# Seed treatment with phytosanitary products and interaction with pre-inoculation in soybean

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## ABSTRACT

Pre-inoculation offers logistical advantages for soybean cultivation by eliminating on-farm inoculation, but little is known about its interaction with phytosanitary products. This study evaluated the effect of seed treatment with pesticides combined with pre-inoculation performed 10 days before sowing, without bacterial protectant, on soybean nodulation and yield. Field trials were conducted in six locations in Paraná and Mato Grosso do Sul states using a randomized block design with five treatments: (1) absolute control (no inoculation or nitrogen fertilization); (2) mineral nitrogen control (200 kg N $ha^{-1}$); (3) standard inoculation with Bradyrhizobium spp. at sowing; (4) pre-inoculation with Bradyrhizobium japonicum + insecticides (imidacloprid + thiodicarb); and (5) pre-inoculation with B. japonicum + fungicide (fludioxonil + metalaxyl-M) + insecticide (thiamethoxam). Parameters assessed included nodulation, plant biomass, nitrogen accumulation in shoots and grains, and yield. Pre-inoculation with pesticides promoted nodulation and productivity comparable or superior to standard inoculation. Across all sites, pre-inoculated treatments outperformed the absolute control, and in most sites they also surpassed mineral nitrogen fertilization. The results demonstrate that pre-inoculation carried out 10 days before sowing, in combination with chemical seed treatment, is an effective strategy to enhance nodulation and yield in soybean cultivation.

***Keywords:*** *Glycine max, pre-inoculation, seed treatment, Bradyrhizobium japonicum, biological nitrogen fixation, bioinputs, crop yield, pesticides.*

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## Highlights of this paper

- Pre-inoculation with liquid Bradyrhizobium japonicum maintains nodulation and nitrogen fixation.
- Yields match or exceed those achieved through traditional inoculation and mineral nitrogen fertilization methods.
- Enhances soybean productivity while reducing mineral nitrogen use and associated costs.

## 1. INTRODUCTION

Considered one of the most important crops in global agriculture, soybean [Glycine max (L.) Merrill] is the primary source of protein for animal feed, while its grains are also rich in lipids, which are used in oil production. In the 2022/23 harvest, Brazil planted slightly over 44 million hectares 6.2% more than in 2021/22 achieving a record production of 154.6 million tons, setting historic highs in planted area, productivity, and total output. In the same harvest, estimated soybean exports exceeded 96 million tons [1].

One of the main factors influencing crop success is the use of high-quality seeds. Maintaining the physiological quality of seeds and minimizing phytosanitary issues can be achieved through seed treatment. This process, which involves applying various products such as chemical pesticides, inoculants, and micronutrients, plays a critical role in crop performance by protecting the seeds and promoting robust early growth, even under adverse conditions [2].

Nitrogen is the most demanded nutrient in soybean cultivation, with approximately 80 kg required to produce 1 ton of grain. Adequate nitrogen supply is essential for physiological processes such as root development, photosynthesis, and assimilate translocation, directly affecting crop growth and yield. Biological nitrogen fixation through inoculation with *Bradyrhizobium japonicum* can supply a significant portion of this requirement, reducing dependence on mineral nitrogen fertilization [3, 4].

As an essential nutrient, its deficiency or imbalance can adversely affect several physiological processes, including root development, photosynthesis, the production and translocation of photoassimilates, and the growth dynamics between leaves and roots [5]. Biological nitrogen fixation (BNF) through inoculation with Bradyrhizobium bacteria via seed treatment can supply a significant portion of the nitrogen required throughout the crop cycle. This practice also reduces production costs by decreasing the need for mineral nitrogen fertilization. Furthermore, inoculation can be applied industrially through a technique called pre-inoculation, in which seeds are delivered to producers already coated with the bacteria, eliminating the need for on-farm inoculation.

The effectiveness of pre-inoculation can be influenced by several factors, including interactions with chemical treatments, which may reduce nodulation parameters [6]. Consequently, further research on the combination of inoculants and pesticides is warranted. This study aimed to evaluate the effects of seed treatment with pesticides and its interaction with pre-inoculation applied 10 days before sowing, without the use of a bacterial protectant, on nodulation parameters and soybean yield.

## 2. MATERIALS AND METHODS

This study was conducted during the 2022/23 harvest season across six experimental sites located in the municipalities of Mandaguaçu, Marialva, Planaltina do Paraná, Munhoz de Melo, and Paranavaí in Paraná, and Itaquiraí in the southern region of Mato Grosso do Sul, Brazil.

The predominant climate in northern Paraná and southern Mato Grosso do Sul is classified as Cfa (humid mesothermal, with abundant summer rainfall, dry winters, and hot summers) according to Köppen. Climate data for the six locations from October 2022 to March 2023 are presented in Figure 1.

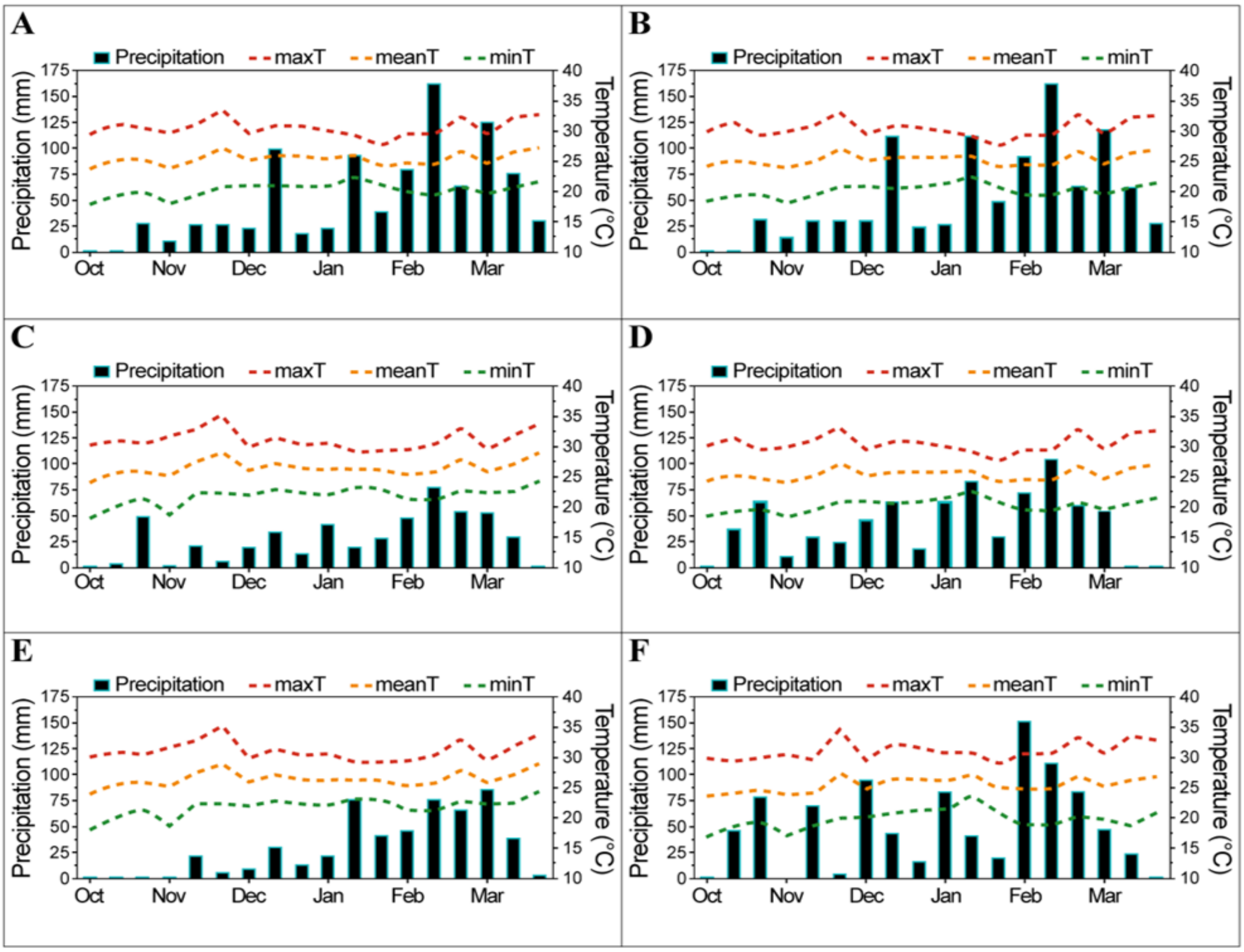


**Figure 1.** Climate data, in ten days, of precipitation and maximum, average, and minimum temperatures, from October 2022 to March 2023, for the municipalities of Mandaguaçu/PR (A), Marialva/PR (B), Planaltina do Paraná/PR (C), Munhoz de Melo/PR (D), Paranavaí/PR (E), and Itaquiraí/MS (F).

The experimental design was a completely randomized block design (CRD) comprising five treatments: CA - Absolute control: without inoculation and without nitrogen fertilization; CN - Mineral nitrogen control (200 kg N ha$^{-1}$) without inoculation; IPS - Standard inoculation with *Bradyrhizobium* spp. at sowing; PII - Pre-inoculation with *Bradyrhizobium japonicum* + insecticides (active ingredients: Imidacloprid 150 g L$^{-1}$ + Thiodicarb 450 g L$^{-1}$); PIFI - Pre-inoculation with *Bradyrhizobium japonicum* + fungicide (active ingredients: Fludioxonil 25 g L$^{-1}$ + Metalaxyl-m 37.5 g L$^{-1}$) + insecticide (active ingredient: Thiamethoxam 350 g L$^{-1}$).

The CA, CN, and IPS treatments did not receive fungicides or insecticides. All inoculated treatments received foliar applications of cobalt (Co) and molybdenum (Mo) at the V4 vegetative stage, at doses of 2.5 g Co ha$^{-1}$ and 20 g Mo ha$^{-1}$, respectively. The liquid inoculant contained *Bradyrhizobium japonicum* SEMIA 5079 and SEMIA 5080 at $7\times10^{9}$ CFU mL$^{-1}$.

Seeds were treated using an Arktos L2K BM machine ('Momesso Indústria de Máquinas Ltda.'), which replicates industrial seed treatment procedures. Seeds were first treated with pesticides and, after drying, received the liquid inoculant. Treated seeds were then stored in the laboratory at a constant temperature (23°C) and relative humidity (70%) for 10 days.

Experimental units consisted of nine 6-m-long rows with 0.45-m row spacing. Harvested areas comprised the four central rows, excluding 1 m from each end. Seeding density was 14 seeds per meter, targeting an established stand of 13 plants per meter, resulting in a final population of approximately 288 plants per hectare, in accordance with Embrapa [7] and the seed provider's guidelines. The cultivar used was BMX FIBRA IPRO (Brasmax Genética), featuring Intacta RR2 PRO® technology, an indeterminate growth habit, and early maturity.

A no-till system was employed at all sites, following chemical and physical soil analyses. Crop establishment and management adhered to Embrapa Soybean Production Technologies recommendations [8] for microregions 201 and 202 (northwest Paraná and southern Mato Grosso do Sul). Sowing occurred between October 16 and November 4, and harvesting between March 3 and 28. Crop cycles ranged from 137 to 151 days, with harvesting performed at 18% grain moisture. Further details on sowing and harvest dates, as well as crop cycle duration by site, are presented in Table 1.

**Table 1.** Sowing, harvesting, and total crop cycle dates in the experimental areas.

| Location | Sowing date | Harvest date | Crop cycle (Days) |
|---|---|---|---|
| Mandaguaçu/PR | 26/10/2022 | 17/03/2023 | 142 |
| Marialva/PR | 24/10/2022 | 18/03/2023 | 145 |
| Planaltina do Paraná/PR | 17/10/2022 | 03/03/2023 | 137 |
| Munhoz de Melo/PR | 23/10/2022 | 19/03/2023 | 147 |
| Paranavaí/PR | 04/11/2022 | 28/03/2023 | 144 |
| Itaquiraí/MS | 16/10/2022 | 04/03/2023 | 151 |

At sowing, all areas received 78 kg ha$^{-1}$ of $P_2O_5$ in the form of ammonium monophosphate and 78 kg ha$^{-1}$ of $K_2O$ as potassium chloride, applied at the V3 stage [9]. Plots were established using manual seeders. Phytosanitary management was consistent across all six experimental sites. From sowing to harvest, the following characteristics were evaluated:

Number of nodules per plant: assessed at the beginning of flowering (R1 stage) in five plants randomly selected from each plot.

Dry mass of nodules: nodules were counted and dried in a forced-air oven at 65°C until a constant mass was achieved.

Dry mass of shoots and roots: determined at the R1 stage in five randomly selected plants per plot, excluding rows designated for yield assessment. Plants were placed in paper bags and dried in a forced-air oven at 65°C until constant mass was reached.

Total nitrogen content in shoots and grains: analyzed by a partner laboratory.

Grain yield is determined by harvesting all plants within the usable area of each experimental unit. Grain moisture was then measured according to the methodology described in the Rules for Seed Analysis [10], and yields were corrected to 13% moisture content.

The data were initially tested for normality and homoscedasticity using the Shapiro-Wilk and Box-Cox tests, respectively, and transformed when necessary. Analysis of variance (ANOVA) was then performed using the F-test ($p \leq 0.05$). When significant differences were detected, means were compared using Tukey's test at the same significance level, using R software (R Core Team, 2020).

## 3. RESULTS AND DISCUSSION

Tables 2-4 present the results of plant nodulation and related performance parameters, including the number and dry mass of nodules (NN and DMN), dry mass of shoots and roots, and thousand-grain weight (DMA, DMR, and TGW), as well as total nitrogen content in shoots and grains (TNA and TNG). These variables were evaluated in response to pre-inoculation treatments with Bradyrhizobium japonicum combined with different pesticides applied via seed treatment.

**Table 2.** Nodulation parameters as a function of seed treatment.

| Trat. | Mandaguaçu/PR | | Marialva/PR | | Planaltina do Paraná/PR | |
|---|---|---|---|---|---|---|
| | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) |
| CA | 27.8 cd | 761.2 cd | 16.3 bc | 619.4 a | 0.1 c | 5.4 b |
| CN | 20.3 d | 708.8 d | 9.2 c | 174.8 b | 0.6 c | 48.2 b |
| IPS | 35.2 bc | 988.4 bc | 23.7 b | 771.0 a | 6.9 a | 384.6 a |
| PII | 43.4 ab | 1133.4 b | 20.1 b | 678.6 a | 2.5 b | 256.2 a |
| PIFI | 49.2 a | 1656.4 a | 41.0 a | 1207.4 a | 7.2 a | 354.4 a |
| Mean | 35.2 | 1049.6 | 22.1 | 690.2 | 3.5 | 209.8 |
| V.C. (%) | 12.8 | 2.2 | 21.4 | 9.1 | 50.8 | 32.5 |
| Trat. | Munhoz de Melo/PR | | Paranavaí/PR | | Itaquiraí/MS | |
| | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) | NN (unid. pl$^{-1}$) | DMN (mg pl$^{-1}$) |
| CA | 11.9 cd | 448.6 bc | 5.0 cd | 137.6 cd | 13.3 bc | 515.2 b |
| CN | 6.8 d | 192.6 c | 3.2 d | 65.6 d | 8.6 c | 315.2 c |
| IPS | 15.6 bc | 579.6 b | 8.5 c | 265.2 bc | 17.3 ab | 668.0 ab |
| PII | 31.4 a | 1072.2 a | 24.3 a | 924.8 a | 18.7 a | 733.8 a |
| PIFI | 24.2 ab | 943.6 a | 14.5 b | 499.4 ab | 21.8 a | 779.2 a |
| Mean | 18.0 | 647.3 | 11.1 | 378.5 | 15.9 | 602.3 |
| V.C. (%) | 18.8 | 25.6 | 16.1 | 11.7 | 15.5 | 14.1 |

Means followed by the same letter in the column do not differ significantly according to Tukey's test at a 5% probability level. Unit pl$^{-1}$: unit per plant; mg pl$^{-1}$: milligrams per plant; g pl$^{-1}$: grams per plant.

**Table 3.** Dry mass and thousand-grain weight parameters as a function of seed treatment.

| | Mandaguaçu/PR | | | Marialva/PR | | |
|---|---|---|---|---|---|---|
| Trat. | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) |
| CA | 80.2 c | 13.6 cd | 98.9 d | 619.4 a | 0.1 c | 5.4 b |
| CN | 80.5 c | 12.8 d | 103.9 cd | 174.8 b | 0.6 c | 48.2 b |
| IPS | 101.6 b | 16.1 bc | 109.0 bc | 771.0 a | 6.9 a | 384.6 a |
| PII | 115.1 b | 17.1 b | 114.7 ab | 678.6 a | 2.5 b | 256.2 a |
| PIFI | 144.1 a | 19.8 a | 118.7 a | 1207.4 a | 7.2 a | 354.4 a |
| Mean | 104.3 | 15.9 | 109.1 | 690.2 | 3.5 | 209.8 |
| V.C. (%) | 10.4 | 14.2 | 2.9 | 9.1 | 50.8 | 32.5 |
| | Planaltina do Paraná/PR | | | Munhoz de Melo/PR | | |
| Trat. | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) |
| CA | 29.3 b | 7.5 c | 133.4 b | 47.5 d | 8.7 bc | 150.7 d |
| CN | 77.9 a | 11.8 bc | 137.9 ab | 62.2 cd | 6.2 c | 165.2 c |
| IPS | 58.8 a | 16.4 a | 148.3 a | 76.9 bc | 12.1 bc | 171.5 bc |
| PII | 47.7 a | 14.5 ab | 141.2 a | 121.0 a | 18.7 a | 184.2 a |
| PIFI | 55.6 a | 15.1 ab | 148.2 a | 89.1 ab | 13.6 ab | 176.8 b |
| Mean | 53.9 | 13.1 | 141.8 | 79.3 | 11.9 | 169.7 |
| V.C. (%) | 7.8 | 17.1 | 23.9 | 5.7 | 26.1 | 12.5 |
| | Paranavaí/PR | | | Itaquiraí/MS | | |
| Trat. | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) | DMA (g pl$^{-1}$) | DMR (g pl$^{-1}$) | TGW (g) |
| CA | 31.4 d | 10.5 c | 125.7 b | 34.4 cd | 6.5 c | 75.1 d |
| CN | 150.0 a | 24.7 a | 143.2 a | 27.2 d | 6.6 c | 80.9 c |
| IPS | 78.6 c | 16.4 b | 130.9 ab | 41.0 bc | 8.7 b | 84.3 bc |
| PII | 114.4 b | 21.9 a | 138.5 ab | 48.8 ab | 10.4 ab | 88.9 ab |
| PIFI | 102.6 bc | 20.4 ab | 135.5 ab | 55.1 a | 12.0 a | 90.8 a |
| Mean | 95.4 | 18.8 | 134.8 | 41.3 | 8.8 | 84.0 |
| V.C. (%) | 18.5 | 13.2 | 6.7 | 14.2 | 12.5 | 3.17 |

Means followed by the same letter in the column do not differ significantly according to Tukey's test at a 5% probability level. Unit $pl^{-1}$: unit per plant; mg $pl^{-1}$: milligrams per plant; g $pl^{-1}$: grams per plant.

**Table 4.** Total nitrogen content parameters as a function of seed treatment.

| **Trat.** | **Mandaguaçu/PR** | | **Marialva/PR** | | **Planaltina do Paraná/PR** | |
|---|---|---|---|---|---|---|
| | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** |
| CA | 344.0 d | 167.1 c | 160.1 d | 131.1 d | 182.8 e | 162.4 d |
| CN | 450.5 c | 192.8 b | 373.2 a | 195.5 b | 280.5 d | 197.9 c |
| IPS | 519.3 bc | 207.5 b | 301.4 b | 165.3 c | 741.0 a | 249.7 a |
| PII | 575.3 b | 227.0 a | 229.2 c | 166.5 c | 390.9 c | 213.1 bc |
| PIFI | 767.2 a | 237.8 a | 386.2 a | 228.5 a | 530.5 b | 230.2 ab |
| Mean | 531.2 | 206.5 | 290.0 | 177.4 | 425.1 | 210.6 |
| V.C. (%) | 6.8 | 12.3 | 11.5 | 6.5 | 2.63 | 6.9 |
| **Trat.** | **Munhoz de Melo/PR** | | **Paranavaí/PR** | | **Itaquiraí/MS** | |
| | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** | **TNA (kg N $ha^{-1}$)** | **TNG (kg N $ha^{-1}$)** |
| CA | 233.1 d | 114.0 d | 193.6 c | 148.6 c | 142.7 d | 62.3 d |
| CN | 296.5 cd | 149.1 c | 927.1 a | 242.0 a | 198.8 c | 82.5 c |
| IPS | 377.3 bc | 177.2 b | 460.9 b | 173.5 bc | 226.3 bc | 94.2 bc |
| PII | 634.0 ab | 234.3 a | 656.9 ab | 240.9 a | 251.6 ab | 110.8 b |
| PIFI | 457.2 a | 199.6 ab | 628.6 ab | 206.0 ab | 299.4 a | 131.7 a |
| Mean | 399.6 | 174.8 | 573.4 | 202.2 | 223.7 | 96.3 |
| V.C. (%) | 3.1 | 6.9 | 15.1 | 15.7 | 11.5 | 9.3 |

Means followed by the same letter in the column do not differ significantly according to Tukey's test at a 5% probability level. unit $pl^{-1}$: unit per plant; mg $pl^{-1}$: milligrams per plant; g $pl^{-1}$: grams per plant.

Figure 2 shows soybean grain yield across the six experimental sites in response to the different seed treatments.

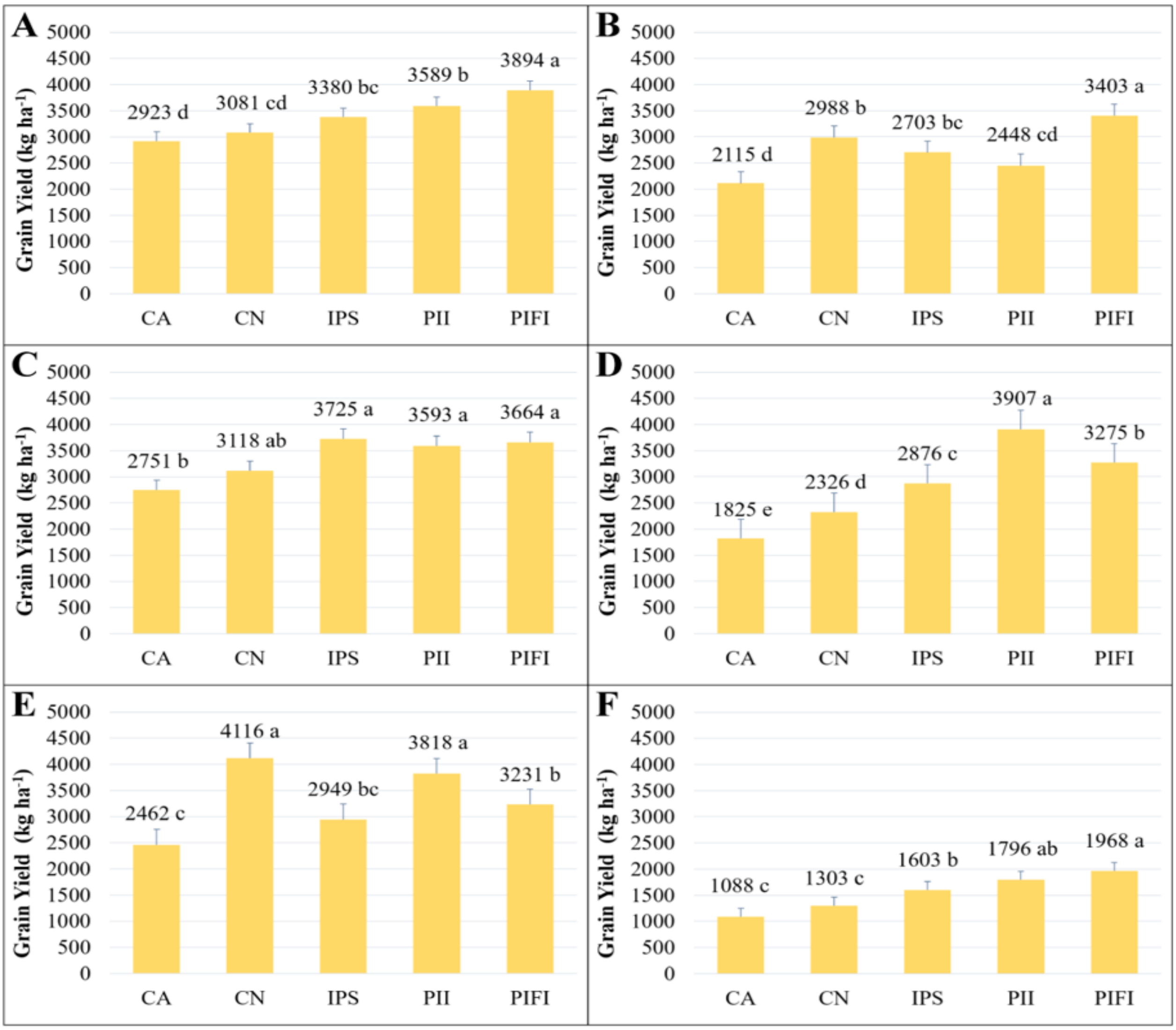


**Figure 2.** Grain productivity, in response to the treatments used, for Mandaguaçu/PR (A), Marialva/PR (B), Planaltina do Paraná/PR (C), Munhoz de Melo/PR (D), Paranavaí/PR (E) and Itaquiraí/MS (F).

Overall, the results of this experiment indicate adequate nodulation, as a dry mass of nodules between 100 and 200 mg per plant at flowering is sufficient to meet the nitrogen requirements for normal soybean development [10]. These findings are consistent with those reported by other authors [11] who observed that plants with 10 to 30 nodules at flowering can fix sufficient nitrogen for optimal growth.

Similarly, these results align with a previous study Silva et al. [12] that evaluated nodulation variables. In that study, pre-inoculation combined with a bacterial protectant was applied alongside four different pesticide treatments (active ingredients: Bifenthrin + Imidacloprid; Pyraclostrobin + Thiophanate-methyl + Fipronil; Metalaxyl-M + Fludioxonil; and Metalaxyl-M + Thiabendazole + Fludioxonil + Thiamethoxam) at intervals of 60, 45, 30, and 15 days. Controls included a treatment with inoculation at sowing and a treatment without inoculation. The study reported significant differences in nodulation parameters, indicating that pre-inoculation combined with seed treatment can influence the dynamics of biological nitrogen fixation.

The results for nodulation-related variables observed in the control with mineral-N application corroborate previous findings [13], which reported that even low doses of nitrogen fertilizer can negatively affect biological nitrogen fixation (BNF) by partially inactivating the nitrogenase enzyme, a process that may be reversible or irreversible.

Similar outcomes were reported by Anghinoni et al. [14], who found that pre-inoculation combined with chemical pesticides Carbendazim + Thiram, Imidacloprid + Thiodicarb, Fludioxonil, and Thiamethoxam allowed seeds to be stored for up to 10 days before sowing without affecting soybean yield components. Other authors have also shown that seed treatment with fungicides such as Metalaxyl and Fludioxonil, and the insecticide Thiamethoxam, together with inoculant in pre-inoculation, can be performed up to 60 days before sowing without compromising nodulation or crop yield [15].

Regarding productivity, it reflects the cumulative effect of all factors influencing the crop throughout its development. However, the lack of a direct yield response to biological nitrogen fixation (BNF) has been reported by several authors. Sanginga et al. [16] demonstrated that inoculant use influenced soybean nodulation but did not affect grain yield. In Brazil, studies have shown that while inoculant application can influence soybean productivity, it does not always correlate with improved nodulation parameters. For instance, a study by Sartori et al. [17] demonstrated that pre-inoculation of soybean seeds for up to 30 days may negatively affect the recovery of colony-forming units, biological nitrogen fixation, plant growth, and the weight of a thousand grains.

In line with previous studies, Da Silva et al. [18] evaluated different methods of inoculating soybean seeds with Bradyrhizobium spp. and Azospirillum brasilense and found that pre-inoculation via seed treatment, combined with foliar application of A. brasilense, was technically feasible for promoting nodulation and maintaining grain yield across diverse edaphoclimatic conditions. These findings support the potential of integrating seed pre-inoculation with compatible bacterial inoculants to optimize biological nitrogen fixation, enhance plant growth, and sustain productivity in soybean crops, even when varying soil and climatic conditions are present. Consistent with the findings of this study, Santos et al. [19] emphasized that achieving high yields in inoculated soybeans depends on the compatibility of pesticides with bacterial inoculants. Conversely, the lack of yield response to seed inoculation under field conditions, even in sandy soils with low organic matter and nutrients, particularly mineral nitrogen, may be related to inoculant quality and the application method used.

Considering the results of this study and previous literature, it can be concluded that the application of inoculants via seed treatment is essential in soybean cultivation. This practice not only enhances crop productivity but also significantly reduces production costs associated with mineral nitrogen fertilization, as suggested by Hungria [20] and Hungria and Nogueira [21].

## 4. CONCLUSIONS

Pre-inoculation with a liquid Bradyrhizobium japonicum inoculant, applied 10 days before sowing in combination with chemical pesticides, resulted in nodulation and soybean yield comparable to or higher than the controls inoculated with Bradyrhizobium spp. at sowing.

Across the six experimental sites, pre-inoculation treatments achieved yields higher than the absolute control (without Bradyrhizobium spp. inoculation), and in five out of six sites, yields also exceeded those of the mineral-N control.

These results indicate that liquid pre-inoculation with Bradyrhizobium japonicum, applied 10 days before sowing alongside various chemical pesticides, is an effective strategy for enhancing both nodulation and soybean yield.

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