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INFLUENCE OF THE DATE OF APPLICATION OF INOCULANTS VIA SEEDS ON BIOLOGICAL NITROGEN FIXATION AND SOYBEAN PRODUCTIVITY

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Abstract: The objective was to evaluate biological nitrogen fixation parameters and grain yield in soybeans submitted to different treatments involving early sowing dates in association with pesticides, inoculants and protector. The experiment was installed in the 2018/19 harvest at the Centro Regional Center Norte, Pindorama –SP. The treatments were: T1= control; T2 = 200 kg ha-1 of N (urea) in installments; T3 = standard inoculation with Biomax ® Premium L Soja (A) on the day of sowing; T4 = Biomax ® 10 (B) + Max Protection (C) on the day of sowing; T5 and T6 = T4 applied respectively, at 7 and 14 days before sowing. The doses of biological inputs applied via seeds corresponded to 100 mL/ 50 kg seeds for A and B and 50 mL/ 50 kg seeds for C, respectively. all seeds received the technological package from Bayer with the commercial products Derosal Plus at a dose of 2 mL/kg of seed and Cropstar (5 mL/kg). The experimental plot consisted of 4 lines of 15 m, with spacing between lines of 0.5 m. The experimental design was DBC with six treatments and 4 replications. After collecting plants in R3, the total number of nodules (NNODT) and dry mass of total nodules (MSNODT), total nitrogen accumulated in the shoot (NPA) were measured; shoot dry mass (MSPA) and root dry mass (MSR). At the time of maturation (R8) the grain yield (PG) and later the total nitrogen accumulated in the grains (NG) were evaluated. It was found that T4 (Biomax ® + Max Protection) applied on the day of sowing promoted increases in NNODT, MSNODT, NPA, MSPA and MSR with respectively 32.67 nodules/plant, 410 mg/plant, 34.45 g.kg-1, 31.17 and 5.44 g/ plant. As for PG, treatment T4 with 3037.50 kg/ha was similar to treatments T5 and T6, whose pre-inoculation time was longer. It is concluded that the longer the storage time of seeds with pesticides, inoculants and protectors, most of the variables related

to biological nitrogen fixation are reduced. The application of inoculant + protector associated with Bayer's technological package is recommended as long as it does not exceed 14 days before sowing the soybean. inoculants and protectors, most variables related to biological nitrogen fixation are reduced. The application of inoculant + protector associated with Bayer's technological package is recommended as long as it does not exceed 14 days before sowing the soybean. inoculants and protectors, most variables related to biological nitrogen fixation are reduced. The application of inoculant + protector associated with Bayer's technological package is recommended as long as it does not exceed 14 days before sowing the soybean.
Keywords: Glycine max, early inoculation, insecticides, fungicides.

INTRODUCTION

The technological use of biological nitrogen fixation (BNF) makes it possible to achieve high productivity in soybean crops by eliminating the application of nitrogen fertilizers. Currently, biological input based on bacteria of the genus Bradyrhizobium is applied to seeds on the day of sowing. This generates extra work and demands additional time and labor at the time of planting, which can lead to a reduction in annual re-inoculation by the soybean farmer (MACHINESKI, 2015).

On the other hand, the interaction of bacteria of the genus Bradyrhizobium with pesticides can modify the efficiency of BNF and reduce nodulation. When seeds are treated with fungicides and insecticides, effects such as delay in the insertion of the bacteria into the plant root, reduction in the number of formed nodules, in the rate of fixation and consequently in the crop yield can be observed (Fox et al., 2007). The reduction in the formation of nodules of the Bradyrhizobium bacterium in seeds treated with pesticides is greater in soils of first cultivation with soybean, and may also vary according to the type of soil, with greater problems presented, particularly in soils with sand ridges (Hungary et al., 2007). Pesticides can harm the association of the legume with Bradyrhizobium, not only reducing nodulation, supply of carbohydrates to existing nodules and inhibition or inactivation of signaling between the bacterium and the host plant for the initiation of nodulation. Inhibition of biochemical signaling between host plant and Bradyrhizobium, and blocking of communication can also be observed, delaying or inhibiting the onset of symbiosis (Anderson et al., 2004; Fox et al. 2007; Pereira et al., 2010; Ahemad and Khan, 2013).

Even so, new technologies are being studied as a way to eliminate the need for concomitant performance of some phases of inoculation by the soybean farmer, among them is the pre-inoculation of seeds with the Bradyrhizobium strains recommended for soybean. The use of these new technologies can eliminate the preparation and application of the inoculant in the seeds at the time of planting, and can increase the efficiency of BNF in soybeans, since the seeds will be commercialized pre-inoculated with the guarantee of the minimum of viable cells of rhizobia (MACHINESKI, 2015).

In search of optimizing the survival of the bacteria, and enabling the practice of early inoculation, osmoprotective products can be used. Such substances provide the formation of a film preventing direct contact with the inoculant and providing substrate for the survival of the bacteria during the period that precedes the symbiosis. Complementing the function of osmoprotectants, inoculants with a higher concentration of bacteria can be used, as well as substances for "communication" between seedlings and bacteria in order to potentiate an early formation of nodules

(FIPKE, 2015).

In view of the above, the objective of the present work is to analyze the productivity and parameters of biological nitrogen fixation in soybeans cultivated in Pindorama-SP, 2018/19 harvest, submitted to different treatments in the seeds, involving inoculants with different concentrations of bacteria associated or not. the use of protector applied 0, 7 and 14 days before sowing, associated with Bayer's technological package.

MATERIAL AND METHODS

The experiment was installed under field conditions, on November 22, 2018 and collected on April 4, 2019 at the Regional Center for Technological Development of Agribusiness in the North Center, linked to the Paulista Agency of Agribusiness Technology -APTA, located in the municipality of Pindorama – SP. The relief of the region is undulating with altitudes ranging from 498 to 594 m, whose geographic coordinates are 21º 13' south latitude and 48º 55' west longitude.

The climate fits, sKöppen Climate Classification (1948), in Aw, defined as a mesothermic climate with dry winter, where the average temperature of the coldest month is below 18 °C and of the warmest month, above 22 °C. . Table 1 shows the monthly meteorological data for the North Central Pole, and the averages for maximum and minimum temperatures were 31.13 and 19.55 °C, respectively, with an average monthly rainfall of 167.3 mm, lower than that of the stride which was 200 mm (CIIAGRO, 2019).

The treatments tested, as well as the doses of inoculants and protector used in the present work are described in Table 2.

The experimental plot consisted of 4 lines of 15 m in length, and the two central lines of 15 m in length and spacing between lines of 0.5 m (15 m^2) were considered as useful area. Thus, the experimental design was randomized blocks composed of the six treatments mentioned above with 4 replications, in a total of 24 experimental plots.

The description of the inoculants used in these experiments follows below:

The) Biomax® Premium Líquido Soja (standard inoculant): liquid inoculant for soybeans, registered and produced by Vittia Fertilizantes e Biológicos S/A, with the guarantee of Bradyrhizobium bacteria (Semia 5080 strain) and (Semia 5079 strain), at a concentration of 7 x 10^9 colony forming units/ mL.

B) Biomax ® 10: Liquid inoculant for soybeans, registered and produced by Vittia Fertilizantes e Biológicos S/A, with a higher concentration of CFU (colony forming units) per liter.• Warranty: 1x10^{10} CFU/mL. • Lowest dosage on the market, reducing the volume of solution used in seed inoculation.

c) Max Protection: Additive for the inoculant that guarantees protection and adherence of bacteria to the seeds during inoculation, guaranteeing greater efficiency in nodulation. It has in its formulation a source of energy for the bacteria, guaranteeing the concentration and potentiating the efficiency of the inoculation.

Before starting the experiment, soil samples were collected from the experimental area for further chemical and granulometric analysis, in addition to counting Bradyrhizobium bacteria and associative diazotrophic bacteria from the soil before sowing. Bacteria counting was performed at the Laboratory of Agricultural Microbiology of FCAV/UNESP, Jaboticabal/SP campus, according to the recommendations of Dobereiner et al. (1995). The values found in the sample were: 6.69 x 10^7 CFU g^{-1} of dry soil of total bacteria, 3.56 x 10^7 CFU g^{-1} of dry soil of Bradyrhizobium bacteria and 3.5 x 10^6 CFU g^{-1} of dry soil of diazotrophic bacteria.

Soil samples for chemical (RAIJ et al.,

MONTH	ABSOL max temp	temp min ABSOL	temp max MONTHLY	Temp Min. MONTHLY	AVERAGE Temp	PRICE	DCCH
	----------°C---------- ----------					mm	days
NOV 2018	34.5	16.2	29.9	19.3	24.6	142.8	17
DEC 2018	36	14	31.9	20.1	26	139.1	16
JAN 2019	36.4	18.7	32.8	20.7	26.8	78.7	14
FEB 2019	36.8	17.3	31	19.9	25.4	254.7	19
MAR 2019	34.4	16.6	30.9	19.4	25.2	242.6	13
APR 2019	33.9	12.3	30.3	17.9	24.1	145.9	10

DCCH = rainy days of the month; precip = precipitation; temp = temperature; absol. = absolute.

Table 1. Monthly meteorological data from Pindorama-SP, referring to the period in which the experiment of inoculation of soybean in pre-sowing was carried out using biological inputs associated with the use of pesticides. Agricultural Year 2018/19. North Central Regional Pole. Pindorama-SP.

Source CIIAGRO: (2019).

Number	Treatments	Inoculants Dose	Max Protection Dose
T1	Witness (no inoculation)	---	----
T2	200 kg ha-1 of N (split at base and top cover)	---	----
T3	Standard Inoculation1 (Biomax® Premium Soy Liquid) applied via seed on the day of sowing	100 mL / 50 kg seeds	----
T4	Inoculation (Biomax®10 + Max Protection) applied via seed on the day of sowing.	100 mL / 50 kg seeds	50 mL / 50 kg seeds
T5	Pre-inoculation (Biomax®10 + Max Protection) applied via seed 7 days before sowing.	100 mL / 50 kg seeds	50 mL / 50 kg seeds
T6	Pre-inoculation (Biomax®10 + Max Protection) applied via seed 14 days before sowing.	100 mL / 50 kg seeds	50 mL / 50 kg seeds

Table 2. Treatments and doses of inoculants and protector used to conduct the pre-sowing soybean inoculation experiment using biological inputs associated with the Bayer technological package. Agricultural Year 2018/19. North Central Regional Pole. Pindorama-SP.

2001) and granulometric (DAY, 1965) characterization were collected in October 2018, in the 0-0.20 m depth layer, and the results obtained were: pH (CaCl2) = 5.80; MO = 10.00 g dm^{-3}; CO = 5.8 g dm^{-3}; P =36.00 mg dm^{-3} ;K =3.1 mmol dm^{-3}; Ca = 26.00mmolc dm^{-3}; Mg = 11.00mmolc dm^{-3}; H + Al =16.00 mmolc dm^{-3}; V = 71%,Total Sand = 892 g kg^{-1} of soil; Clay = 72 g kg^{-1} of soil and Silt = 36 g kg-of soil, with conventional soil preparation.

Sowing fertilization was carried out with fertilizer formulated 4-30-16, at a dose of (350 kg. ha^{-1}). Only in Treatment T2 (200 kg ha^{-1} of Nitrogen) the remaining dose of N was manually applied, being half in the base and half in coverage with the use of the urea source, at 35 days after emergence.

The soybean cultivar used was BRS 7380 RR. This cultivar is one of the highlights of the new generation of RR cultivars from Embrapa's genetic improvement program, being transgenic, free of technological fee by patent, and has an early cycle, maturity group 7.3, resistance to the herbicide glyphosate, and associates resistance to races 3, 4, 6, 9, 10 and 14 of the soybean cyst nematode (Heterodera glycines) with resistance to the two root-knot nematodes, Meloidogyne incognita and Meloidogyne javanica, as well as a low multiplication factor to the nematode Pratylenchus brachyurus. Due to these characteristics of multiple resistance to nematodes and its early cycle, it allows its use in the production system in succession of cultures in regions whose soils have a history of problems with these nematodes,

Thirty-five m^{-1} seeds were sown, in order to obtain 16 plants per linear meter. For this, thinning was carried out in order to obtain the final average population of 320,000 plants ha^{-1}.

Thus, in the laboratory, before sowing,The procedures aimed at treating the seeds were carried out with the Bayer technological package: composed of the commercial products Derosal Plus at a dose of 2 mL per kg of seed and Cropstar at a dose of 5 mL per kg of seed, this package being commonly used for six treatments tested.In On Farm Treatment, care must be taken to maintain a good quality in the process, not harming the functioning of the products, and consequently, the emergence of plants (BAYER BRASIL, 2019). It takes a trained professional, in addition to specific and well-regulated machines. Subsequently, to compose the different treatments regarding the use of biological inputs from the company Vittia Fertilizantes e Biológicos S/A, only treatments T3, T4, T5 and T6 were prepared according to the procedures described in Table 1, and treatments T1 and T2 had no the addition of inoculants or additives.

Some precautions were taken to ensure greater efficiency of inoculants, such as seed inoculation carried out in the shade and uniform distribution of inoculants in all seeds. Thus, there was no direct contact between the inoculants and the fungicides used in the seed treatment.

Fertilizer containing the micronutrients cobalt and molybdenum was applied via foliar spraying at the V_5 phenological stage (FEHR; CAVINESS, 1977), in all treatments, including the control. Disease and pest control was also carried out using fungicides and insecticides when necessary.

All soybean cultivation techniques, such as cultivar choice, sowing time, plant population, weed control, insects and diseases followed the technical recommendations for soybean cultivation by EMBRAPA (2013).

At full flowering, 3 plants were collected per experimental plot. Thus, the parameters evaluated were: total number of nodules (NNODT); total dry nodule mass (MSNODT) in mg $plant^{-1}$, shoot dry mass (MSPA) and root (MSR) in g $plant^{-1}$, in addition to plant height at flowering (APF) in cm and nitrogen

content accumulated in shoot (NPA) in g. kg^{-1} determined by the methodology described by Bataglia et al. (1983). And at the time of maturation (R8) it was also evaluatedgrain yield (PG) = harvestedon the two central lines of 15 m in length and spacing between lines of 0.5 m. From the average values referring to the production of the plots of each treatment, the productivity was calculated, being expressed in kg ha^{-1} (values corrected for 13% of humidity). Subsequently, it was determinedtotal nitrogen content in grains in g.kg^{-1} (NTG), following the methodology described by Bataglia et al. (1983).

For the parameters, the estimated and applied Box-Cox transformations were performed as proposed by Hawkins and Weisberg (2017), with the average values maintained in the original scale. The variances, standard deviations, coefficients of variation, DMS, analysis of variance and mean comparisons were calculated with the transformed data. Subsequently, the normality of the residues was verified by the Shapiro-Wilk test at 5% probability (ROYSTON, 1995). And also Homoscedasticity through the homogeneity of variances by Levene's test at 5% probability (GASTWIRTH et al., 2009). When significant differences were detected in the analysis of variance, the means were compared by Tukey's test at 5% probability. In the specific case of this test in the MSPA and MSR parameters and for PG, there were indications of inhomogeneity of variances by Levene's test at 5% probability. Analyzes of variance were then performed by the Feasible Weighted Least Squares method with White's correction (1980), as described by Long and Ervin (2000). Mean comparisons were performed using the Games-Howell (1976) non-parametric test. The analyzes were performed with the aid of the AgroEstat Software, online version (MALDONADO JUNIOR, 2019).

RESULTS AND DISCUSSION

Table 3 shows the average results obtained in the parameters evaluated at flowering. The F test detected highly significant statistical significance ($p < 0.01$) for all variables analyzed.

For the total number of nodules, it was verified that the treatment that used the inoculation with Biomax® 10 + Max Protection applied in the seed treatment on the day of sowing, that is, the treatment (T4) was the highlight in terms of the number of nodules, presenting the highest average value of 32.67 nodules $plant^{-1}$, being this treatment statistically superior to the others tested in the present work. Still in relation to the number of nodules, it is observed that with intermediate values, treatments T3 and T5 were positioned, which were statistically equivalent to each other, with respectively (8.92 and 10.59 nodules) and superior to treatments T1 and T2, which differed statistically. with each other presenting respectively 6.17 and 3.50 nodules $plant^{-1}$. The results obtained in the present experiment corroborate those of Fagan et al. (2007) who report thatSoybean plants submitted to chemical nitrogen fertilization have a significant reduction in the development of nodules. Already, Silva et al. (2019) found in a study involving nodulation in soybean plants subjected to different fertilization, no effect of inoculation on the number of nodules when plants were kept under chemical nitrogen fertilization.

In the initial phase, between 10 and 15 days after emergence, about 4 to 8 nodules per plant must be found and in the flowering stages (R1 and R2), in well nodulated plants, there must be 15 to 30 nodules per root system (HUNGARY et al., 2007). In this work, the plants that were collected were already in the full flowering stage, however, they presented a reduced number of nodules, that is, a little below the recommended for the general

average of the experiment, which was (11.50 total nodules planted^{-1}). Only for T4, an average value of NNODT was found above the recommended range (32.67 nodules. plant^{-1}). Silva et al. (2019) also obtained a lower number of nodules than recommended in full flowering and report that this fact is possibly linked to the plants having been grown at an inappropriate sowing time for the cultivar, directly affecting the photoperiod. According to Alcantara Neto et al. (2012) this fact significantly alters the stages of development and productivity. On the other hand, Deaker et al. (2004) describe that a well performed inoculation protects bacterial cells, providing the maintenance of a large amount of viable cells. Thus, resulting in greater number of nodules and survival of bacteria in the soil under normal seeding conditions, where crop emergence occurs within the expected time and normal conditions. (2012) this fact significantly alters the stages of development and productivity. On the other hand, Deaker et al. (2004) describe that a well performed inoculation protects bacterial cells, providing the maintenance of a large amount of viable cells. Thus, resulting in greater number of nodules and survival of bacteria in the soil under normal seeding conditions, where crop emergence occurs within the expected time and normal conditions. (2012) this fact significantly alters the stages of development and productivity. On the other hand, Deaker et al. (2004) describe that a well-performed inoculation protects bacterial cells, providing the maintenance of large numbers of viable cells. Thus, resulting in higher nodule numbers and survival of bacteria in the soil under normal seeding conditions, where crop emergence occurs within the expected time frame and normal conditions.

Another consideration is that T4 increased by 67.49% the amount of nodules compared to T5, thus, certainly, the longer storage time of inoculated seed (7 days) in pre-planting associated with pesticides certainly decreased the development of nodules of bacteria contained in the inoculant Biomax® 10, even with the use of the protector Max Protection. According to Fox et al. (2007) the interaction of bacteria of the genus Bradyrhizobium with pesticides can modify the efficiency of biological nitrogen fixation and reduce nodulation. When seeds are treated with fungicides and insecticides, effects can be observed such as delayed insertion of the bacteria into the plant root, reduction in the number of nodules formed. In research conducted by Cardoso et al. (2019) in evaluations performed on plants that had the bacteria exposed to treatments for 24 hours, it was possible to observe a decrease in the number of nodules for all treatments, indicating little action of pesticides, but marked action of the waiting time for sowing.

In relation to MSNODT, T4 was superior to the other treatments, obtaining 410 mg.plant^{-1}, and with intermediate average values came treatments T5 (173.50 mg) and T6 (191.50 mg) that did not differ from each other, and soon after, treatment T3 with 135 mg.plant^{-1}, which in turn differed statistically from treatments T1 and T2, which were statistically equivalent and had respectively the lowest average for this parameter, which was 79.80 and 69.30 mg planta^{-1} . In general, the T2 treatment with 69.30 mg of MSNODT also showed a smaller increase in the weight of nodules. Compared to other studies, mass between 100 and 200 mg is sufficient to guarantee the supply of N required by a soybean plant for its normal development (HUNGRIA et al., 2007; BRANDELERO et al., 2009, BARBARO et al., 2009).

Still in relation to MSNNOT, it is noted that T4 showed respectively 57.68 and 53.29 % more nodule dry mass when compared to treatments T5 and T6, whose inoculation

was anticipated in 7 and 14 days respectively. According to Zilli et al. (2010) seed treatments with fungicides and inoculant in pre-seeding can reduce the number of nodule and nodule mass, when compared with the results obtained for the control, which had the seeds treated only with inoculant.

For the accumulation of nitrogen in the aerial part (NPA), it can be seen in Table 3, that the treatment T2 which used only chemical nitrogen fertilization showed the highest content of nitrogen in the aerial part (34.45 g.kg-1) being statistically superior to the others tested in this work. This result infers that the correct position in terms of nitrogen accumulation in the aerial part is due to the fact that treatments T3 and T4, because they consist of applying soybean inoculant on the day of sowing, probably act favoring a greater survival of the symbiotic bacteria that provide the plant with nitrogen produced by the BNF process. In turn, the treatment T5 that consisted of the use of Biomax ® 10 + Max Protection applied seven days before sowing was statistically inferior to the above treatments showing 29.25 $g.kg^{-1}$ and following this same logic of survival of bacteria it was statistically superior to T6 whose advance application of inputs were 14 days before planting. With the worst value of nitrogen accumulation in the aerial part, as expected, was the treatment T1, non-inoculated control, with 23.65 $g.kg^{-1}$. The N-fertilizer is easily absorbed by the plant, because it is readily available. However, in the case of biological N, the energy expenditure of the plant is higher, because it requires a greater expenditure of initial energy for the formation of nodules (HUNGRIA et al., 2007).

When analyzing the DMPA, similar to nodulation, it was noted that the treatment (T4) that made use of inoculant with a higher concentration of bacteria and protector applied on the day of sowing was statistically superior to the other treatments, with 31.17 gplant-1. The smallest increases in leaf dry mass were obtained in treatments T1, T3 and T6. Also in relation to this parameter, it was found that T5 that made use of this inoculant with higher concentration of bacteria associated with the protector only applied 7 days before sowing increased in a statistically intermediate way when compared to the other treatments tested being statistically inferior to T4 and T2 and on the other hand superior to treatments T1, T3 and T6. Cardoso et al. (2019) verified that the plants with the highest mean MSPA were those from seeds sown immediately after the treatment performed only with inoculant.

For RSM, T4 held 5.44 $gplant^{-1}$ and thus, promoted greater root dry mass and did not differ statistically from treatments T2 and T5, the other treatments tested being statistically inferior in relation to this parameter and statistically equivalent among themselves (Table 3).

In Table 4, analyzing the content of nitrogen accumulated in grains (NG) it is noted in this work that the non-inoculated control (T1) corresponded to the treatment with the lowest average value (39.00 $g.kg^{-1}$), although not statistically different from T6 and T3, which showed an average of 41.40 and 41.50 $g.kg^{-1}$, respectively. Treatments T4, T2 and T5 stood out in this evaluation with the highest averages. The overall average of this parameter in the experiment was 43.22 $g.kg^{-1}$. The N-fertilizer is easily absorbed by the plant because it is readily available. However, in the case of biological N, the energy expenditure of the plant is higher, because it requires a greater expenditure of initial energy for the formation of nodules (HUNGRIA et al., 2007). Thus, it becomes important to identify soybean genotypes with a highly efficient symbiosis, aiming to obtain a performance equal, or superior, to that of a plant supplied with synthetic nitrogen fertilizers (LIBORIO,

2019). Zilli et al. (2010) in a study on the effectiveness of Bradyrhizobium inoculation in soybean pre-seeding, observed in the grains that all inoculated treatments, and also the treatment with nitrogen fertilizer, provided greater N accumulation than the control.

In relation to grain yield (PG), (Table 4) an overall average of 2837.92 kg.ha^{-1} was observed for the treatments, showing a low performance of soybean culture in the 2018/19 crop year; the national and São Paulo State productivity average for the 2017/18 crop was respectively 3362 and 3440 kg ha^{-1} (CONAB, 2019). This result obtained was linked to the veranico that occurred in the month of January 2019 that probably interfered negatively in grain productivity (CIIAGRO, 2019). According to Pastore (2016), the known "Veranicos" are events that make it difficult to obtain maximum yields, because they impact nodulation, biological fixation, and depending on the phase in which they occur in relation to the crop, they hit it at the time when it requires the greatest water needs, making the crop unable to overcome the momentary stress in periods such as flowering and grain filling.

Treatment T4, which made use of the inoculant Biomax ® 10 + Max Protection applied on the day of sowing was responsible for the highest yield of 3037.50 kg ha^{-1} not statistically different from treatments T3, T5 and T6. In turn, it is important to note that the treatments T1 and T2 had equal statistics and showed grain yields of 2710 and 2745 kg ha^{-1} In terms of increases in PG, it is noted in this study that T4 increased productivity by 327.50 kg ha^{-1} or 5.46 bags ha^{-1} when compared to T1. On the other hand, there is also a significant increase in productivity when comparing, for example, the treatment T5, which used the same inputs used in T4, but with inoculation performed 7 days prior to planting, which provided an increase of 132.50 kg ha^{-1} or 2.21 bags ha^{-1} when compared to T1, the non-inoculated control.

CONCLUSIONS

The period of storage of soybean seeds with Bayer's technological package, inoculated before planting, can reduce the development of nodules and other parameters of biological nitrogen fixation, even with the use of protector;

In terms of grain yield, the use of pesticides in association with the commercial inoculant Biomax® 10 and Max Protection must be applied via seed as close to sowing and not more than fourteen days in advance.

treat	NNODT[1]	NPA	MSPA*[1]	MSR*[1]	MSNODT[1]
	Unit plant-[1]	g.kg-[1]	g plant-[1]		mg plant-[1]
T1	6.17 d	23.65 and	20.83 d	3.68 b	79.80 d
T2	3.50 and	34.45 to	26.50 b	5.27 to	69.30 d
T3	8.92 BC	31.95 b	21.42 CD	3.88 b	135.00 c
T4	32.67 to	31.95 b	31.17 to	5.44 to	410.00 to
T5	10.59 b	29.25 c	23.33 c	5.25 to	173.50 b
T6	7.17 cd	26.90 d	21.17 cd	3.74 b	191.50 b
F	584.10**	126.81**	103.20**	139.47**	222.55**
CV (%)	7.53	0.88	2.73	2.11	2.72
Average	11.50	29.69	24.07	4.54	176.50

Mean of four repetitions followed by the same lowercase letters in the column do not differ from each other by Tukey's test at 5%; * = Comparison of means by the nonparametric test of Games-Howell (1976); T1 = Control (no inoculation); T2 = Nitrogen fertilization with 200 kg nitrogen ha^{-1} (in installments); T3 = Inoculation Standard[1] (Biomax® Premium Liquid Soybean) applied via seed on the day of sowing; T4 = Inoculation (Biomax® 10 + Max Protection) applied via seed on the day of sowing; T5 = Pre-inoculation (Biomax®10 + Max Protection) applied via seed 7 days before sowing; T6 = Pre-inoculation (Biomax®10 + Max Protection) applied via seed 14 days before sowing; NNODT = total number of nodes; NPA = total nitrogen accumulated in the shoot; MSPA = shoot dry mass; MSR = root dry mass; MSNODT=total dry mass of nodules;[1] Average respectively of three plants per repetition.

Table 3. Parameters evaluated at floweringof the experiment of inoculation of soybean in pre-sowing using biological inputs and protector associated with the Bayer technological package. Agricultural Year 2018/19. North Central Regional Pole. Pindorama-SP.

Treatments	NTG	*PG[2]
	g.kg-[1]	kg ha-[1]
T1	39.00 c	2710.00 b
T2	45.60 to	2745.00 b
T3	41.50 BC	2852.50 ab
T4	46.75 to	3037.50 to
T5	45.05 ab	2842.50 ab
T6	41.40 BC	2840.00 ab
F	13.20**	16.21**
CV (%)	0.36	0.94
Average	43.22	2837.92

[2,3] Means followed by the same lower case letters in the column do not differ by Tukey test at 5%; * = comparison of means by non-parametric test of Games-Howell (1976); T1 = Witness (no inoculation); T2 = Nitrogen fertilization with 200 kg nitrogen ha^{-1} (split); T3 = Standard Inoculation[1] (Biomax® Premium Liquid Soy) applied via seed on the day of sowing; T4 = Inoculation (Biomax® 10 + Max Protection) applied via seed on the day of sowing; T5 = Pre-inoculation (Biomax®10 + Max Protection) applied via seed with 7 days before sowing; T6 = Pre-inoculation (Biomax®10 + Max Protection) applied via seed with 14 days before sowing; NTG = nitrogen content accumulated in the grains and PG = grain yield; 2 = average of 4 repetitions, values corrected to 13% humidity.

Table 4. Nitrogen accumulated in grains and yield evaluated in soybean inoculation experiment inpre-sowing using biological inputs from associated with the Bayer technological package. Agricultural Year 2018/19. North Central Regional Pole. Pindorama-SP.

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