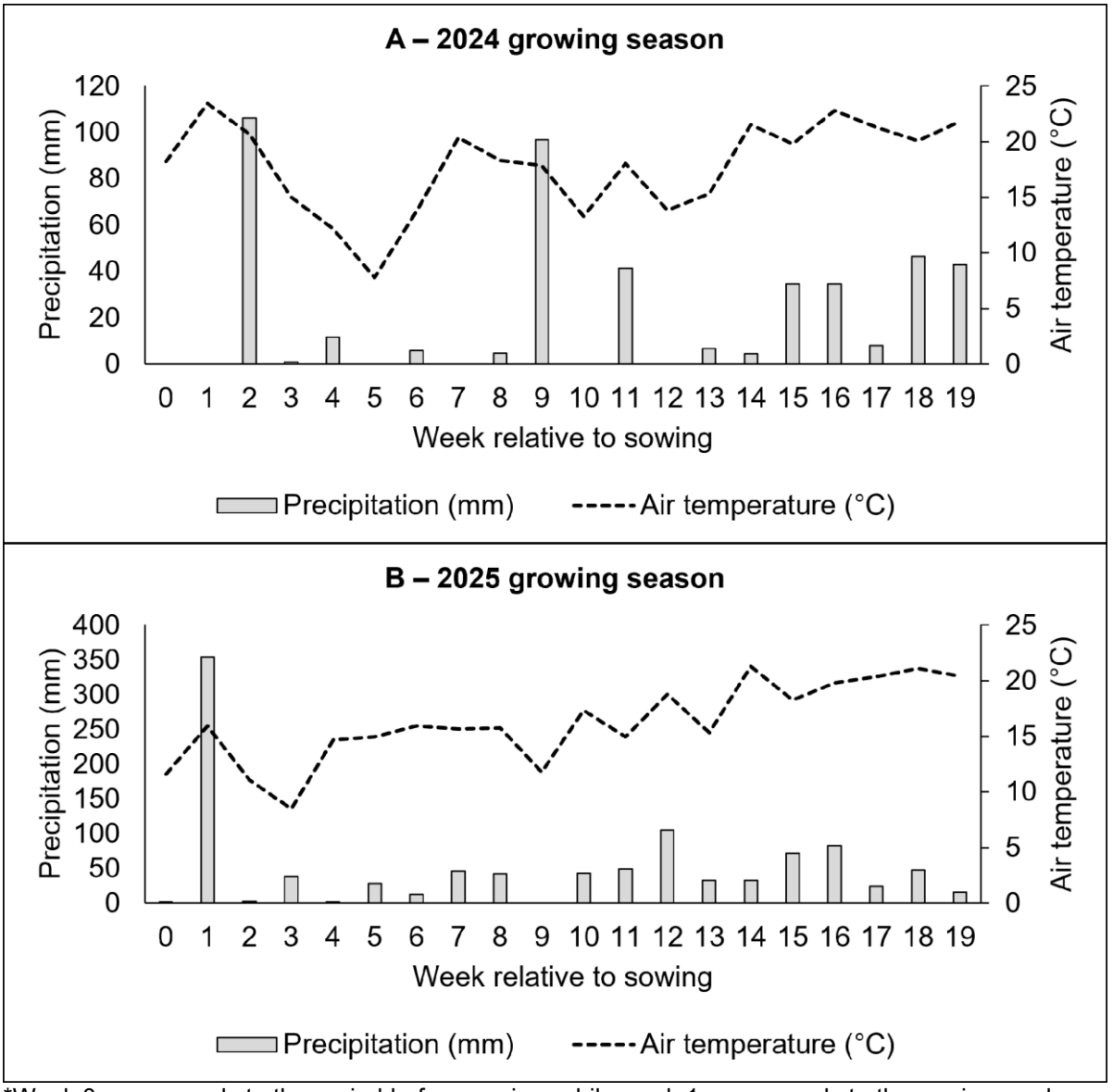
Figure 1. Accumulated precipitation and average air temperature during the canola growing cycle in the 2024 (A) and 2025 (B) seasons in São Borja, RS.



*Week 0 corresponds to the period before sowing, while week 1 corresponds to the sowing week.

Growth and productivity

Analysis of variance showed distinct responses between the two independent experiments regarding urea rates and *A. brasilense* inoculation (Table 1). In 2024, no significant effects of the factors or their interaction on shoot green biomass, leaf area index, and grain yield were observed. In 2025, only urea rates had a statistically detectable effect on grain yield, whereas no statistically detectable effects were

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observed for *A. brasilense* inoculation or the interaction between the factors. The *A. brasilense* application strategies differed between the experiments, with furrow and furrow + foliar applications used in 2024 and foliar applications at different rates used in 2025.

Table 1. Summary of the analyses of variance for shoot green biomass (SGB), Leaf area index (LAI), and grain yield (GY) of canola in two independent experiments conducted in 2024 and 2025 in São Borja, RS.

Experiment	Variable	Urea	AZ	Urea × AZ	CV (%)
1 (2024)	SGB	ns	ns	ns	22.35
	LAI	ns	ns	ns	23.38
	GY	ns	ns	ns	7.9
2 (2025)	GY	**	ns	ns	11.79

ns = not significant; * and ** indicate significance at the 5% and 1% levels, respectively, by the F-test.

In the 2024 season, SGB ranged from 44.7 to 79.6 g per plant, and LAI ranged from 1.5 to 2.4 across treatments. The highest numerical values for SGB (79.6 g per plant) and LAI (2.4) were observed with 35 kg N ha^{-1} of urea combined with 2 + 2 L ha^{-1} of *A. brasilense* (furrow + V4); however, the differences were not significant.

The lack of growth responses to urea rates contrasts with results obtained in other studies. Kaefer et al.(2014), for example, observed increases in canola dry mass and leaf area with rising N availability, with maximum responses occurring at approximately 73-74 kg ha^{-1} of N. This difference may be related to the environmental conditions and the availability of other limiting factors, given that crop response to N can vary across environments (Liyanage et al., 2022; Wen et al.,2023).

Grain yield in 2024 ranged from 1,011.7 to 1,138.3 kg ha^{-1}, with no significant effect of urea rates, *A. brasilense* inoculation, or the interaction between factors (Figure 2). The lowest value was observed in the treatment with no application of urea or *A. brasilense* (1,011.7 kg ha^{-1}), while the highest occurred with *A. brasilense* in the absence of urea (1,138.3 kg ha^{-1}). Even with numerical differences between

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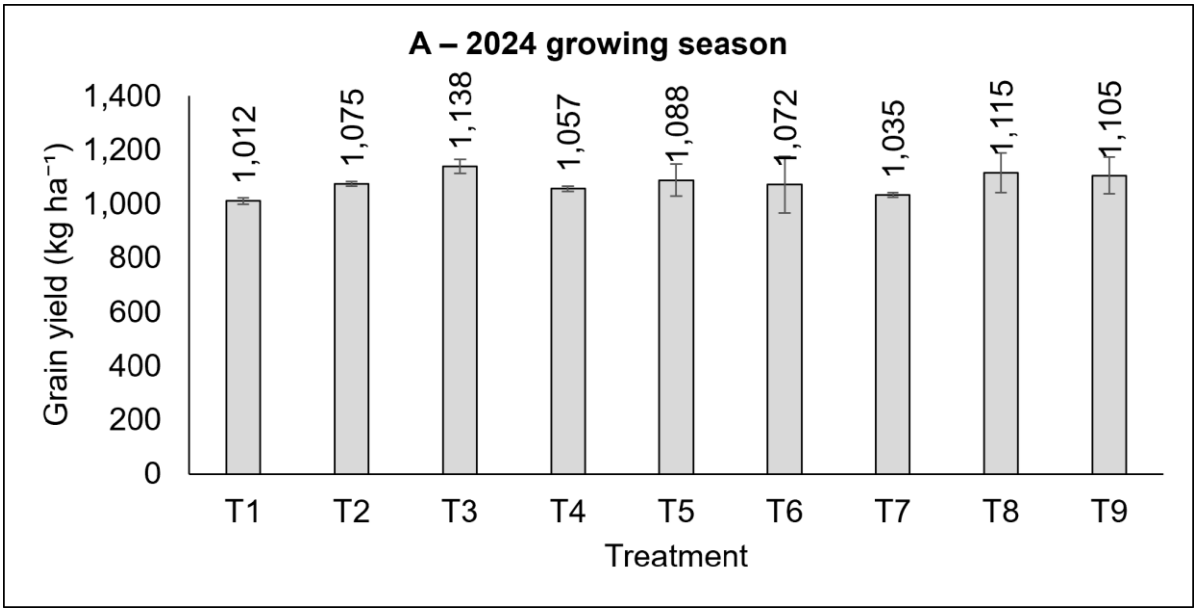
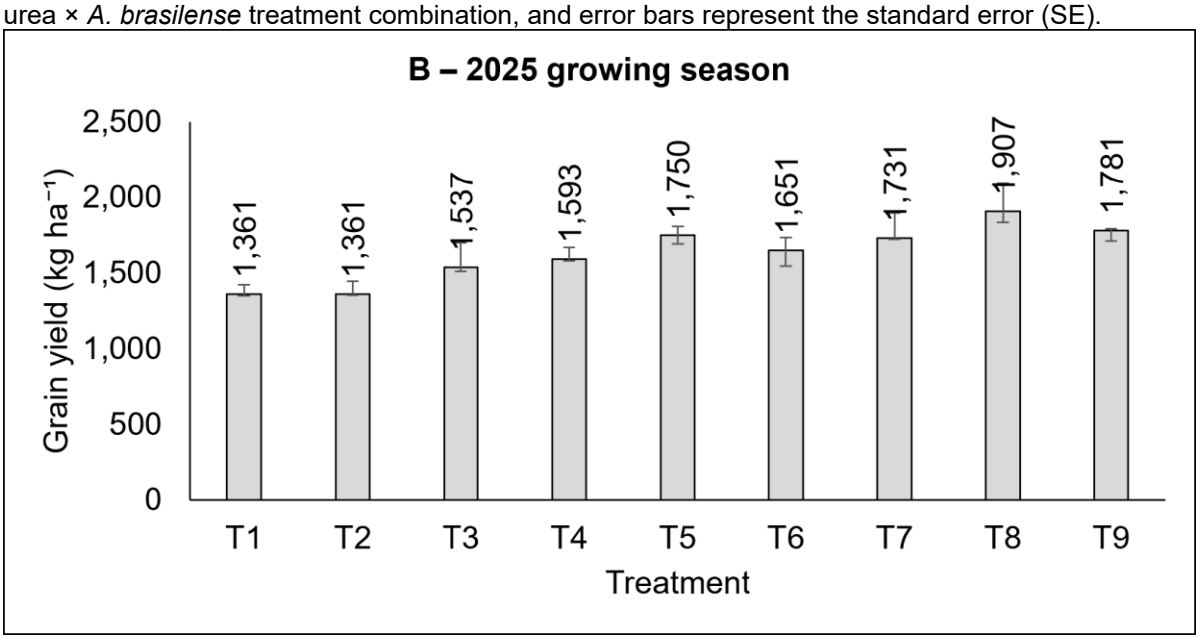
treatments, the absence of a statistical effect indicates that these variations were not consistent across replicates.

Figure 2. Canola grain yield in response to combinations of urea rates and *A. brasilense* in the experiments conducted in 2024 (A) and 2025 (B) in São Borja, RS.



T1: Control; **T2:** *A. brasilense* 2 L ha⁻¹ (furrow); **T3:** *A. brasilense* 2 + 2 L ha⁻¹ (furrow + V4); **T4:** Urea 75 kg ha⁻¹; **T5:** Urea 75 kg ha⁻¹ + *A. brasilense* 2 L ha⁻¹ (furrow); **T6:** Urea 75 kg ha⁻¹ + *A. brasilense* 2 + 2 L ha⁻¹ (furrow + V4); **T7:** Urea 150 kg ha⁻¹; **T8:** Urea 150 kg ha⁻¹ + *A. brasilense* 2 L ha⁻¹ (furrow); **T9:** Urea 150 kg ha⁻¹ + *A. brasilense* 2 + 2 L ha⁻¹ (furrow + V4). Bars represent the means of each urea × *A. brasilense* treatment combination, and error bars represent the standard error (SE).



T1: Control; **T2:** *A. brasilense* 4 L ha⁻¹; **T3:** *A. brasilense* 8 L ha⁻¹; **T4:** urea 75 kg ha⁻¹; **T5:** urea 75 kg ha⁻¹ + *A. brasilense* 4 L ha⁻¹; **T6:** urea 75 kg ha⁻¹ + *A. brasilense* 8 L ha⁻¹; **T7:** urea 150 kg ha⁻¹; **T8:**

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urea 150 kg ha^{-1} + *A. brasilense* 4 L ha^{-1}; **T9:** urea 150 kg ha^{-1} + *A. brasilense* 8 L ha^{-1}. Bars represent the means of each urea × *A. brasilense* treatment combination, and error bars represent the standard error (SE).

The lack of a productive response to urea application in 2024 may be associated with the specific environmental and nutritional conditions of this growing season. Accumulated precipitation was considerably lower than that recorded in 2025 and showed an irregular distribution throughout the cycle (Figure 1), with periods of low rainfall interspersed with more intense events. Water availability influences nutrient uptake and utilization and may limit the crop's response to N fertilization. Ma et al. (2026) also observed that environmental conditions, including periods of water deficit, altered the canola crop's response to N fertilization. In addition, basal fertilization differed between the experiments, with 200 kg ha^{-1} of 05-10-10 applied in 2024 and 100 kg ha^{-1} of 05-20-20 plus 100 kg ha^{-1} of ammonium sulfate applied in 2025. The ammonium sulfate supplied an additional source of sulfur in the 2025 experiment, which may have contributed to differences in the nutritional conditions under which the crop responded to increasing urea rates. Nitrogen and sulfur nutrition are closely related in canola, and the balance between these nutrients can affect nutrient use and crop productivity (Ma et al., 2024; Ma et al., 2026). However, because water availability and basal nutrient supply differed simultaneously between the experiments, their individual contributions to the contrasting responses to urea cannot be separated. The absence of pre-sowing soil chemical analysis is also a limitation of the study, as soil fertility status and nutrient availability could not be characterized before the experiments. Therefore, the potential contribution of initial soil conditions to the responses observed cannot be assessed.

Therefore, the contrast between the two experiments suggests that environmental and nutritional conditions may have contributed to the different responses to N fertilization, but the available data do not allow their individual effects to be distinguished. Kaefer et al. (2014) and Wen et al. (2023) also demonstrated that canola yield response to N can vary across years and locations, supporting the importance of considering the environmental conditions when determining nitrogen management.

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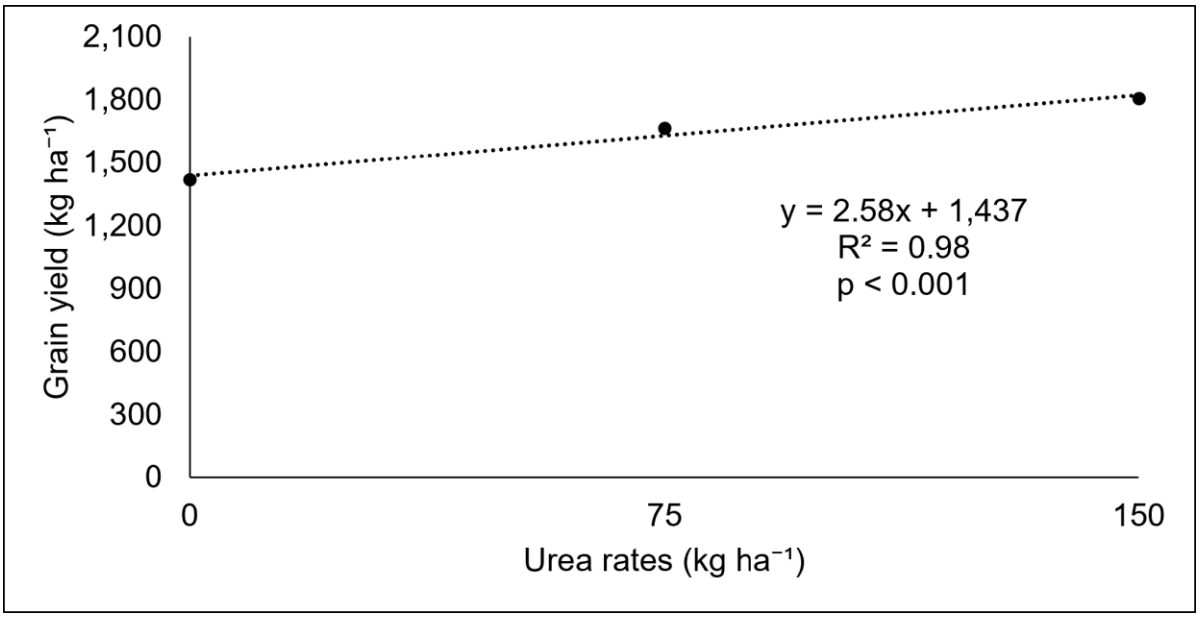
Pages: 1-21

Response to urea fertilization in Experiment 2 (2025)

Unlike what was observed in 2024, canola yield in 2025 increased linearly with urea rates (Figure 3), rising from approximately 1,420 kg ha^{-1} with no urea application to 1,665 and 1,807 kg ha^{-1} with 75 and 150 kg ha^{-1} of urea, respectively. The linear model showed a high coefficient of determination (R^2 = 0,98; p = 0.001), indicating a strong association between the increase in urea rate and yield under the conditions of this crop.

Figure 3. Canola grain yield in response to urea rates applied at the V4 stage during the 2025 growing season in São Borja, RS.



The points represent the mean productivity values. The line represents the linear regression fit (y = 2.58x + 1,437); R^2 = 0.98; p = 0.001.

The response observed in 2025 occurred under different environmental and nutritional conditions from those observed in 2024, including greater accumulated precipitation and differences in basal fertilization. More frequent rainfall may have favored conditions for N availability and uptake; however, the contribution of water availability cannot be distinguished from the other environmental and nutritional differences between the experiments. Wen et al. (2023) observed that the magnitude of the canola response to nitrogen fertilization varies across environments, while

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Vol: 13.20
DOI: 10.66104/vpaa8v69
Pages: 1-21

Liyanage et al. (2022) highlight the interaction among N availability, water, and environmental conditions as a determinant of nutrient use efficiency.

The linear response observed in this study also differs from results in which productivity showed a quadratic response to N fertilization. Kaefer et al. (2014), for example, estimated maximum productivity at approximately 88-89 kg ha^{-1} of N. In the present study, grain yield increased linearly within the range of urea rates evaluated (0–150 kg ha^{-1}, equivalent to 0–69 kg N ha^{-1} supplied as topdressing). Therefore, the linear response observed should not be extrapolated beyond the range of rates tested. Nitrogen management should consider not only the applied rate but also water availability and yield potential under the conditions of each production environment (Wen et al., 2022; Ma et al., 2026).

Effect of *Azospirillum brasilense*

Within each experiment, the *A. brasilense* application strategies did not result in statistically significant differences in grain yield. Because the application strategies differed between the experiments, the results are presented and interpreted separately for each experimental configuration. In 2025, average yields were 1,561.7, 1,672.8, and 1,656.4 kg ha^{-1} for the no-application, 4 L ha^{-1}, and 8 L ha^{-1}, respectively. Although the application of 4 L ha^{-1} resulted in a numerical increase of approximately 7.1% compared to the non-inoculated treatment, this difference was not statistically detectable. The 8 L ha^{-1} treatment also did not differ statistically from the non-inoculated treatment.

The lack of significant response does not necessarily indicate an absence of activity or agronomic potential of *A. brasilense.* According to the literature, *A. brasilense* can act through various mechanisms, including phytohormone production, modulation of root development, enhancement of nutrient acquisition, and biological N fixation (Pelagio-Flores et al., 2025). However, the magnitude of these responses depends on the interaction among the microorganism, the plant, the soil, and environmental conditions. Microbial colonization was not directly assessed in the present study; therefore, it was not possible to determine whether differences in bacterial establishment contributed to the observed responses.

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Vol: 13.20
DOI: 10.66104/vpaa8v69
Pages: 1-21

Results obtained specifically with *B. napus* corroborate this variability. Aquino et al. (2023) observed increased yield with *A. brasilense*, whereas no significant effects were detected in the present study. Świątczak et al. (2024) also observed distinct responses to plant growth-promoting bacteria depending on cultivation conditions. These differences may be related to genotype, the native microbial community, nutrient availability, or environmental conditions, as well as the dosage, timing, and method of inoculant application.

The non-significant interaction between urea and *A. brasilense* further indicates that, under the conditions evaluated, the inoculant did not consistently modify canola yield in response to N rates. Therefore, the results did not support the hypothesis that the inoculation strategies employed enhanced grain yield or compensated for N availability under the conditions of this experiment.

Partial gross margin

The partial gross margin (PGM) showed a distinct pattern between the two experiments (Figure 4). In 2024, since urea application rates did not result in a significant yield increase, the higher investment in N reduced the PGM, dropping from BRL 2,318.17 ha^{-1} (without urea) to BRL 2,124.64 and BRL 1,964.83 ha^{-1} with 75 and 150 kg ha^{-1}, respectively. In this scenario, the additional fertilizer cost was not offset by a proportional increase in revenue.

In 2025, on the other hand, the productivity gain associated with urea rate application resulted in a numerical increase in PGM, from BRL 3,054.13 ha^{-1} in the absence of urea to BRL 3,517.27 ha^{-1} with 150 kg ha^{-1}. Thus, under the prices considered in this experiment, the yield response to urea was sufficient to offset the additional fertilizer cost.

In Experiment 2 (2025), the *A. brasilense* treatments showed numerical differences in PGM, with mean values of BRL 3,196.23, BRL 3,414.99, and BRL 3,357.31 ha^{-1} for the control, 4 L ha^{-1}, and 8 L ha^{-1} treatments, respectively. In Experiment 1 (2024), the highest numerical PGM was obtained with 2 L ha^{-1} applied in the furrow + 2 L ha^{-1} at V4, in the absence of urea (BRL 2,444.39 ha^{-1}). These values should not be interpreted as a direct comparison of inoculation strategies

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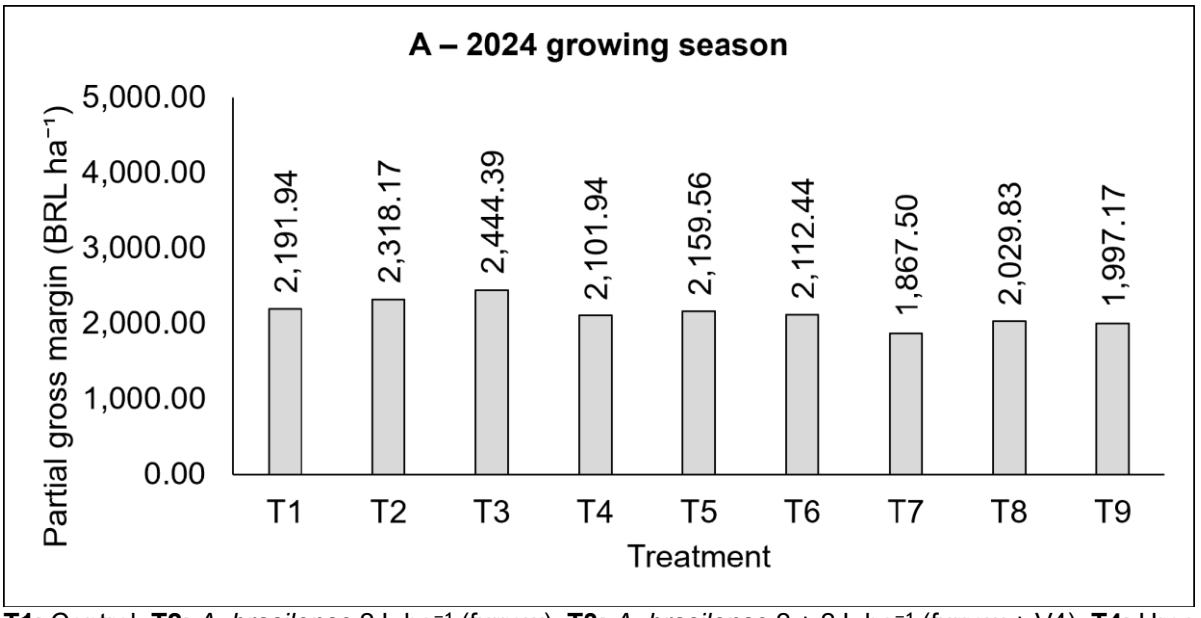
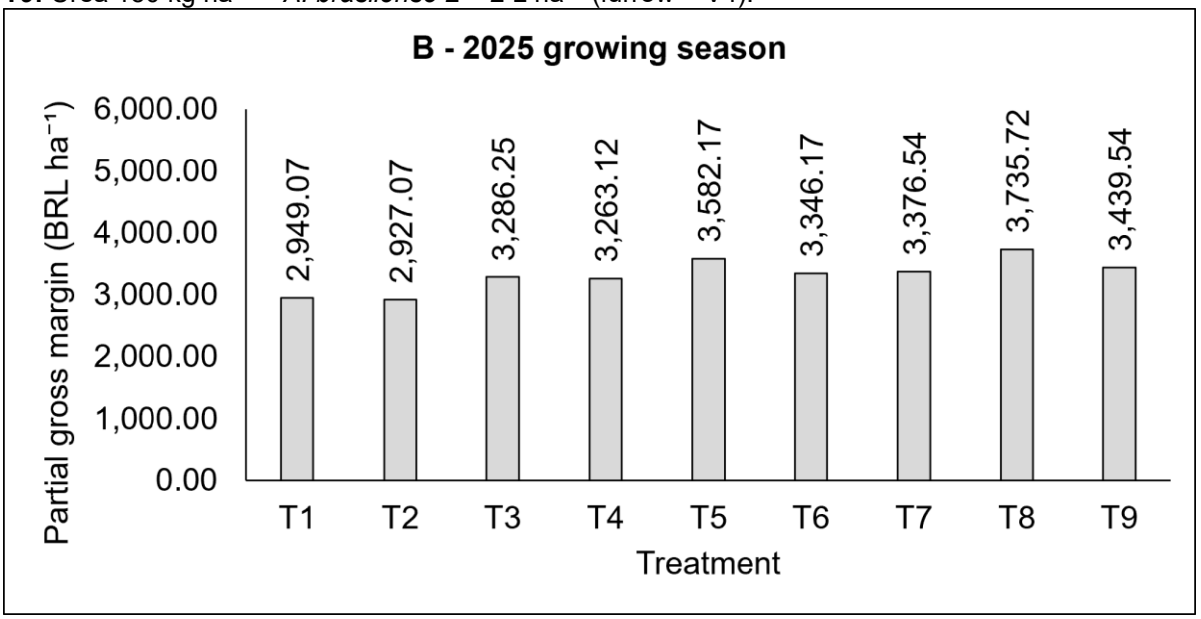
Vol: 13.20
DOI: 10.66104/vpaa8v69
Pages: 1-21

between experiments, because the application methods and rates differed between them.

Figure 4. Partial gross margin (PGM) based on urea and *A. brasilense* application strategies in canola crops during the 2024 and 2025 seasons, considering market value and the cost of products used in the treatments.



T1: Control; **T2:** *A. brasilense* 2 L ha^{-1} (furrow); **T3:** *A. brasilense* 2 + 2 L ha^{-1} (furrow + V4); **T4:** Urea 75 kg ha^{-1}; **T5:** Urea 75 kg ha^{-1} + *A. brasilense* 2 L ha^{-1} (furrow); **T6:** Urea 75 kg ha^{-1} + *A. brasilense* 2 + 2 L ha^{-1} (furrow + V4); **T7:** Urea 150 kg ha^{-1}; **T8:** Urea 150 kg ha^{-1} + *A. brasilense* 2 L ha^{-1} (furrow); **T9:** Urea 150 kg ha^{-1} + *A. brasilense* 2 + 2 L ha^{-1} (furrow + V4).



T1: Control; **T2:** *A. brasilense* 4 L ha^{-1}; **T3:** *A. brasilense* 8 L ha^{-1}; **T4:** urea 75 kg ha^{-1}; **T5:** urea 75 kg ha^{-1} + *A. brasilense* 4 L ha^{-1}; **T6:** urea 75 kg ha^{-1} + *A. brasilense* 8 L ha^{-1}; **T7:** urea 150 kg ha^{-1}; **T8:**

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Vol: 13.20
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Pages: 1-21

urea 150 kg ha^{-1} + *A. brasilense* 4 L ha^{-1}; **T9:** urea 150 kg ha^{-1} + *A. brasilense* 8 L ha^{-1}.

The relationship between productivity and economic returns was also reported by Khakbazan et al. (2021), who found wide variation in canola yield and economic returns across locations and years. Ma et al. (2026) likewise highlighted that determining economically appropriate N rates must simultaneously consider yield response, nutrient availability and environmental conditions.

In the present study, this relationship differed between the two independent experiments: in 2024, the lack of a yield response to N meant that increased fertilization reduced the partial gross margin, whereas in 2025, the yield increase offset the additional cost of urea. These results reinforce that nitrogen management must jointly consider yield response, environmental conditions, and input costs, while the use of *A. brasilense* showed no statistically significant effect on yield, and its numerical differences in partial gross margin were not sufficient to support an economic advantage.

These results should be interpreted considering the prices adopted in each experiment, as changes in grain and input prices may alter the partial gross margin associated with each treatment.

4. Conclusion

Canola's response to urea application differed between the two independent field experiments. Urea rates did not affect growth variables or grain yield in the 2024 experiment, whereas in the 2025 experiment, grain yield showed a linear response to increasing urea rates up to 150 kg ha^{-1}.The *Azospirillum brasilense* application strategies did not significantly affect the growth variables evaluated in the 2024 experiment or grain yield in either experiment. Growth variables were not evaluated in the 2025 experiment. A descriptive economic analysis indicated that increasing the urea rate did not necessarily result in a higher partial gross margin, highlighting the importance of considering environmental conditions, yield response, and input costs when evaluating nitrogen management. These findings should be interpreted within the specific conditions of the study, including the location in São Borja, RS, the Diamond hybrid, and the environmental conditions of the 2024 and 2025 experiments,

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Vol: 13.20
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Pages: 1-21

and should not be extrapolated directly to other locations, hybrids, or growing conditions.

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Vol: 13.20
DOI: 10.66104/vpaa8v69
Pages: 1-21

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