

# Agronomic Efficiency of Signum Inoculant in Pre-inoculation of Soybean at 35 and 20 Days Before Sowing in Treated Seeds

Gabriela S. Machineski<sup>1</sup>, Andrea S. Scaramal<sup>1</sup>, Maria A. Matos<sup>1</sup>, Jonatas F. Langame<sup>2</sup>, Giovana G. Gaiser<sup>2</sup>,  
Oswaldo Machineski<sup>1</sup> & Arnaldo Colozzi Filho<sup>1</sup>

<sup>1</sup> Laboratory of Soil Microbiology, Institute of Rural Development of Paraná, Londrina, Brazil

<sup>2</sup> School of Agronomy, University UNOPAR-PITAGORAS, Londrina, Brazil

Correspondence: Gabriela S. Machineski, Laboratory of Soil Microbiology, Institute of Rural Development of Paraná, Londrina, Brazil. Tel: 55-43-3376-2300. E-mail: gabymachine@yahoo.com

Received: February 26, 2022

Accepted: April 20, 2022

Online Published: May 15, 2022

doi:10.5539/jas.v14n6p141

URL: <https://doi.org/10.5539/jas.v14n6p141>

## Abstract

Pre-inoculating soybean seeds can make sowing faster and provide additional benefits to farmers. However, it needs to guarantee the nitrogen supply to maintain the viability and sustainability of the technique. In this study, we evaluated the agronomic efficiency of SIGNUM<sup>®</sup> inoculant in the pre-inoculation at 20 and 35 days before sowing chemically treated soybean seeds. Experiments were conducted in four field experiments located at Paraná, Brazil, with a history of soybean cultivation managed under no-tillage systems, with crop rotation according to regional edaphoclimatic conditions. Agronomic efficiency in fields were compared with standard inoculation with a registered product used by farmers. Chemical treatment of soybean seeds with Standak Top<sup>®</sup> or Maxin XL<sup>®</sup> + Cruiser<sup>®</sup> associated with pre-inoculation of the inoculant SIGNUM<sup>®</sup> for 25 and 30 days reduced the concentration of viable *Bradyrhizobium* cells recovered from seeds. However, no significant difference was observed regarding nodulation, biological nitrogen fixation, and yield between the soybean inoculated with standard inoculation on farm or pre-inoculation with SIGNUM<sup>®</sup> in most studied areas.

**Keywords:** biological nitrogen fixation, fungicides, insecticides, pre-sowing, rhizobia

## 1. Introduction

Soybean [*Glycine max* (L.) Merrill] is among the most important crops in the world. In Brazil, soybean is the main crop in national agribusiness and, according to the National Supply Company—CONAB, Brazil is the world's largest soybean producer, with a record production of 135.9 million tons, a cultivated area of 38.5 million hectares, and a mean yield of 3,527 kg per hectare in the 2020/21 growing season (CONAB, 2022).

Crop nitrogen (N) demand is high to ensure high yields. Hungria et al. (2007) estimated that the crop needs 80 kg of N for every 1000 kg of grain produced, resulting in approximately 270 kg ha<sup>-1</sup> of N to the soil to supply plants needs and reach the national mean grain production, *i.e.*, 3,527 kg ha<sup>-1</sup> (CONAB, 2022). Thus, without nitrogen fixation the chemical nitrogen fertilizers required to meet the demand would make soybean production drastically more expensive. Alves and Aguila (2020) cited that Brazilian soybean production would be economically unfeasible if it depended entirely on chemical fertilization.

The highest contribution to supplying N demand in soybean is given by biological nitrogen fixation (BNF). In this process, there is a symbiosis between bacteria of the genus *Bradyrhizobium* and soybean, forming structures called nodules in the roots. In these structures, bacteria convert atmospheric N<sub>2</sub> into ammonia and transfer it directly to plant tissues, which in turn provide organic compounds to the bacteria (Van Rhyn & Vanderleyden, 1995).

The inoculation of these bacteria is necessary to optimize the BNF process in agricultural areas of soybean production. The inoculation of selected BNF-efficient strains ensures the presence of a high number of bacteria that can reach the roots and supply the plant N demand (Hungria et al., 2007). In this sense, different inoculation strategies have emerged for soybean. Traditionally, inoculation is carried out on the seed at the time of sowing. However, some studies have shown the possibility of inoculation directly into the soil (Meert et al., 2020; Pedrozo, Oliveira, & Alberton, 2018; Zilli et al., 2010) or the pre-inoculation of seeds days before sowing

(Hungria et al., 2020; Machineski et al., 2018; Silva et al., 2018; Anghinoni et al., 2017; Araujo et al., 2017; Zilli et al., 2010).

Pre-inoculation allows farmers to dedicate themselves to the sowing operation, without having to deal with inoculation on the day of sowing, which is usually time-consuming and labor-intensive (Hungria et al., 2020). Some studies have reported the success of pre-inoculation practice in soybean. Anghinoni et al. (2017) and Silva et al. (2018) results indicated the storage of pre-inoculated seeds up to 10 days without affecting yield components; Hungria et al. (2020) reported the feasibility of pre-inoculation for a period of 15 days in soybean; and Machineski et al. (2018) reported the pre-inoculation of soybean seeds up to 60 days before sowing. However, the practice needs to guarantee the presence of viable bacteria at adequate concentrations in the seeds at the time of sowing, even after the necessary chemical treatment and seed storage.

SIGNUM<sup>®</sup> inoculant, according to manufacturer (Rizobacter of Brazil), is based on bioinducer technology, which stimulates bacteria to produce multiple determinants of nodulation, improving communication between bacteria and the root, and osmoprotection technology, that improves the physiological state and the resistance of bacteria, increasing their survival on the seed. Thus, this study aimed to evaluate the agronomic efficiency of SIGNUM<sup>®</sup> inoculant in pre-inoculation at 20 and 35 days before sowing chemically treated soybean seeds.

## 2. Materials and Methods

### 2.1 Viability and Purity of Inoculants

The inoculant SIGNUM<sup>®</sup> (Rizobacter do Brasil, Paraná, Brazil), which consists of a liquid inoculant composed of the strains Semia-5079 and Semia-5080, with a concentration of viable cells, according to the manufacturer's information, of  $1.0 \times 10^9$  colony forming units (CFU) of *Bradyrhizobium japonicum* per mL of inoculant, was tested in pre-inoculation of soybean. The inoculant RIZOLIQ<sup>®</sup> (Rizobacter do Brasil, Paraná, Brazil), consisting of *Bradyrhizobium japonicum* (Semia-5079 and Semia-5080 strains), with a concentration of viable cells of  $5.0 \times 10^9$  CFU mL<sup>-1</sup> of inoculant, according to the manufacturer's information, was used as a standard control inoculant.

The quality of inoculants was evaluated by counting viable cells by the droplet scattering method and counting *Bradyrhizobium japonicum* colony-forming units in YMA (yeast, mannitol, and agar) solid culture medium in Petri dishes. Analyses of pH and the presence of contaminants in the inoculants were also carried out by determining CFU of contaminants in two dilution series, as recommended in the Normative Instruction SDA/MAPA 30/2010, article 4 (DOU 11/17/2010) (MAPA, 2010).

### 2.2 Description, Characterization, and Preparation of Experimental Areas

Four experimental areas with different edaphoclimatic conditions but suitable for soybean cultivation in the 2017/2018 agricultural season were selected. The experimental areas are located in the municipalities of Londrina (23°21'26"S and 51°10'08"W, 565 m altitude), Santa Tereza do Oeste (25°05'19"S and 53°35'19"W, 750 m altitude), Pato Branco (26°07'32"S and 52°38'56"W, 720 m altitude), and Ponta Grossa (25°09'04"S and 50°09'14"W, 865 m altitude), and belong to the Instituto de Desenvolvimento Rural do Paraná IAPAR-EMATER (IDR-PR), with a history of soybean cultivation managed under the no-tillage system, with crop rotation according to regional edaphoclimatic conditions. Climatic data of all experimental areas are in Figure 1.

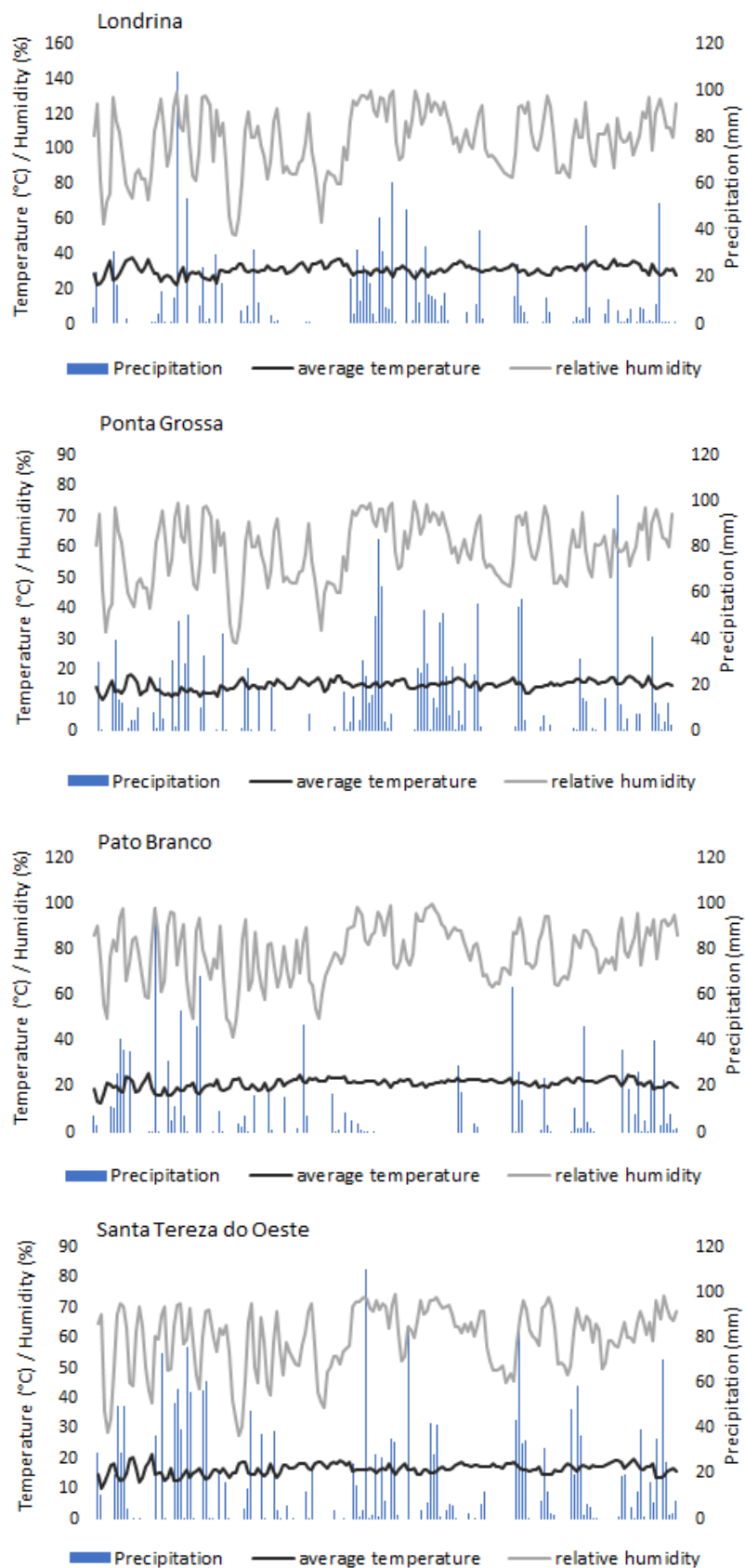


Figure 1. Climatic data of average temperature (°C), relative humidity (%) and Precipitation (mm) of experimental areas located in Londrina, Ponta Grossa, Pato Branco and Santa Tereza do Oeste, Brazil

Soil chemical characterization of each location was carried out at the Laboratory of Soil and Tissue of IDR-PR, following the methodology of Pavan et al. (1992) (Table 1). Soil correction and fertilization were carried out based on the chemical analysis results and according to the technical recommendation for soybean fertilization (Moreira et al., 2017). Soil fertilization was performed with NPK fertilizer (0-20-20).

Table 1. Soil chemical analysis of the experimental areas according to the municipality location

Location	P <sup>1</sup>	C <sup>5</sup>	pH <sup>4</sup>	Al <sup>3</sup>	H+Al	Ca <sup>2</sup>	Mg <sup>2</sup>	K <sup>1</sup>
	mg dm <sup>-3</sup>	g dm <sup>-3</sup>				cmol <sub>c</sub> dm <sup>-3</sup> soil		
Londrina	11.90	19.12	5.30	0.00	4.27	5.65	2.71	0.30
Santa Tereza	19.88	31.95	4.60	0.17	10.45	5.67	2.02	0.61
Pato Branco	18.40	31.75	5.00	0.04	7.20	6.10	3.53	0.27
Ponta Grossa	8.30	12.89	4.90	0.03	3.97	2.37	1.11	0.14

Note. <sup>1</sup> P and K: Mehlich I; <sup>2</sup> Ca and Mg: KCl; <sup>3</sup> Al: SMP; <sup>4</sup> pH: 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub>; <sup>5</sup> C: Walkley-Black.

The naturalized population of soybean-nodulating rhizobia present in the soil was evaluated in all areas by the infection method in plants grown in nutrient solution and conducted in a greenhouse, according to MAPA Normative Instruction No. 30 of 2010 (DOU 11/17/2010) (MAPA, 2010). One negative control, composed of uninoculated soybean seedlings, and one positive control, with inoculation of seedlings with the SEMIA 5080 strain, recommended for soybean inoculation, were added to these assays.

### 2.3 Treatments, Inoculation, Sowing, and Conduction of Experiments

The experimental design in all areas was randomized blocks with four replications and plots of 4.05 × 6 m (24.3 m<sup>2</sup>) with nine sowing rows of 6 m in length, spaced at 0.45 m and density of 12 plants per linear meter. The treatments consisted of T-I Control without chemical seed treatment and without inoculation; T-II Standard inoculation with RIZOLIQ<sup>®</sup> on the day of sowing in untreated seeds; T-III Standard inoculation with RIZOLIQ<sup>®</sup> on the day of sowing in seeds treated with Standak Top<sup>®</sup>; T-IV Standard inoculation with RIZOLIQ<sup>®</sup> on the day of sowing in seeds treated with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-V Inoculation with SIGNUM<sup>®</sup> at 35 days before sowing in seeds treated with Standak<sup>®</sup> Top; T-VI Inoculation with SIGNUM<sup>®</sup> at 35 days before sowing in seeds treated with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-VII Inoculation with SIGNUM<sup>®</sup> at 20 days before sowing in seeds treated with Standak<sup>®</sup> Top; and T-VIII Inoculation with SIGNUM<sup>®</sup> at 20 days before sowing in seeds treated with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>.

The soybean varieties were BMX Potência RR in the assays carried out in Londrina and Pato Branco and BMX Apolo RR in the assays carried out in Santa Tereza do Oeste and Ponta Grossa, both indicated for cultivation in these regions of the Paraná State. The seeds showed a germination vigor above 85%. The seeds were chemically treated with the fungicide and insecticide Standak<sup>®</sup> Top (pyraclostrobin, thiophanate methyl, and fipronil; 225, 250, and 25 g ai L<sup>-1</sup>, respectively, SF, Basf) at a dose of 2 mL kg<sup>-1</sup> seed; the fungicide Maxin XL<sup>®</sup> (metalaxyl-M and fludioxonil; 10 and 25 g ai L<sup>-1</sup>, respectively, FS, Syngenta) at a dose of 1 mL kg<sup>-1</sup> seed; and the insecticide Cruiser<sup>®</sup> 350FS (thiamethoxam; 350 g ai L<sup>-1</sup>, FS, Syngenta) at a dose of 2 mL kg<sup>-1</sup> seed. The seeds were inoculated according to the manufacturer's instructions described on the inoculant package. The treated seeds from treatments V to VIII were pre-inoculated at 20 and 35 days pre-sowing, as defined for each treatment, stored in paper bags, and maintained at room conditions (25±3 °C) protected from sunlight and humidity until the time of sowing.

The experiments were set up following the agroclimatic zoning of each region (MAPA, 2015) and meeting the soil moisture conditions suitable for sowing. Sowing was carried out on October 27 in Londrina, November 1 in Santa Tereza, November 6 in Pato Branco, and November 26, 2017, in Ponta Grossa. Cultural practices to manage weeds, pests, and diseases were carried out in accordance with agronomic practices recommended for soybean cultivation (Pas Campo, 2005).

### 2.4 *Bradyrhizobium* sp. Survival in Inoculated Seeds

The survival of *Bradyrhizobium* sp. inoculated to the seeds was determined at the time of sowing by evaluating the recovery of viable cells of *Bradyrhizobium* sp. on the seed surface through plating in semi-selective Ikuta culture medium (Ikuta, 1995) and CFU counting, as recommended (MAPA, 2010). The data were transformed into Log<sub>10</sub> for statistical analysis.

## 2.5 Assessments

Five soybean plants were collected at flowering (phenological stage R1) from each treatment and the number of nodules per plant, nodule dry mass per plant, root and shoot dry mass, and shoot nitrogen (N) concentration were evaluated according to the methodology described by Miyazawa et al. (1992). Grain production in the experimental plots and N concentration in grains (Miyazawa et al., 1992) were evaluated after 50% of the plant population reached the R8 phenological stage. Yield and nitrogen exported by the crop in the grains were calculated using a population density of 200 thousand plants ha<sup>-1</sup>. Grain moisture was corrected to 13% moisture and grain yield was expressed in kg ha<sup>-1</sup>. The variables were analyzed according to MAPA Normative Instruction No. 30 of 2010 (DOU 11/17/2010) (MAPA, 2010).

The results were subjected to analysis of variance ( $p \leq 0.05$ ). The means of viable bacterial cells in the seeds, nodulation (number of nodules and nodule dry mass), biomass (root and shoot dry mass), N concentration in shoots and grains, and yield were compared pairwise with the treatment standard inoculation (treatment II: inoculation at seeding with RIZOLIQ<sup>®</sup> without chemical treatment) by bilateral Dunnett's test ( $p \leq 0.05$ ) using SAS software (SAS Institute Inc. 2019).

## 3. Results

### 3.1 Inoculant Quality

The evaluation of viable cells (CFU—colony forming units) of *Bradyrhizobium japonicum* in the inoculants used in the experiment is shown in Table 2.

Table 2. Viable cells (colony forming units—CFU) of *Bradyrhizobium japonicum* in the inoculants RIZOLIQ<sup>®</sup> and SIGNUM<sup>®</sup>, produced by Rizobacter and used in field experiments in the 2017/2018 growing season

Inoculant	Inoculation	CFU mL <sup>-1</sup>
RIZOLIQ <sup>®*</sup>	Sowing	$2.70 \times 10^{10}$
SIGNUM <sup>®*</sup>	20 days pre-sowing	$2.85 \times 10^{10}$
SIGNUM <sup>®</sup>	35 days pre-sowing	$2.93 \times 10^{10}$
Mean		2.83

Note. \**Bradyrhizobium japonicum* (Semia 5079 and Semia 5080 strains).

The mean number of *Bradyrhizobium japonicum* cells was  $2.83 \times 10^{10}$  CFU mL<sup>-1</sup> of inoculant, meeting the requirement of Brazilian legislation, which establishes a minimum concentration of  $1.0 \times 10^9$  (CFU) per gram or milliliter of product for inoculants with nitrogen-fixing bacteria for symbiosis with legumes, according to art. 1 of the Normative Instruction No. 13, of March 24, 2011. The inoculants RIZOLIQ<sup>®</sup> (standard inoculation at sowing), SIGNUM<sup>®</sup> (inoculation at 20 days pre-sowing), and SIGNUM<sup>®</sup> (inoculation at 35 days pre-sowing) presented pH values of 7.6, 7.6, and 7.5, respectively. The inoculants did not present contaminants above  $10^2$  CFU mL<sup>-1</sup> in two dilution series, meeting the standard described in art. 1, item IV, in Normative Instruction No. 13, of March 24, 2011, which requires inoculants free of unspecified microorganisms at a concentration of  $1.0 \times 10^5$  mL<sup>-1</sup> of inoculant.

### 3.2 Naturalized Population of Rhizobia in the Experimental Areas

All areas showed a high naturalized population of soybean-nodulating rhizobia, ranging from 1.08 to  $8.37 \times 10^6$  bacteria g<sup>-1</sup> soil (Table 3). The history of regular cultivation of inoculated soybean in these areas may be related to this high population of rhizobia in the soil.

Table 3. Naturalized population (MPN—most probable number) of soybean-nodulating rhizobium in the soil of the locations where the experiments were carried out referring to the inoculant SIGNUM<sup>®</sup> in pre-inoculation in the 2017/18 growing season

Location	MPN g <sup>-1</sup> Soil
Londrina	$8.37 \times 10^6$
Santa Tereza Oeste	$2.03 \times 10^6$
Pato Branco	$6.95 \times 10^6$
Ponta Grossa	$1.08 \times 10^6$

### 3.3 Survival of *Bradyrhizobium* sp. in Inoculated Seeds

The recovery of viable *Bradyrhizobium* sp. cells on the surface of inoculated seeds is shown in Table 4.

Table 4. Concentration of *Bradyrhizobium* cells recovered on soybean seeds treated with different fungicides/insecticides and inoculated on the day of sowing with RIZOLIQ<sup>®</sup> or pre-inoculated at 20 and 35 days before sowing with SIGNUM<sup>®</sup>. Means of three replications

Inoculant	Inoculation	Seed Treatment	CFU seed <sup>-1</sup>
RIZOLIQ <sup>®1</sup>	Sowing	Untreated	$2.60 \times 10^5$
RIZOLIQ <sup>®</sup>	Sowing	ST <sup>2</sup>	$2.91 \times 10^5$
RIZOLIQ <sup>®</sup>	Sowing	MADV+C <sup>3</sup>	$1.60 \times 10^5$
SIGNUM <sup>®1</sup>	20 days pre-sowing	ST	$1.05 \times 10^3$ *
		MADV+C	$2.60 \times 10^3$ *
	35 days pre-sowing	ST	$0.95 \times 10^3$ *
		MADV+C	$4.25 \times 10^3$ *

Note. <sup>1</sup> *Bradyrhizobium japonicum* (Semia 5079 and Semia 5080 strains); <sup>2</sup> Standak Top<sup>®</sup>; <sup>3</sup> Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>. Means followed by \* differ significantly from the RIZOLIQ<sup>®</sup> treatment in the sowing without seed treatment by bilateral Dunnett's test at the 5% probability level. The data were transformed into Log<sub>10</sub> for statistical analysis.

Recovery of viable *Bradyrhizobium* sp. cells on the surface of seeds inoculated with RIZOLIQ<sup>®</sup> on the day of sowing without treatment or with chemical treatments with Standak Top<sup>®</sup> or Maxim XL<sup>®</sup> + Cruiser<sup>®</sup> ranged from 1.6 to  $2.91 \times 10^5$  CFU seed<sup>-1</sup>. Seeds pre-inoculated with SIGNUM<sup>®</sup> at 20 and 35 days before sowing showed values of *Bradyrhizobium japonicum* cell recovery on seeds ranging from 0.95 to  $4.25 \times 10^3$  CFU seed<sup>-1</sup>, regardless of the chemical treatment, which is statistically lower than the values observed in seeds inoculated with RIZOLIQ<sup>®</sup> in the sowing without treatment (Table 4).

### 3.4 Agronomic Efficiency in the Field

In the field experiment carried out in Londrina, the variables number of nodules, nodule dry mass, root dry mass, shoot dry mass, shoot nitrogen, nitrogen exported in the grains, and yield showed no significant difference between treatments compared with the standard inoculation (Table 5). Only the nitrogen content of soybean grains pre-inoculated with SIGNUM<sup>®</sup> at 35 days before sowing and treated with Standak Top<sup>®</sup> (T-V) was significantly higher than the content observed in the standard inoculation treatment (T-II).

Table 5. Nodulation (number of nodules—No. and dry mass—DM), root and shoot dry mass, nitrogen in the shoot and grains, and yield of soybean grown from seeds treated with pesticides and pre-inoculated with SIGNUM<sup>®</sup> at 35 and 20 days before sowing. Londrina, PR, Brazil, 2017/18 growing season. Means of four replications

Treatment	Nodulation		Dry Mass		Nitrogen			Yield
	No.	DM	Root	Shoot	Shoot	Grains	Total exported	
	No. plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
T-I	72	0.23	2.47	19.74	36.71	59.28	260.40	4393
T-II	100	0.22	2.28	17.86	40.47	59.16	262.20	4429
T-III	86	0.20	2.15	19.82	42.54	60.67	278.61	4589
T-IV	106	0.19	2.12	16.35	41.32	61.77	269.67	4365
T-V	90	0.27	2.19	17.36	42.96	63.04*	286.83	4549
T-VI	81	0.31	2.29	17.35	40.35	60.63	273.82	4518
T-VII	63	0.19	1.74	18.14	44.48	58.20	245.73	4220
T-VIII	83	0.26	2.16	19.74	41.26	60.93	275.53	4521
P > F	0.0711	0.0103	0.1203	0.5144	0.4942	0.0031	0.2270	0.7111
CV (%)	22	19	14	14	11	2	8	7
LSD	37.862	0.0894	0.6087	5.0155	9.5192	2.9258	42.129	604.45

*Note.* T-I: no seed treatment and no inoculation; T-II: standard inoculation at sowing with RIZOLIQ<sup>®</sup>; T-III: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Standak Top<sup>®</sup>; T-IV: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-V: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VI: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-VII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VIII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>. Means followed by \* differ significantly from treatment T-II (standard inoculation) by bilateral Dunnett's test at  $p \leq 0.05$ .

Santa Tereza do Oeste also showed that the variables number of nodules, nodule dry mass, shoot dry mass, and nitrogen in grains (g kg<sup>-1</sup>) did not present significant differences between treatments compared to the standard inoculation (Table 6). Soybean inoculated with RIZOLIQ<sup>®</sup> (standard inoculation T-III) in seeds treated with Standak Top<sup>®</sup> showed a reduction in root dry mass compared to that which received standard inoculation in seeds without treatment (T-II). Treatments T-I, T-III, T-V, T-VII, and T-VIII resulted in lower shoot nitrogen contents compared to the values observed in T-II. However, treatments T-I, T-V, and T-VIII showed higher contents of N exported by grains (kg ha<sup>-1</sup>). Treatments T-I, T-III, T-V, T-VI, and T-VIII promoted higher grain yields compared to T-II (Table 6).

Table 6. Nodulation (number of nodules—No. and dry mass—DM), root and shoot dry mass, nitrogen in the shoot and grains, and yield of soybean grown from seeds treated with pesticides and pre-inoculated with SIGNUM<sup>®</sup> at 35 and 20 days before sowing. Santa Tereza do Oeste, PR, Brazil, 2017/18 growing season. Means of four replications

Treatment	Nodulation		Dry Mass		Nitrogen			Yield
	No.	DM	Root	Shoot	Shoot	Grains	Total exported	
	No. plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g planta <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
T-I	62	0.26	1.37	14.35	31.46*	61.72	261.54*	4239*
T-II	54	0.23	1.78	15.87	42.58	60.30	235.24	3902
T-III	63	0.31	1.31*	13.91	34.45*	60.91	257.06	4219*
T-IV	61	0.28	1.46	12.83	39.69	59.29	247.17	4168
T-V	58	0.24	1.36	14.77	32.68*	62.38	269.43*	4321*
T-VI	67	0.26	1.62	15.49	35.02	59.22	251.39	4245*
T-VII	64	0.27	1.39	13.39	32.58*	61.99	253.61	4091
T-VIII	69	0.29	1.56	14.50	30.81*	62.50	266.25*	4259*
P > F	0.5919	0.6408	0.0644	0.1811	0.0036	0.0011	0.0068	0.0316
CV (%)	17	21	14	11	11	2	4	4
LSD	21.45	0.11	0.43	3.20	7.94	2.27	22.33	315.77

*Note.* T-I: no seed treatment and no inoculation; T-II: standard inoculation at sowing with RIZOLIQ<sup>®</sup>; T-III: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Standak Top<sup>®</sup>; T-IV: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-V: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VI: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-VII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VIII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>. Means followed by \* differ significantly from treatment T-II (standard inoculation) by bilateral Dunnett's test at  $p \leq 0.05$ .

In Ponta Grossa, the variables number of nodules, nodule dry mass, root dry mass, nitrogen in the shoot, nitrogen exported by the grains, and soybean yield showed no significant difference between treatments compared to the standard inoculation. Soybean presented lower shoot dry mass than in treatment T-II only in treatment T-VIII (Table 7).



Table 7. Nodulation (number of nodules—No. and dry mass—DM), root and shoot dry mass, nitrogen in the shoot and grains, and yield of soybean grown from seeds treated with pesticides and pre-inoculated with SIGNUM<sup>®</sup> at 35 and 20 days before sowing. Ponta Grossa, PR, Brazil, 2017/18 growing season. Means of four replications

Treatment	Nodulation		Dry Mass		Nitrogen			Yield
	No.	DM	Root	Shoot	Shoot	Grains	Total exported	
	No. plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
T-I	40	0.16	1.15	7.36	49.45	54.63	207.30	3807
T-II	40	0.13	1.34	8.57	49.15	57.54	247.31	4298
T-III	34	0.15	1.23	6.78	51.30	60.32	236.65	3921
T-IV	36	0.11	1.54	7.47	47.90	60.52	271.26	4484
T-V	42	0.16	1.50	7.43	49.69	61.04	259.28	4246
T-VI	42	0.12	1.60	7.04	47.40	60.95	264.90	4343
T-VII	39	0.14	1.14	7.05	45.88	59.89	246.79	4120
T-VIII	38	0.13	1.10	5.72*	40.61	62.04	267.77	4320
P > F	0.5778	0.1345	0.0041	0.1214	0.5071	0.0038	0.0082	0.1631
CV (%)	15	20	15	16	14	4	9	8
LSD	12.10	0.06	0.39	2.33	13.77	4.59	43.47	705.41

*Note.* T-I: no seed treatment and no inoculation; T-II: standard inoculation at sowing with RIZOLIQ<sup>®</sup>; T-III: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Standak Top<sup>®</sup>; T-IV: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-V: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VI: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-VII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VIII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>. Means followed by \* differ significantly from treatment T-II (standard inoculation) by bilateral Dunnett's test at  $p \leq 0.05$ .

In Pato Branco, the variables nodule dry mass, shoot dry mass, nitrogen in the shoot, nitrogen exported by the crop, and soybean yield did not present significant differences between treatments compared with the standard inoculation (T-II) (Table 8). Soybean with seeds treated with Standak Top<sup>®</sup> and inoculated with RIZOLIQ<sup>®</sup> at sowing (T-III) had a higher number of nodules than the soybean under standard inoculation (T-II). Treatments T-IV, T-VI, and T-VII promoted a significant reduction in soybean root dry mass. Treatment T-VI significantly increased the N content of soybean grains.

Table 8. Nodulation (number of nodules—No. and dry mass—DM), root and shoot dry mass, nitrogen in the shoot and grains, and yield of soybean grown from seeds treated with pesticides and pre-inoculated with SIGNUM<sup>®</sup> at 35 and 20 days before sowing. Pato Branco, PR, Brazil, 2017/18 growing season. Means of four replications

Treatment	Nodulation		Dry Mass		Nitrogen			Yield
	No.	DM	Root	Shoot	Shoot	Grains	Total exported	
	No plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
T-I	110	0.38	2.20	26.53	56.39	61.41	177.10	2884
T-II	93	0.44	2.29	21.73	49.43	64.13	187.85	2932
T-III	143*	0.46	2.30	20.88	50.28	61.42	191.71	3122
T-IV	106	0.37	1.76*	16.91	50.19	63.01	197.81	3143
T-V	109	0.39	2.35	21.27	50.38	65.81	200.86	3053
T-VI	123	0.36	1.88*	19.77	50.55	68.73*	196.41	2860
T-VII	109	0.40	1.90*	18.37	53.43	65.19	186.38	2857
T-VIII	106	0.30	2.17	21.80	49.81	65.19	190.32	2920
P > F	0.1326	0.1092	0.0003	0.0037	0.1317	0.0001	0.0355	0.0561
CV (%)	19	18	8	13	7	2	5	5
LSD	44.15	0.14	0.34	5.46	7.07	3.00	18.39	303.51

Note. T-I: no seed treatment and no inoculation; T-II: standard inoculation at sowing with RIZOLIQ<sup>®</sup>; T-III: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Standak Top<sup>®</sup>; T-IV: standard inoculation with RIZOLIQ<sup>®</sup> and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-V: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VI: pre-inoculation with SIGNUM<sup>®</sup> at 35 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>; T-VII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Standak Top<sup>®</sup>; T-VIII: pre-inoculation with SIGNUM<sup>®</sup> at 20 days before sowing and seed treatment with Maxin XL<sup>®</sup> + Cruiser<sup>®</sup>. Means followed by \* differ significantly from treatment T-II (standard inoculation) by bilateral Dunnett's test at  $p \leq 0.05$ .

#### 4. Discussion

The four studied areas had the establishment of a naturalized population of *Bradyrhizobium* sp. capable of nodulating soybean in the order of  $10^6$  bacteria  $g^{-1}$  soil (Table 3) due to the history of annual soybean production. Soybean is a species introduced in Brazil from Asia, hindering the occurrence of native rhizobia of species symbiotic to soybean (Lima et al., 1998). The symbiosis is the result of an evolutionary process lasting millions of years between the bacterium and the plant, and it is at its center of origin (Asia) that native bacteria are also found (Hungria et al., 2007).

However, intensive and continuous cultivation with inoculated seeds, as in the experimental areas used in this study, may allow bacteria from the inoculant to survive in the soil, establish and “naturalize,” forming a population capable of competing for sites of root infection with those inoculated and nodulate soybean (Mendoza-Suárez et al., 2021), fixing nitrogen for the plant (Campos & Gnatta, 2006).

In this study, the control treatments (without inoculation) presented nodulation, BNF, and yields similar to the other inoculated treatments (Tables 4 to 7). These results are similar to other studies where soybean inoculation did not increased yields in sites where soybean was previously grown (Carciochi et al., 2019; De Bruim et al., 2010). Mendes et al. (2014) suggested that soybean selects a specific microbial community that inhabits the rhizosphere based on functional characteristics, which may be related to benefits to the plant, such as growth and nutrition promotion, such as rhizobia. Furthermore, the authors reported that long-term cultivation in an area strengthens this selective power of the crop for beneficial microorganisms for the plant.

Although the rhizobium naturalization in soils occurs with soybean cultivation, Hungria, Campo, and Mendes (2001) emphasized the need for inoculation with selected and efficient strains throughout the season even in areas with a high population of rhizobia in the soil. In this case, many soil microorganisms are inactive and environmental limitations can prevent symbiosis from occurring. Thus, the presence of efficient bacteria for BNF needs to be guaranteed through inoculation.

The *Bradyrhizobium japonicum* concentration cells of the inoculants tested in this study were higher than what is specified and required for inoculants with Rhizobium, according to Article. 1 of Normative Instruction No. 13, of March 24, 2011. However, the number of bacterial cells recovered from pre-inoculated seeds (Table 4) was

below the recommended by the research ( $6.0 \times 10^5$  cells per seed) to guarantee the success of nodulation and BNF (Hungria et al., 2007; RELARE, 2014). Although, in Brazil there is no official definition of a minimum limit value of bacterial cells recovered from seeds established by the Ministry of Agriculture, Livestock, and Food Supply. The storage of seeds inoculated with SIGNUM<sup>®</sup> technology and treated with Standak Top<sup>®</sup> or Maxin XL<sup>®</sup> + Cruiser<sup>®</sup> in pre-inoculation treatments at 20 and 35 days before sowing reduced the survival of bacterial cells in the seeds. This reduction in the number of cells recovered from treated seeds is a concern that has been reported in other studies (Costa et al., 2013; Pereira et al., 2010). Some authors have reported that this reduction is related to the toxicity of phytosanitary products used in seed treatment for bacteria present in inoculants (A. S. F. D. Araújo & R. S. Araújo, 2006; Hartley et al., 2012; Marks et al., 2013), characterized as a challenge to be overcome to guarantee the efficiency of the soybean inoculation technology.

The low recovery of cells in seeds in all treatments did not reflect losses to nodulation. Soybean nodulation (number of nodules and nodule dry mass) was adequate in all locations (Tables 4 to 7). According to Hungria et al. (2007), a well-nodulated soybean plant at the time of flowering should have between 15 to 30 nodules or 100 to 200 mg of dry nodules per plant.

Although some treatments in all locations showed differences in the root or shoot dry mass compared to the standard inoculation, all treatments showed yield and nitrogen exported in the grains similar or even higher than the standard inoculation (Tables 4 to 7). These results indicate that pre-inoculation with SIGNUM<sup>®</sup> technology promoted plant development, BNF, nodulation, and soybean production. Furthermore, all treatments in the experiments of Londrina, Ponta Grossa, and Santa Tereza do Oeste showed higher yields than the national mean for the 2017/18 growing season (*i.e.*, 3,394 kg ha<sup>-1</sup>) and the mean for the Paraná State (*i.e.*, 3,508 kg ha<sup>-1</sup>), which has the highest national yield. Although the yield in Pato Branco was above 2,000 kg ha<sup>-1</sup>, as recommended to validate tests with soybean by MAPA Normative Instruction No. 30 of 2010 (DOU 11/17/2010) (MAPA, 2010), the values were below the national and state means. In this case, yield may have been affected by the 25-day drought period between December 2017 and January 2018, a critical period for soybean, as flowering and the beginning of grain filling occur during this period, which may have led to a reduction in production.

Pre-inoculation technologies have already been reported as successful in several studies, aiming to optimize the sowing process and stimulate and expand the use of inoculants in soybean cultivation (Gemell et al., 2005; Zilli et al., 2010; Anghinoni et al., 2017; Machineski et al., 2018; Hungria et al., 2020). In this study, pre-inoculation with SIGNUM<sup>®</sup> at 20 or 35 days before planting did not differ from the treatment with standard inoculation, regardless of the used seed treatment.

## References

- Alves, A. C. O., & Aguila, L. S. H. D. (2020) *A importância da fixação biológica para a cultura da soja*. Embrapa Clima Temperado-Artigo em Anais de Congresso (ALICE), Semana Integrada UFPel, 6, Congresso de Iniciação Científica, 19, Pelotas.
- Anghinoni, F. B. G., Braccini, A. L., Scapim C. A., Anghinoni G., Ferri, G. C., Suzukawa, A. K., & Tonin, T. A. (2017). Pre-Inoculation with *Bradyrhizobium* spp. in industrially treated soybean seeds. *Agricultural Sciences*, 8(7), 582-590. <https://doi.org/10.4236/as.2017.87044>
- Araújo, A. S. F. D., & Araújo, R. S. (2006). Sobrevivência e nodulação do *Rhizobium tropici* em sementes de feijão tratadas com fungicidas. *Ciência Rural*, 36(3), 973-976. <https://doi.org/10.1590/s0103-84782006000300039>
- Araujo, R. S., Cruz, S. P., Souchie, E. L., Martin, T. N., Nakatani, A. S., Nogueira, M. A., & Hungria, M. (2017). Preinoculation of soybean seeds treated with agrichemicals up to 30 days before sowing: Technological innovation for large-scale agriculture. *International Journal of Microbiology*, 2017, Article ID 5914786. <https://doi.org/10.1155/2017/5914786>
- Campos, B. H. C. D., & Gnatta, V. (2006). Inoculantes e fertilizantes foliares na soja em área de populações estabelecidas de *Bradyrhizobium* sob sistema plantio direto. *Revista Brasileira de Ciência do Solo*, 30, 69-76. <https://doi.org/10.1590/s0100-06832006000100008>
- Carciochi, W. D., Rosso, L. H. M., Secchi, M. A., Torres, A. R., Naeve, S., Casteel, S. N., & Ciampitti, I. A. (2019). Soybean yield, biological N<sub>2</sub> fixation and seed composition responses to additional inoculation in the United States. *Scientific Reports*, 9(1), 1-10. <https://doi.org/10.1038/s41598-019-56465-0>
- CONAB (Companhia Nacional de Abastecimento). (2017). *Acompanhamento da safra brasileira: Grãos. Décimo segundo levantamento*. CONAB: Brasília, Brazil. Retrieved from: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos?start=50>

- Costa, M. R., Cavalheiro, J. C. T., Goulart, A. C. P., & Mercante, F. M. (2013). Sobrevivência de *Bradyrhizobium japonicum* em sementes de soja tratadas com fungicidas e os efeitos sobre a nodulação e a produtividade da cultura. *Summa Phytopathologica*, 39, 186-192. <https://doi.org/s0100-54052013000300007>
- De Bruin, J. L., Pedersen, P., Conley, S. P., Gaska, J. M., Naeve, S. L., Kurle, J. E., ... Abendroth, L. J. (2010). Probability of yield response to inoculants in fields with a history of soybean. *Crop Science*, 50(1), 265-272. <https://doi.org/10.2135/cropsci2009.04.0185>
- EMBRAPA. (2020). *Fixação biológica de nitrogênio—Perguntas e respostas*. Embrapa, Brasília. Retrieved from <https://www.embrapa.br/tema-fixacao-biologica-de-nitrogenio/perguntas-erespostas>
- Gemell, L. G., Hartley, E. J., & Herridge, D. F. (2005). Point-of-sale evaluation of preinoculated and custom-inoculated pasture legume seed. *Australian Journal of Experimental Agriculture*, 45, 161-169. <https://doi.org/10.1071/ea03151>
- Hartley, E. J., Gemell, L. G., & Deaker, R. (2012). Some factors that contribute to poor survival of rhizobia on preinoculated legume seed. *Crop & Pasture Science*, 63(9), 858-865. <https://doi.org/10.1071/CP12132>
- Hungria, M., & Nogueira, M. A. (2020) *Fixação biológica de nitrogênio*. Embrapa Soja-Capítulo em livro científico (ALICE).
- Hungria, M., Campo, R. J., & Mendes, I. D. C. (2001). *Fixação biológica do nitrogênio na cultura da soja*. Embrapa Soja-Circular Técnica (INFOTECA-E), Brasília, Brazil.
- Hungria, M., Campo, R. J., & Mendes, I. D. C. (2007). *A importância do processo de fixação biológica do nitrogênio para a cultura da soja: Componente essencial para a competitividade do produto brasileiro* (Documentos/Embrapa Soja, n. 283, p. 80). Londrina, Brazil: Embrapa Soja: Embrapa Cerrados.
- Ikuta, N. (1995). *Desenvolvimento de métodos de identificação e quantificação de Bradyrhizobium japonicum* (p. 90, PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre).
- Lima, S. C., Lopes, E. S., & Lemos, E. G. M. (1998). Caracterização de rizóbios (*Bradyrhizobium japonicum*) e produtividade da soja. *Scientia Agricola*, 55, 360-366. <https://doi.org/10.1590/S0103-90161998000300003>
- Machineski, G. S., Scaramal, A. S., Matos, M. A., Machineski, O., & Colozzi Filho, A. (2018). Efficiency of pre-inoculation of soybeans with *Bradyrhizobium* up to 60 days before sowing. *African Journal of Agricultural Research*, 13(24), 1233-1242. <https://doi.org/10.5897/ajar2018.13108>
- MAPA (Ministério da Agricultura, Pecuária e Abastecimento), Secretaria De Defesa Agropecuária. (2010). Instrução Normativa, nº 30, 12 Nov. 2010. *Estabelecer os métodos oficiais para análise de inoculantes, sua contagem, identificação e análise de pureza na forma desta Instrução Normativa* (2 Seção 1, p. 28). Diário Oficial [da] República Federativa do Brasil, Brasília.
- MAPA (Ministério da Agricultura, Pecuária e Abastecimento), Secretaria De Defesa Agropecuária. (2015). Ato Portaria Nº 177, 30 Jul 2015. *Aprovar o Zoneamento Agrícola de Risco Climático para a cultura de soja no Estado do Paraná, anosafra 2015/2016* (p. 9). Diário Oficial [da] República Federativa do Brasil, Brasília.
- Marks, B. B., Bangel, E. V., Tedescom V., Silva, S. L. C., Ferreira, S. B., Vargas, R., & Silva, G. M. (2013). Avaliação da sobrevivência de *Bradyrhizobium* spp em sementes de soja tratadas com fungicidas, protetor celular. *Revista Internacional de Ciências*, 3(1). <https://doi.org/10.12957/ric.2013.7063>
- Meert, L., Muller, M. M. L., Genú, A. M., Espindola, J. S., Aragão, G. N., & Figueiredo, A. S. T. (2020) Different inoculating, forms of inoculation and their effects on the agronomic characteristics of soy culture. *Research, Society and Development*, 9(10), e2969108499. <https://doi.org/10.33448/rsd-v9i10.8499>
- Mendes, L., Kuramae, E., Navarrete, A., van Veen, J. A., & Tsai, S. M. (2014). Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME Journal*, 8, 1577-1587. <https://doi.org/10.1038/ismej.2014.17>
- Mendoza-Suárez, M., Andersen, S. U., Poole, P. S., & Sánchez-Cañizares, C. (2021). Competition, Nodule Occupancy, and Persistence of Inoculant Strains: Key Factors in the Rhizobium-Legume Symbioses. *Front. Plant Sci.*, 12, 690567. <https://doi.org/10.3389/fpls.2021.690567>
- Miyazawa, M., Pavan, M. A., & Bloch, M. F. M. (1992). *Análise química de tecido vegetal* (Circular 74, p. 17). Londrina: IAPAR.

- Moreira, A., Motta, A. C. V., Costa, A., Muniz, A. S., Cassol, L. C., Zanão Júnior, L. A., ... Pauletti, V. (2017). *Fertilization and liming manual for the state of paran* (p. 482). Curitiba: SBCS.
- PAS CAMPO. (2005). *Manual de seguran e qualidade para a cultura da soja* (p. 69). Braslia: EMBRAPA, Transferncia de Tecnologia. Retrieved from <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/116424/1/MANUALSEGURANCAQUALIDADEParaaculturadesoja.pdf>
- Pavan, M. A., Bloch, M. F., Zempulski, H. C., Miyazawa, M., & Zocoler, D. C. (1992). *Manual de anlise qumica de solo e controle de qualidade* (Circular 76, p. 40). Londrina, IAPAR.
- Pedrozo, A., Oliveira, N. J. G., & Alberton, O. (2018). Biological nitrogen fixation and agronomic features of soybean (*Glycine max* (L.) Merr.) crop under different doses of inoculant. *Acta Agron.* 67(2), 297-302. <https://doi.org/10.15446/acag.v67n2.56375>
- Pereira, C. E., Oliveira, J. A., Costa Neto, J., Moreira, F. M. D. S., & Vieira, A. R. (2010). Tratamentos inseticida, peliculizao e inoculao de sementes de soja com rizbio. *Revista Ceres*, 57, 653-658. <https://doi.org/10.1590/s0034-737x2010000500014>
- RELARE. (2014). *Reunio da Rede de Labortorios para Recomendao, Padronizao e Difuso de Tecnologia de Inoculantes Microbianos de Interesse Agrcola* (Documentos 350, p. 80). EMBRAPA Soja, Braslia.
- SAS Institute Inc. (2019). *SAS/STAT Product Documentation*. SAS Institute Inc., Cary, NC. Retrieved from <http://support.sas.com/documentation/onlinedoc/stat>
- Silva, K., Silva, E. E., Farias, E. N. C., Chaves, S., Albuquerque, C. N. B., & Cardoso, C. (2018). Agronomic efficiency of *Bradyrhizobium* preinoculation in association with chemical treatment of soybean seeds. *African Journal of Agricultural Research*, 13(14), 726-732. <https://doi.org/10.5897/AJAR2018.13016>
- Van Rhyn, P., & Vanderleyden, J. (1995). The Rhizobium-plant symbiosis. *Microbiological Reviews*, 59, 124-142. <https://doi.org/10.1128/mr.59.1.124-142.1995>
- Zilli, J. E., Campo, R. J., & Hungria, M. (2010). Eficcia da inoculao de *Bradyrhizobium* em pr-semeadura da soja. *Pesquisa Agropecuria Brasileira*, 45(3), 335-338. <https://doi.org/10.1590/S0100-204X2010000300015>
- Zilli, J. E., Gianluppi, V., Campo, R. J., Rouws, J. R. C., & Hungria, M. (2010). Inoculao da soja com *Bradyrhizobium* no sulco de semeadura alternativamente  inoculao de sementes. *Revista Brasileira de Cincia do Solo*, 34(6), 1875-1881. <https://doi.org/10.1590/S0100-06832010000600011>

## Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).