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Effect of biostimulants and plant population densities on soybean yield components

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ABSTRACT

This study aimed to analyze the effects of applying to analyze the effects of applying the biostimulants Stimulate® and Fertiactyl® and varying plant population densities on canopy closure, yield components, and grain yield in soybean crops. The experiment was conducted during the 2014/2015 growing season at the State University of Mato Grosso do Sul – Aquidauana University Unit. A randomized block design in a 3 x 3 factorial scheme was used. The soybean cultivars SYN 9070 RR and AS 3610 IPRO were used, with treatments including seed treatment with Fertiactyl® and Stimulate®, and a control without biostimulant application, combined with plant populations of 100,000, 300,000, and 500,000 plants ha^{-1}. Canopy closure was assessed at the V3 and V6 growth stages. At the R8 stage, the yield components: fertile branches, number of pods, number of grains, and yield were measured. No significant difference was observed between the analyzed variables for yield components and yield across cultivars when growth stimulants were applied. However, plant population density significantly affected the yield components. This was especially true for the cultivar AS 3610 IPRO, which showed increased number of pods, number of grains, and yield at a population density of 300,000 plants ha^{-1}. The absence of bioestimulant effects on the cultivars is attributed to the favorable climatic conditions - temperature, precipitation distribution, and adequate soil fertility - which provided good plant development and may have masked the potential biostimulant responses.

Keywords: Plant population density, Plant growth stimulants, *Glycine max.*

Efeito de bioestimulantes e densidades populacionais nos componentes de rendimento da soja

RESUMO

O objetivo do trabalho foi analisar o efeito da aplicação dos bioestimulantes Stimulate®, Fertiactyl® e densidade populacional, analisando as respostas no fechamento das entrelinhas, nos componentes de rendimento e rendimento da cultura soja. O experimento foi realizado a campo na safra 2014/2015, na Universidade Estadual de Mato Grosso do Sul–Unidade Universitária de Aquidauana. Conduzido em delineamento de blocos casualizados em fatorial 3x3. Utilizando as cultivares SYN 9070 RR e AS 3610 IPRO, sendo realizado o tratamento de sementes com Fertiactyl®, Stimulate® e sem aplicação do bioestimulante, em populações de 100.000, 300.000 e 500.000 plantas ha^{-1}. Os parâmetros analisados foram o fechamento das entrelinhas nos estádios V3 e V6. Em R8 os componentes de rendimento ramos férteis, número de legumes, número de grãos e produtividade. Não houve diferença significativa entre as variáveis analisadas para os componentes de rendimento e produtividade nas cultivares quando realizado o tratamento com estimulantes de crescimento. Porém a densidade populacional foi significativa sobre os componentes de rendimento. Principalmente para a cultivar AS 3610 IPRO proporcionando incremento no número de legumes, número de grãos e produtividade na população de 300.000 plantas ha^{-1}. Em relação ao não efeito dos bioestimulantes nas cultivares está associado às boas condições climáticas, temperatura, distribuição das precipitações e fertilidade adequada do solo que proporcionaram o bom desenvolvimento das plantas, não sendo possível observar o efeito dos bioestimulantes.

Palavras-chave: Densidade populacional, Estimulantes de crescimento vegetal, *Glycine max*.

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1. Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important agricultural crops and commodities for the global economy. Its grains are an excellent source of protein concentrates and vegetable oil, utilized in the agribusiness sector for producing animal feed. These characteristics justify the financial and technological investment in expanding the crop's production (Matos and Caires, 2022).

Soybean cultivation in Brazil has shown increasing production. For the 2024/2025 season, a production of 168.3 million tons was projected, representing an increase of 14.25% compared to the 2023/2024 season, which yielded 147.3 million tons of grain. The average yield for 2024/2025 is 3,614 kg ha^{-1}, resulting in 60.23 sc ha^{-1} (Conab, 2025). Improved crop management, combined with genetic breeding and the use of synthetic or natural biostimulants applied via soil, foliage, or seed treatment, has enhanced the development of yield components, leading to increased productivity (Souza et al., 2023).

Biostimulants are mixtures of natural or synthetic organic compounds that can modulate and regulate morpho-physiological processes, promoting plant development by improving flowering, plant growth, fruiting, crop yield, as well as increasing nutrient use efficiency and enhancing tolerance to a wide range of abiotic stresses (Campos et al., 2020).

Under unfavorable edaphoclimatic conditions, the use of biostimulants can improve yield. Satisfactory results have been achieved when plants under stress were sprayed with stimulants, leading to greater development of yield components and increased productivity (Souza et al., 2023).

For soybeans, biostimulants such as Aminospeed Raiz®, Stimulate®, Ultraseed®, and Nobrico Super CoMo® are recommended for seed treatment (Santini et al., 2015; Hermes et al., 2015). The use of biostimulants in this crop has been shown to increase the number of pods and number of grains in seed treatment and foliar applications, resulting in a yield increase (Bertolin et al., 2010).

However, the results are not conclusive regarding the method of application and the timing for using these products relative to the crop's development stage and their effect on yield components and the consequent yield increase. Therefore, the objective of this study was to analyze the effect of applying the stimulants Stimulate® and Fertiactyl® at different plant population densities on canopy closure, yield components, and soybean yield.

2. Material and Methods

The experiment was conducted in the plant production area of the State University of Mato Grosso do Sul, Aquidauana University Unit (UEMS/UUA), located at latitude 20º20’S, longitude 55º48'W, and an average altitude of 178 m, within the Pantanal Biome. The region's climate, according to the Köppen classification, is Aw type (Tropical with a dry winter season and summer rains), with an average annual precipitation of 1,200 mm, and average temperatures of 33 ºC maximum and 19.6 ºC minimum. The soil was classified as a Distrophic Red-Yellow Argisol. A chemical analysis of the soil at 0-0.20 m was performed (Table 1), and temperature and precipitation data were collected during the crop's development (Figure 1).

The conventional tillage system was used, the soil was tillage at 0.3 m depth with disc harrows with 50 cm. No corrective or base fertilization was necessary. Sowing was carried out manually on November 15, 2014. The seeds were treated with Standak Top® at a rate of 200 mL of commercial product per 100 kg of seeds and inoculated with *Bradyrhizobium*.

During the experiment, cultural practices followed the recommendations for pest management by Staback et al. (2020), with three applications of the insecticide Nomolt® for caterpillar control and three applications of the insecticide Engeo Pleno® for stink bug control. For disease control, according to the methodology of Godoy et al. (2021), three applications of the fungicides Priori Xtra®, Nativo®, and Fox® were performed. Weed control followed the methodology of Martin et al. (2022), using the herbicide Round Up® once.

The cultivars used were SYN 9070 RR, which has an indeterminate growth habit, maturity group 7, and a medium cycle of 130 to 135 days, recommended for the northern regions of Paraná, Goiás, and southern Mato Grosso do Sul; and AS 3610 IPRO, with maturity group 6.1, a super early cycle, a medium cycle of 125 days, and an indeterminate growth habit, recommended for the West of Paraná, southern Mato Grosso do Sul, and West of São Paulo.

The experimental design was a randomized block design, totaling three blocks containing 27 experimental units, each consisting of five rows 3 m long, with a spacing of 0.45 m, in a factorial scheme of three populations (100,000, 300,000, and 500,000 plants ha^{-1}) and the application of Fertiactyl® (micronutrients and organic carbon), Stimulate® (Kinetin 0.009%, gibberellic acid 0.005%, and indole-3-butyric acid 0.005%) and no biostimulant application. The biostimulants Stimulate® and Fertiactyl® were applied as a seed treatment at the manufacturer's recommended doses of 7.5 and 2 mL kg^{-1} of seed, respectively.

Table 1. Chemical soil analysis of the experimental area in Aquidauana (MS) (latitude 20º 20'S, longitude 55º 48'W), at 0-0.20 m depth, October 2014.

pH	O.M.	P	H+Al	Al	Ca	Mg	Na	K	SB	CTC	V%
-	($g.dm^{-3}$)	($mg.dm^{-3}$)	($cmol_c dm^{-3}$)								
5.3	62.9	70.2	3.2	0.0	5.3	1.2	-	0.4	6.9	10.1	68.5

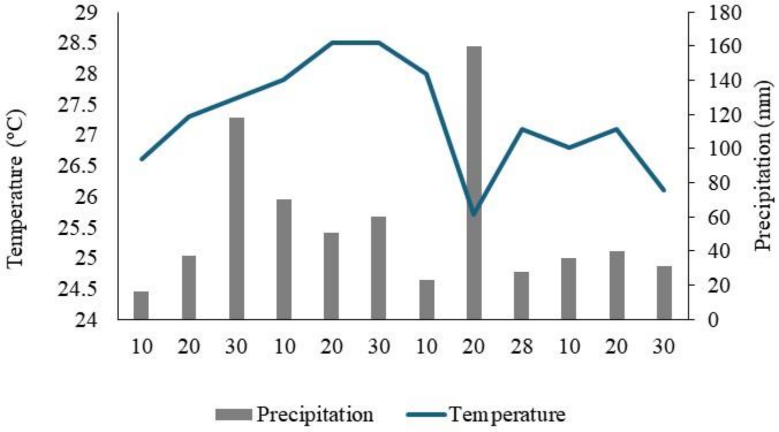

Figure 1. Mean temperature and precipitation data in Aquidauana-MS from December to March in 2014/2015. (Aquidauana-MS).

The germination rate of the cultivars used was 80%. Thus, sowing was adjusted so that the viable seed capacity per hectare corresponded to the tested populations: 125,000, 375,000, and 625,000 viable seeds ha^{-1} for the populations of 100,000, 300,000, and 500,000 plants ha^{-1}, respectively. These populations were chosen because the average range of less dense or more dense plant populations used in commercial fields follows this pattern.

Canopy closure was evaluated at the V3 and V6 growth stages by measuring, in each experimental unit, the distance between the plant base of one row and the other, and the distance between the projections of the aerial parts (canopy). Ten evaluations were performed in each experimental unit. Canopy closure was expressed as a percentage calculated using Equation (1):

$$\text{Canopy closure(\%)}=\left(1-\frac{\text{Distance between canopies}}{\text{Distance between rows}}\right)\times 100 \qquad \text{(Eq. 1)}$$

At the R8 stage, the two central rows of each plot were harvested over a 1 m linear length, and the number of fertile branches, number of pods, and number of grains were measured. Grain yield was estimated using Equation (2) (Solano and Yamashita, 2011; Komatsu et al., 2010; Rambo et al., 2002):

$$\text{Grain Yield }\left(\text{kg ha}^{-1}\right)=\frac{\text{grains nº}\times 100\text{-grain weight(g)}\times 10{,}000}{\text{Area harvested(m}^2)\times 1{,}000}$$

(Eq.2)

Data were subjected to analysis of variance (ANOVA), and means were compared using the Tukey test at 5% significance. For plant populations, regression analysis was performed when appropriate. Each cultivar was analyzed separately, considered as a distinct experiment. Principal component analysis (PCA) was applied to the matrix formed by data of canopy closure, number of branches, pods, grains, and yield, and the eighteen combinations of biostimulants and plant population, and cultivars. The similarity between the data was analyzed by clustering using the k-means method with PROC Cluster and PROC Tree in SAS 9.3 software (SAS Institute Inc. Cary, NC, USA).

3. Results and Discussion

The soil nutritional levels in the experimental area met the requirements for soybean development (Table 1) (Souza et al., 2016). Key factors such as base saturation met the crop's demand, exceeding 50%; phosphorus was above 12 mg dm^{-3}, the adequate level for crop development in the Cerrado region; and potassium was above 0.08 cmol$_c$ dm^{-3}, as required for crop development in regional soils (Oliveira et al., 2023). Therefore, chemical correction with fertilizers or lime was unnecessary.

During soybean seed germination, water absorption equivalent to 50% of its weight is necessary for germination. In the subsequent phenological stages, there is a demand of 8 mm day^{-1} for crop development. During the experiment, from sowing to harvest, there was an accumulated rainfall of 700 mm over the entire cycle and daily average temperatures of 25 ºC and no shading over the crop, allowing for maximum utilization of solar radiation to achieve maximum soybean yield (Figure 1). These data align with those determined by Neumaier et al. (2020) for soybean cultivation, with air temperatures between 20 and 30 ºC; 25 ºC being the ideal value for rapid and uniform seedling emergence. For high productive potential, soybeans require 450 to 800 mm of water throughout their development cycle.

No significant effect of the biostimulants on canopy closure was observed in any of the evaluated cultivars at the V3 and V6 growth stages (Table 2).

Conversely, there was an effect of population ($p < 0.001$) in all evaluations. The average canopy closure was approximately 30% for both cultivars at the V3 stage. At the V6 stage, the values were higher, close to 55% for the cultivar SYN 9070 RR and 64% for the cultivar AS3610 IPRO, with no statistical difference between them. The effect of plant population on canopy closure was significant (Table 2) for both cultivars. In both cultivars, the population of 500,000 plants ha^{-1} showed the highest closure when compared to the population of 100,000 plants ha^{-1} at the V3 and V6 stages (Figure 2). These results are similar to those found by Moro et al. (2021) and Menegon et al. (2024), who observed that higher plant populations led to faster row closure compared to lower densities, an effect attributed to population densification. This response is likely associated with increased competition for light among plants, which favors the upward growth of branches toward the light. This is common in higher population densities, as reported by Xu et al. (2021).

Table 2. Effect of biostimulant application on canopy closure in soybean crops sown at different plant populations.

	Cultivar SYN 9070 RR (%)		**Cultivar AS 3610 IPRO (%)**	
Treataments	**V3**	**V6**	**V3**	**V6**
Fertactyl	30 a*	53 a	35 a	64 a
No application	31 a	52 a	35 a	64 a
Stimulate	32 a	56 a	37 a	64 a
F_{cal}.**Bioestimul.**	0.45 (p = 0.647)	0.48 (p = 0.063)	0.26 (p = 0.777)	0.09 (p = 0.910)
F_{cal}.**Population**	**11.86 (p <0.001)**	**15.25 (p <0.001)**	**10.11 (p <0.001)**	**11.23 (p <0.001)**
F_{cal}.**Bio. x Pop.**	0.37 (p = 0.830)	0.62 (p = 0.658)	0.88 (p = 0.499)	0.61 (p = 0.663)
F_{cal}.**Block**	2.08 (p = 0.157)	0.62 (p = 0.055)	3.09 (p = 0.073)	**8.58 (p = 0.002)**
CV (%)	16	18	15	12

*Means followed by the same letter do not differ statistically by the Tukey test at 5%; Bio.: Stimulants; Pop.: Population. Bold values are significant by the *F test* at 5%.

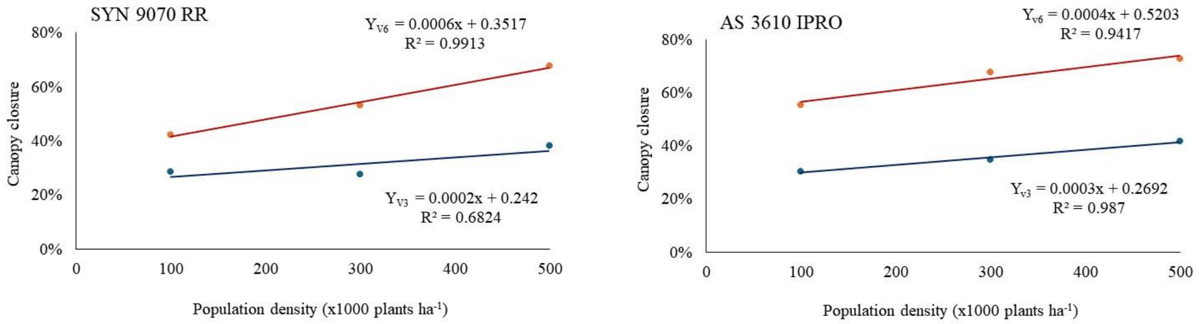

Figure 2. Canopy closure in soybean at the V3 (Blue) and V6 (Red) growth stages sown at different populations with biostimulant application.

The use of biostimulants Stimulate® and Fertactyl® in the cultivars SYN 9070 RR and AS 3610 IPRO did not increase yield components compared to the treatment without application (Table 3).

When comparing the effects of stimulants only in the cultivar SYN 9070 RR, the number of branches and number of pods using Stimulate® was higher than under Fertactyl®, however, this difference did not translate into higher yield, and both treatments were statistically similar to the non-application control. Similar results were observed by Araújo et al. (2021), who, when using biostimulants Proggib®, Stimulate®, and Biozyme® in soybean, reported an increase in the yield components: number of branches and pods, but also observed that yield gain was associated with genotypic differences among cultivars.

Regarding the number of pods, Mattos and Caires (2022) did not observe an increase in this yield component when using a soil biostimulant.

Similarly, Cavalcante et al. (2020), testing five biostimulants individually, found no statistical difference in the number of pods or the total number of grains compared with plants grown without a biostimulant.

Hermes et al. (2015), after seed treatment with the biostimulant Nobrico® Super CoMo®, observed no increase in the number of pods and number of grains of the soybean cultivar V-Max RR.

Table 3. Effect of plant stimulants on yield components in soybean crops at different plant populations.

Treataments	**Branches m^{-2}**	**Pods m^{-2}**	**Grains m^{-2}**	**Yield Kg ha^{-1}**
		Cultivar SYN 9070 RR		
Fertactyl	74.19 b*	1,545.80 b	3,072.59 a	3,803.40 a
No application	80.61 ab	1,770.61 ab	3,609.50 a	4,543.20 a
Stimulate	90.12 a	1,904.07 a	3,794.19 a	4,964.10 a
F_{cal}.**Bioestimul.**	3.65 (0.050)	**3.77 (0.045)**	2.40 (0.122)	2.85 (0.087)
F_{cal}.**Population**	**31.11 (0.001)**	0.84 (0.448)	0.76 (0.482)	0.21 (0.810)
F_{cal}.**Bio x Pop**	3.65 (0.800)	3.77 (0.153)	1.63 (0.122)	0.78 (0.554)
F_{cal}.**Block**	**6.69 (0.008)**	**3.77 (0.003)**	**5.33 (0.170)**	4.70 (0.554)
CV (%)	15	16	21	25
		Cultivar AS 3610 IPRO		
Fertactyl	59.26 a*	1,304.32 a	2,618.00 a	4,042.70 a
No application	48.40 a	1,265.56 a	2,604.70 a	4,034.20 a
Stimulate	54.69 a	1,205.80 a	2,424.01 a	3,709.40 a
F_{cal}.**Bioestimul.**	0.52 (p = 0.605)	0.51(p = 0.609)	0.58 (p = 0.569)	0.59 (p = 0.566)
F_{cal}.**Population**	**4.63 (p = 0.025)**	**4.51 (p = 0.028)**	**4.68 (p = 0.025)**	**4.98 (p = 0.020)**
F_{cal}.**Bio. x Pop.**	0.46 (p = 0.767)	0.45 (p = 0.772)	0.29 (p = 0.878)	0.17 (p = 0.949)
F_{cal}.**Block**	2.82 (p = 0.089)	1.86 (p = 0.187)	1.28 (p = 0.304)	0.83 (p = 0.452)
CV (%)	42	16	16	18

*Means followed by the same letter do not differ statistically by the Tukey test at 5%; Bio.: biostimulants; Pop.: Population. Bold values are significant by the F test at 5%.

Marques et al. (2014) also detected no increase in the number of grains with the use of a biostimulant based on *Sarganum spp.* algae extracts. Regarding yield, the use or non-use of biostimulants in both cultivars did not increase yield, as yield components remained unchanged in both cases. Hermes et al. (2015) and Araújo et al. (2021) suggested that the absence of response may be related to favorable environmental conditions, as temperature, precipitation, and photoperiod were satisfactory during the cultivation period and may have altered crop behavior, thereby influencing the results.

For both cultivars, the evaluated yield components - number of branches, number of pods, number of grains, and yield - were altered by the effect of the plant population (Figures 3 and 4) rather than by biostimulant application.

The number of branches was affected by plant population densities for the cultivar SYN 9070 RR (Figure 3), with the 100,000 plants ha^{-1} population yielding the highest values of 100 branches m^{-2}, and the 500,000 plants ha^{-1} population yielding the lowest values of 55 branches m^{-2}.

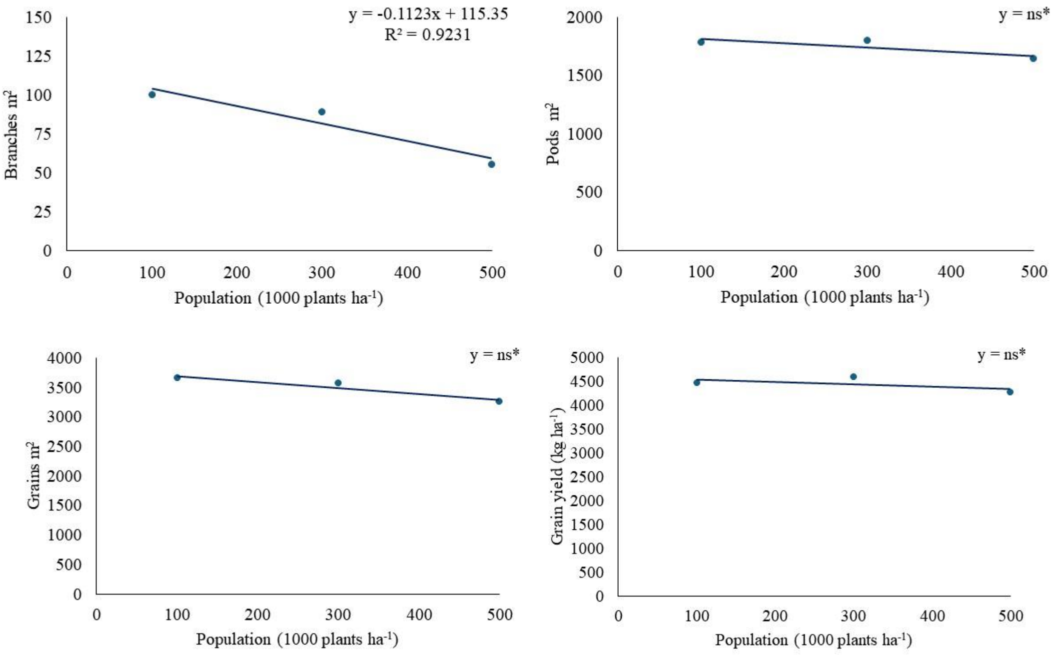

Figure 3. Soybean yield components as a function of plant population densities for the cultivar SYN 9070 RR. Means of two biostimulants and one non-application treatment.

It is important to note that soybeans can alter their architecture in response to competition or to compensate for population losses. As noted by Xu et al. (2021), light competition in denser populations favors accelerated vertical growth, contrasting with observations by Menegon et al. (2024) in less dense populations.

The other yield components on cultivar SYN 9070 RR were not affected by the population effect; the number of pods showed an average of 1,800 pods m^{-2}, and number of grains averaged value of 3,500 grains m^{-2}. Consequently, the yield for this cultivar did not change with population, resulting in a linear adjustment (Figure 3). Similar results were obtained by Moro et al. (2021), who found that varying population densities of cultivar Monsoy 8374 did not influence the number of branches, grains, thousand-grain weight, or yield, although pod number per plant decreased. The authors explain that, regardless of population density, plant architecture and resource utilization remained unchanged.

Plant population had a significant effect on branches for the cultivar AS 3610 IPRO (Figure 4). At a population of 100,000 plants ha^{-1}, values of 61 branches m^{-2} were obtained compared to the 500,000 plants ha^{-1} population, where the number of branches was 35 m^{-2}. However, the 300,000 plants ha^{-1} population yielded 65 branches m^{-2}. In this same population, the number of pods was 1,428 pods m^{-2}, and the number of grains was 1,428 grains m^{-2}. Regarding yield, the 300,000 plants ha^{-1} population provided the highest yield with 4,551 kg ha^{-1}, which followed a quadratic response pattern (Figure 4).

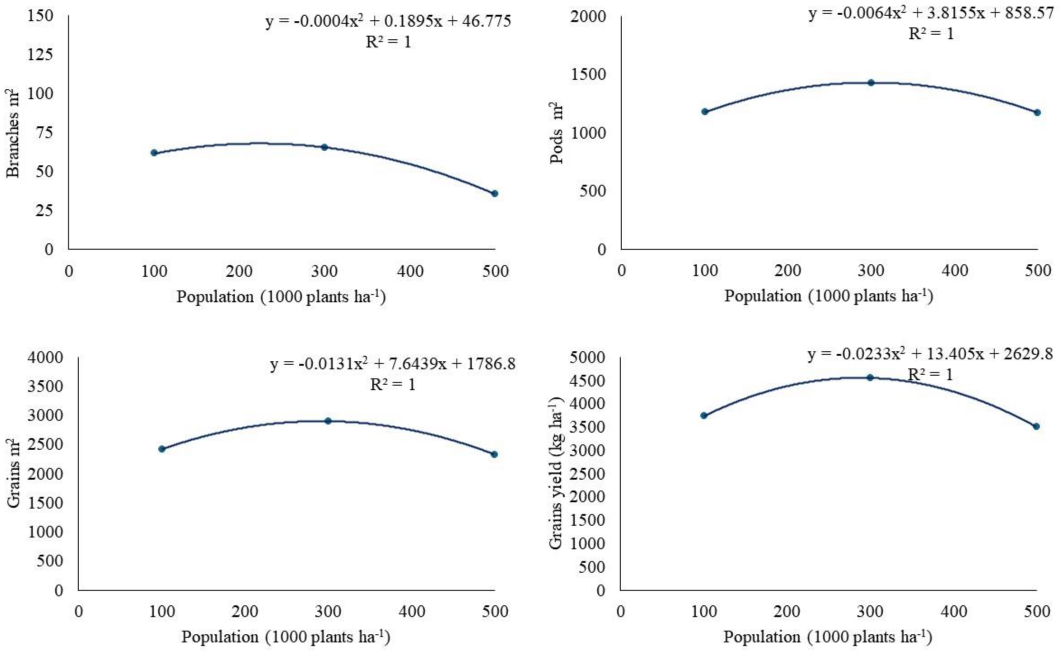

Figure 4. Yield components as a function of plant population densities for the cultivar AS 3610 IPRO. Means of two biostimulants and one non-application treatment.

The population effect for AS 3610 IPRO was similar to that observed by Menegon et al. (2024), who found that a population density of 250,000 plants ha^{-1} resulted in better yields in pod number, grain number, and yield for cultivar 98R30 CE. The authors explain that higher yields at lower densities are related to improved plant development and compensation capability, a response favored by favorable climatic conditions, including adequate temperature and well-distributed precipitation during crop development, enabling maximum phenotypic expression and supporting plant growth.

Principal component analysis (PCA) (Figure 5) showed that the first two components accounted for 86.62% of the total variance, with Component 1 explaining 59.58% and Component 2 explaining 27.04%. These components captured the main effects of population density on canopy closure, yield component formation, and overall yield variation.

The biplot in Figure 5 shows that Component 1 had a greater influence on the number of branches, number of pods, number of grains, and yield, while its effect on canopy closure was minimal. In contrast, Component 2 was more strongly associated with canopy closure at growth stages V3 and V6.

The three population densities were distinctly plotted in the two-dimensional space of the PCA, indicating that they promoted divergent effects on the composition of canopy closure, yield components, and soybean yield. Clear group formation and direct correlations were observed based on the measured variables.

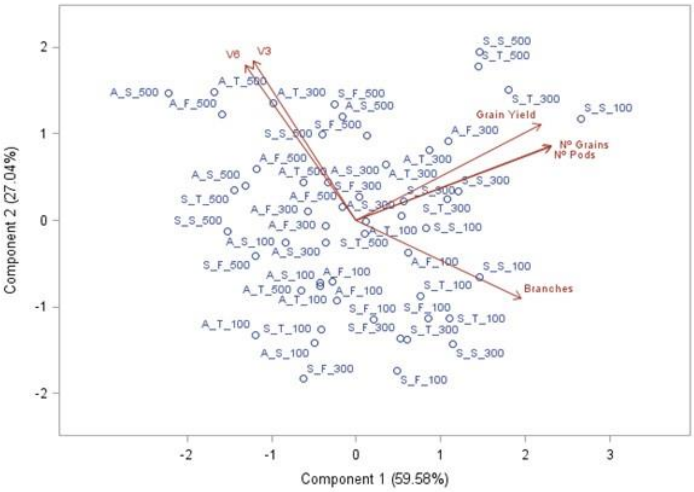

Figure 5. Two-dimensional dispersion of population densities, biostimulants, and cultivars according to the scores of the principal components, obtained with six biometric variables (Biplot Component 1 × Component 2: 86.6%).

The 500,000 plants ha^{-1} population had a direct correlation with canopy closure at the V3 and V6 growth stages. For the 100,000 plants ha^{-1} population, a direct correlation is observed in the formation of the number of branches. The components number of pods, number of grains, and yield have a direct correlation with the 300,000 plants ha^{-1} population.

Hierarchical clustering using the Ward k-means method formed three distinct groups from the dataset (Figure 6).

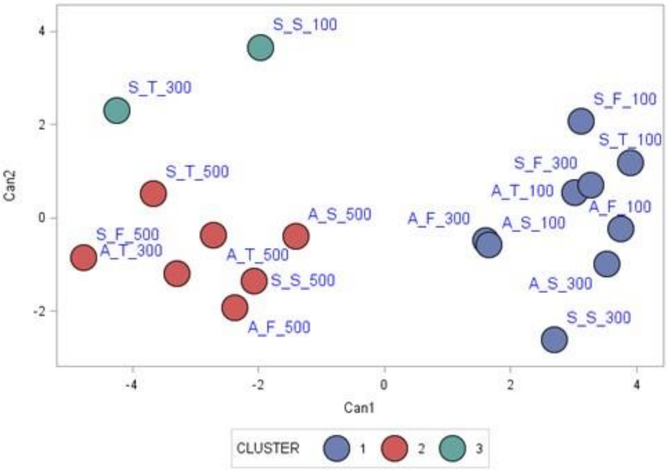

Figure 6. K-means clustering of the combination of soybean cultivars sown at different populations with the use of different Growth Stimulants. A (AS 3610 IPRO); S (SYN 9070 RR); T (without stimulant application); F (Fertiactil); S (Stimulate); 100, 300, and 500 = plant populations (× 1,000 plants ha^{-1}).

The 500,000 plants ha^{-1} population had a direct correlation with canopy closure at the V3 and V6 growth stages. For the 100,000 plants ha^{-1} population, a direct correlation is observed in the formation of the number of branches. The components number of pods, number of grains, and yield have a direct correlation with the 300,000 plants ha^{-1} population.

Hierarchical clustering using the Ward k-means method formed three distinct groups from the dataset (Figure 6). This analysis indicated that the treatments involving biostimulants were similar to each other but differed from the three plant population densities regarding canopy closure, yield component formation, and yield.

4. Conclusions

For the AS 3610 IPRO cultivar, the plant population density of 300,000 plants ha^{-1} resulted in an increase in the number of pods, number of grains, and yield.

In the SYN 9070 RR cultivar, the lowest population density resulted in a higher number of fertile branches.

The growth stimulants Fertiactyl® and Stimulate®, applied to the AS 3610 IPRO and SYN 9070 RR cultivars under the edaphoclimatic conditions of the study site and year, did not exert significant effects on the development of yield components or grain yield. Favorable environmental conditions likely supported crop development regardless of stimulant application.

Additional field studies are recommended, particularly those involving seed treatment and a broader range of cultivars and biostimulants, to better understand their potential effects under varying environmental conditions.

Authors' Contribution

Conceptualization and methodology: Felipe André Sganzerla Graichen. Data collection and curation: Felipe André Sganzerla Graichen, Cristiano Moreira. Statistical analysis: Felipe André Sganzerla Graichen. Data interpretation and writing: Vittor Gomes Cavalcanti, Neder Henrique Martinez Blanco, Felipe André Sganzerla Graichen, Cristiano Moreira. Original draft preparation and reviewing: Vittor Gomes Cavalcanti, Cristiano, Neder Henrique Martinez Blanco. Review and editing: Vittor Gomes Cavalcanti, Neder Henrique Martinez Blanco, Felipe André Sganzerla Graichen, Cristiano Moreira. All authors read and approved the final version of the manuscript.

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