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Response of soybean crop with different combinations of seed treatment and application of nitrogen, cobalt, and molybdenum topdressing

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Respuesta del cultivo de soja con diferentes combinaciones de tratamiento de semillas y aplicación de nitrógeno, cobalto y molibdeno como cobertura

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Luciano Moro[1]*, Maik Fernando Franz[1], Martios Ecco[1], Milciades Ariel Melgarejo Arrúa[2] and Marlon Akiyama Ribas[1]

ABSTRACT

Nitrogen is the element most demanded by the soybean crop, and the biological fixation of atmospheric nitrogen is the main means to supply it. In contrast, micronutrients and chemical treatments applied on seeds together with the inoculant can alter the phenomenon of biological fixation of atmospheric nitrogen. This work aimed to evaluate the effect of chemical products, micronutrients, and nitrogen fertilization on the nodulation, development, and yield of soybean. The experiment was developed in a field and a greenhouse in the municipality of Toledo, Brazil. A randomized block with four repetitions was used as an experimental design. This design had eight treatments, namely: T1 - Control (seeds treated with insecticide); T2 - Seeds treated with insecticides and inoculated with *Bradyrhizobium*; T3 - Untreated seeds inoculated with *Bradyrhizobium*; T4 - Seeds treated with insecticides and cobalt-molybdenum (CoMo), inoculated with *Bradyrhizobium*; T5 - Seeds with CoMo inoculated with *Bradyrhizobium*; T6 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with foliar application of CoMo; T7 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with the application of nitrogen in cover; T8 - Seeds treated with nitrogen by broadcast. No significant differences were observed between treatments on the nodules numbers, stem diameter, plant height, root length, the mass of 1000 grains, and yield. The application of nitrogen at the R2 stage (a plant with an open flower in one of the two uppermost nodes of the main stem with a fully developed leaf) and in association with the inoculant + CoMo without seed treatment provided a greater number of nodes, pods, and grains per plant.

Keywords:
Biological nitrogen fixation
Bradyrhizobium japonicum
Glycine max L.
Nodules
Seed treatment

RESUMEN

El nitrógeno es el elemento que presenta mayor demanda por parte del cultivo de soja, y la fijación biológica del nitrógeno atmosférico el principal medio para abastecerlo. En cambio, micronutrientes y tratamientos químicos aplicados a la semilla conjuntamente con el inoculante pueden alterar dicha fijación biológica de nitrógeno. El objetivo de este trabajo fue evaluar el efecto de productos químicos, micronutrientes y de la fertilizacion nitrogenada, en la nodulacion, desarrollo y rendimiento de la soya. El experimento fue desarrollado en campo y en invernadero en el municipio de Toledo, Brasil. El diseño experimental utilizado fue el de bloques al azar, con cuatro repeticiones. Los tratamientos fueron: T1 - Control (semillas tratadas con insecticida); T2 - Semillas tratadas con insecticidas e inoculadas con *Bradyrhizobium*; T3 - Semillas no tratadas inoculadas con *Bradyrhizobium*; T4 - Semillas tratadas con insecticidas y cobalto-molibdeno (CoMo), inoculadas con *Bradyrhizobium*; T5 - Semillas con CoMo inoculadas con *Bradyrhizobium*; T6 - Semillas tratadas con insecticidas, inoculadas con *Bradyrhizobium* y con aplicación de CoMo vía foliar; T7 - Semillas tratadas con insecticidas, inoculadas con *Bradyrhizobium* y con aplicación de nitrógeno en cubierta; T8 - Semillas tratadas con nitrógeno al voleo. No se observaron diferencias significativas entre los tratamientos sobre el número de nódulos, diametro de la tallo principal, altura de planta, longitud de raiz, masa de 1000 granos y rendimiento. La aplicacion de nitrógeno en la etapa R2 y la asociación del inoculante + CoMo sin tratamiento de semilla proporcionó mayor número de nudos, vainas y granos por planta.

Palabras clave:
Fijación biológica de nitrógeno
Bradyrhizobium japonicum
Glycine max L
Nódulos
Tratamiento de semillas

[1] Pontificia Universidade Católica do Paraná, Toledo campus, Paraná, Brazil. lucianomoro23@hotmail.com, maikfranz@hotmail.com, ecco.martios@pucpr.br, marlon_ribas@hotmail.com.
[2] Universidad Nacional de Canindeyú - UNICAN, Salto del Guairá campus, Paraguay. milciadesmelgarejo1@gmail.com
* Corresponding author

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One of the main agents of productivity increase in recent years has been the research on soil fertility and the scientific and technological innovations that have allowed the efficient use of agricultural soil corrective and fertilizers (Balafoutis *et al.,* 2017).

Nitrogen (N) is one of the most important elements that was introduced in large quantities as fertilizer, increasing agricultural production by up to 40%, since it is a constituent of amino acids, proteins, nucleic acids, among others (de Mello-Prado, 2021).

In plants, N is the component responsible for several reactions, besides being part of the structure of chlorophyll, enzymes, and proteins. Being an essential element, N has an impact on root formation, photosynthesis, production and translocation of photoassimilates, and the growth rate between leaves and roots, being primarily involved the leaf growth (Bang *et al.,* 2021).

According to Hungria *et al.* (2006), the rates of N obtained through biological fixation of atmospheric nitrogen (BNF) are between 60 and 250 kg ha^{-1}, which represents between 70 to 85% of all N accumulated in the soybean plant. N constitutes 80% of the gases of the atmosphere that is also in the rhizosphere, in the pores of the soil; however, plants cannot easily take it, due to the triple bond between the atoms of N. Meanwhile, some bacteria such as *Bradyrhizobium* through the dinitrogenase enzyme can break this connection, providing the reduction until ammonia (NH_3), even when N is supplied by chemical products.

These bacteria are chemically attracted by molecules exuded by germinating seeds and the root system of the soybean plants, which subsequently, penetrate these roots and stimulate the growth of cells in the host plant forming the nodules, where they remain (Santi *et al.*, 2013).

Ions of hydrogen (H^+) that are abundant in bacteria cells are incorporated into the ammonia forming ammonium (NH_4), which are distributed to host plants and incorporated for the formation of various substances (Parente *et al.*, 2015).

The use of these bacteria in the composition of commercial agricultural inoculants is an efficient way to increase agricultural production, which guarantees productivity to supply the growing demand for food and also the need to reduce dependence on chemical fertilizers, thus, promoting sustainable agriculture (Nosheen *et al.*, 2021). According to Hungria *et al.* (2006), the main Fabaceae commercially planted in Brazil, totally free of nitrogen fertilization, under normal growing conditions, can be supplied with the N requirements of the crop by BNF.

In Brazil, BNF is the main form to provide N; nevertheless, the peak fixation occurs in grain filling, and after this stage, there is a considerable reduction of BNF, given that, the preferential absorption process becomes the translocation of N from leaves and stems to pods (Moreno *et al.,* 2018).

Excess of nutrients can trigger negative environmental impacts that cause contamination and eutrophication of water bodies, and reduction of microflora and native microfauna. Also, the acidification of the soil, especially when it has the availability of elements in different forms (Sharpley, 2016). This negative interference can occur not only with the supply of N but also with other nutrients that participate in the BNF process such as molybdenum (Mo) and cobalt (Co). For both, there are variable responses of soybean to complementary fertilization, even with the importance of these nutrients for the process of symbiotic fixation of N, there are doubts in its application to obtain a higher yield of grains in the crop (Marcondes and Caires, 2005).

For BNF to be effective, bacteria must be highly dependent on these micronutrients. Mo acts on the enzyme nitrate reductase that is responsible for the reduction of nitrate to nitrite in the cellular cytoplasm and for participating in nitrogen metabolism as a cofactor of the enzymes nitrogenase and nitrite reductase (Marcondes and Caires, 2005). Co is part of the structure of vitamin B12 that is fundamental for the synthesis of leghemoglobin, which determines the activity of nodules, also has an influence on the nitrogen absorption through symbiotic (Marcondes and Caires, 2005). Concerning the forms of fertilization of the crop, there is uncertainty about the mineral and biological fertilization consortium. According to Embrapa (2008), the application of nitrogen fertilizer at any stage of plant development, in addition to the reduction in nodulation and the effectiveness of biological nitrogen fixation, does not cause productivity increases.

In this context, this study aimed to evaluate the interference of chemicals products, micronutrients, and nitrogen fertilization on the efficiency of biological nitrogen fixation in soybean inoculated with *Bradyrhizobium.*

MATERIALS AND METHODS

This study was carried out in the experimental farm of the Pontifícia Universidade Católica do Paraná PUCPR, Toledo campus, located at the coordinates 24°43'14"S and 53°43'56"W, with an average altitude of 557 m, in the agricultural year 2016/17. The local climate is subtropical humid (Cfa) according to Koppen, with hot summers and infrequent frosts with trends in rainfall concentrations in the summer months, without a defined dry season, and the soil is classified as a Dystropherric Red Latosol (Embrapa, 2013).

The research was developed in two scenarios: directly in the field and in a pot arrangement under a protected environment. In the field, the soil was collected using the sampling methodology proposed by Raij *et al*. (2001) and showed the following results in the shallow layers from 0 to 20 cm: pH ($CaCl_2$) 4.75; 4.71 $cmol_c\ dm^{-3}$ of H^++Al^{3+}; 4.36 $cmol_c\ dm^{-3}$ of Ca^{2+}; 1.88 $cmol_c\ dm^{-3}$ of Mg^{2+}; 0.26 $cmol_c\ dm^{-3}$ of K^+; 6.39 mg dm^{-3} of P (Mehlich-1); and 57.98% of saturation per base.

The experimental area was approximately 555 m², previously planted with oats. Treatments were conducted in plots of 22.05 m² (3.15×7.0 m), arranged in randomized blocks with three replications and eight treatments (Table 1).

For the implantation of the experiment were used seeds without treatments and seeds previously treated with 6 mL kg^{-1} of Tiodicarbe 45 % + Imidacloprid 15%, the inoculation was performed shortly before sowing by mixing the inoculant with the seed in a plastic bag. A mixed liquid inoculant was used, which was composed of *B. japonicum* CPAC 15 (SEMIA 5079) and *B. diazoefficiens* CPAC 7 (SEMIA 5080) at the concentration of 6×10^9 colony-forming units mL^{-1} with a dose of 2 mL kg^{-1} of seed. The application of CoMo consisted of 3 mL of the commercial product composed of 15% of Mo and 1.5% of Co kg^{-1} of seed before inoculation using a plastic bag, and also, 240 mL ha^{-1} via foliar at R2 stage. N was applied manually by the end of the afternoon, the source used was ammonium sulfate composed of 21% N and 24% S, the dose was 100 kg ha^{-1}.

Table 1. Treatments applied to soybean crops via seed, foliar, and cover.

T1 - Control (seeds treated with insecticide)
T2 - Seeds treated with insecticides and inoculated with *Bradyrhizobium*
T3 - Untreated seeds inoculated with *Bradyrhizobium*
T4 - Seeds treated with insecticides and CoMo, inoculated with *Bradyrhizobium*
T5 - Seeds with CoMo inoculated with *Bradyrhizobium*
T6 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with the application of CoMo via foliar
T7 - treated with insecticides, inoculated with *Bradyrhizobium* and with the application of nitrogen in cover
T8 - treated with nitrogen by broadcast

The sowing of the soybean was carried out manually on October 01, 2016, in which the cultivar Monsoy 5947 IPRO was used, with space between lines of 0.45 m using 36 seeds m^{-1}. Two days before sowing, the area was fertilized using a no-tillage seeder with the same space between lines using 300 kg ha^{-1} of the formula 00-20-18 of N-P-K. At the V3 stage (a plant with three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes), a reduction was carried out maintaining 10 to 12 plants m^{-1}. The average air temperature and rainfall that occurred during the crop cycle were 24.7 °C and 1125 mm, respectively.

The cultural treatments were according to the need of the crop. Two applications of herbicide, the first one at V3 with 2 L ha^{-1} of glyphosate 48% + 0.45 L ha^{-1} Cletodim

24% and the second one with 2 L ha^{-1} of glyphosate 48% at pre-flowering. Three applications of fungicides were also made, the first at R2, the second at R4, and the third at R5.4; all with 200 g ha^{-1} of Azoxystrobin 30% + Benzovindiflupir 15% as well as four applications of insecticide for bedbugs control.

In the first application, 500 g ha^{-1} of 75% acephate at the R2 stage was used, the second one, 1 L ha^{-1} of imidacloprid 10% + beta cyfluthrin 1.25% was applied at R4, the third application was performed at the R5.4 stage using 200 g ha^{-1} of 75% acephate and the last application at R6 using 200 g ha^{-1} of 75% acephate, for caterpillar it was not necessary to apply since the cultivar was resistant.

All applications were performed at the freshest times of the day, early in the morning, or late afternoon, with a yellow Teejet spray tip with an application angle of 110° and a flow rate of 0.46 to 0.91 L min^{-1}.

After maturation, the three central lines were collected manually with a length of 5 m in each plot, totaling 6.75 m² of useful area, discarding 1 m of each end of the plot.

The material was threshed, pre-cleaned, and moisture determined on a digital electronic device. After being weighed, the result of each plot was adjusted to 13% moisture and extrapolated to bags ha^{-1}. The evaluation of the production components: the number of pods and the number of grains plant^{-1} was performed using 10 plants of each plot collected at harvest time. The mass of 1000 grains was determined according to the Seed Analysis Rule. Stem diameter was measured using a caliper, the height of the plant was measured by measuring tape. The number of pods, grains, and nodes per plant were counted manually, then the mean of each variable was calculated.

In the other scenario, 5 L of capacity perforated polyethylene pots were used, performing the same treatments that were carried out in the field; however, without fertilization and leaving only one plant per pot after emergence. The substrate used was soil from the field experiment site and sand in a ratio 4:1, respectively. Pots were kept in the university's greenhouse (PUCPR) and irrigated daily, in which they were distributed in a completely randomized experimental design with three replications. From the pot experiment, some variables were analyzed such as the number of nodules, which after washing the roots, the nodules were detached and counted one by one, and root length, which was measured by a measuring tape.

The data were tabulated and submitted to analysis of variance, according to the level of 5% of significance by Test F, and the qualitative averages grouped by the Tukey test at 5% probability. The analyzes were performed using the statistical software SISVAR 5.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

According to Table 2, there were no significant effects among treatments (P>0.05), mainly highlighting that the variable number of nodules did not have a significant variation, proving that the experimental area may have had seeds with *Bradyrhizobium* strains previously. That is, the bacterium was not native to the soil after the first inoculation, given that the results of subsequent inoculations were lower. According to Gris *et al*. (2005), the untreated seeds and those treated with the commercial inoculant may not present a significant difference due to the presence of bacterial populations already existing in the soil, generating good nodulation and BNF.

Santos *et al*. (2013) observed that seeds treated with thiamethoxam, fludioxonil + metalaxyl-m, and thiabendazole molecules have a phytotoxic effect on bacteria and can inhibit the biological fixation or decrease inoculant viability. Also, Silva *et al*. (2011) affirm that the smaller number of nodules may be related to the active principles used in the treatment of seeds as fungicides, insecticides, and inoculants, compromising the diazotrophic bacteria results that differ from those found in this study.

According to Embrapa (2008), the presence of nitrogen fertilizers can reduce the efficiency of bacteria, and N, in mineral form affects the fixation and also the nodules in the plants. This is related to the decrease in available oxygen used in nodular respiration and also to the limitation of carbohydrates in the nodule. Another factor that may compromise nodulation is soil salinization, which was observed by Velagaleti *et al*. (1990) who reported that the salinity affects the infection process and initial development of the nodules, this may be due to the inhibition of calcium absorption by excess salts, which

Table 2. Averages of the variables number of nodules, stem diameter, and plant height with different combinations of seed treatment and application of N, Co, and Mo. Crop 2015/16. Toledo, PR.

Treatment	Number of nodules *	Stem diameter (mm)	Plant height (cm)
T1 - Control	260.0	6.76	99.6
T2 **-** *Bradyrhizobium*+Treatment	273.7	7.26	99.5
T3 - *Bradyrhizobium*+untreatment	283.2	7.56	105.3
T4 - *Bradyrhizobium*+Treatment+CoMo	250.0	7.63	105.3
T5 - *Bradyrhizobium*+CoMo+untreatment	223.0	7.96	112.0
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	277.7	7.03	105.7
T7 - *Bradyrhizobium*+Treatment+N in R2	219.5	8.33	110.7
T8 - Treatment+N in R2	268.7	8.10	110.6
mean	257	7.58	106.1
F value	0.813 ^{ns}	3.456^{ns}	1.893^{ns}
CV (%)	21.03	6.75	5.74

ns= non-significant; CV= coefficient of variation
* greenhouse experiment

reduces the growth of root and root hairs and decreases the potential for *Bradyrhizobium* infection.

According to Santos *et al*. (2013), the presence of the active ingredients in the rhizosphere causes the exudates of the roots to change, thus decreasing the emission of molecular signals, generating fewer nodules and lower BNF.

For the stem diameter, there was no statistical difference (P>0.05) possibly because only one cultivar was evaluated with the same population density, maintaining a standard of stem diameters.

For plant height, there was no significant difference (P>0.05) This occurs because the soil could have supplied the plant requirements of N through the satisfactory availability of this nutrient in the environment. However, if T5 (seeds with CoMo inoculated with *Bradyrhizobium*) is compared to T1 (control), the difference in plant height is 12.4 cm (Table 2). A similar result was found by Parente *et al*. (2015), who verified that the plant height in BRS Valiosa RR cultivar did not present a significant difference, but its height was above the average stipulated by the genotype providers.

T5, T7, and T8 had a higher number of nodes, pods, and grains (P<0.05) than the rest of the other treatments, according to Table 3. The application of N at the R2 stage of T7 and T8 could have provided a possible BNF deficiency, complementing the demand of the plant and resulting in a greater vegetative development as shown by the greater number of nodes.

Regarding the treatments of seeds, T5, T7, and T8 no differences were observed, that is, the treatments of seeds with different chemicals did not have influence, which could lead to the reduction of symbiotic microorganisms, and decrease the number of pods and grains. A different result was presented by Santos *et al*. (2013), who observed that, when the seeds are treated with chemical insecticides and fungicides, and then the inoculation of bacteria is used, the viability of the seeds is lower, and the inoculated bacteria cannot perform their function of BNF. The non-inoculation with *Bradyrhizobium* in T8 (only the chemical treatment of the seeds and application of nitrogen topdressing was carried out) allowed similar results to the other two treatments in which the inoculation was performed (T5 and T7). Therefore, after the first inoculations, the results are inferior, however, in many cases, they can be economically efficient.

Vieira *et al*. (2017) in their work on reinoculation of the soybean crop in a no-tillage area, observed that there was no difference with the control without inoculant. This result may be due to the efficiency of the natural *Bradyrhizobium* population of the soil, which can supply the nitrogen needs of the crop.

Table 3. Averages of the variables number of nodes, number of pods, and number of grain with different combinations of seed treatment and application of N, Co, and Mo. Crop 2016/17. Toledo, PR.

Treatment	Number of nodes	Number of pods	Number of grain
T1 - Control	31.93 b	51.66 b	127.96 b
T2 - *Bradyrhizobium*+Treatment	34.03 b	56.96 b	133.26 b
T3 - *Bradyrhizobium*+untreatment	35.30 b	59.20 b	143.53 b
T4 - *Bradyrhizobium*+Treatment+CoMo	35.93 b	55.70 b	138.73 b
T5 - *Bradyrhizobium*+CoMo+untreatment	40.42 a	64.66 a	159.36 a
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	35.77 b	55.53 b	135.53 b
T7- *Bradyrhizobium*+Treatment+N at R2	41.13 a	65.60 a	158.30 a
T8 - Treatment+N at R2	43.57 a	69.10 a	166.40 a
mean	37.26	59.80	145.38
F Value	5.342*	4.120*	2.795*
CV (%)	8.03	10.4	10.07

*: significant at the 5% probability level by the F test. Means followed by the same letter do not differ by Tukey test at 5% significance; CV= coefficient of variation

In the variables root length, the mass of a thousand grains, and productivity, the application of Co and Mo was not highlighted (Table 4). It can be stated that the soil is possibly supplied with these two micronutrients, without limiting the viability of the nodules.

Pessoa *et al*. (1999) found no significant difference in their work, but when the authors applied a dose of 80 g ha^{-1} of Mo, there was an increase in yield, although it did not differ from the other doses tested.

Although the number of nodes presented a significant difference, pods and grains plant^{-1} did not differ in terms of productivity, which can be explained by the fact that there was no significance for the mass of 1000 grain. Another factor that may favor a similar result is the low

Table 4. Averages of the variables root length, the mass of 1000 grain, and productivity regarding the seed treatments and the application of N, Co, and Mo. Crop 2016/17. Toledo, PR.

Treatments	Root length* (cm)	Mass of 1000 grain (g)	Productivity (kg ha^{-1})
T1 - Control	21.87	149.23	3.097
T2 - *Bradyrhizobium*+Treatment	17.12	156.36	4.680
T3 - *Bradyrhizobium*+untreatment	19.37	152.66	4.619
T4 - *Bradyrhizobium*+Treatment+CoMo	16.25	156.10	4.358
T5 - *Bradyrhizobium*+CoMo+untreatment	21.75	159.43	4.618
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	32.50	152.40	4.912
T7 - *Bradyrhizobium*+Treatment+N at R2	25.87	157.06	4.440
T8 - Treatment+N at R2	24.62	153.66	4.139
mean	44.48	154.61	4.483
F value	1.117 ^{ns}	1.027 ^{ns}	0.459 ^{ns}
CV (%)	22.42	3.57	15.91

ns= not significant; CV= coefficient of variation
* greenhouse experiment

pH of the soil since according to Farias *et al.* (2016) a higher pH, produced by liming and soil cultivation, seems to trigger ecological modifications, which benefit the appearance of strains of *Rhizobium* spp.

Furthermore, Parente *et al*. (2015), also found no significant difference when applying N at R1 for the variables mass of 1000 grains and yield of BMX Potência cultivar. These results corroborate those obtained by Aratani *et al*. (2008), who did not obtain an increase in productivity with the application of N regardless of the application stage. Bahry *et al*. (2013) in their work with nitrogen fertilization in soybean crop coverage concluded that there was no higher yield of grains in the crop, a similar result to this work. Still, Pessoa *et al*. (1999) did not obtain a significant difference for soybean yield after Mo application and did not find a significant interaction between inoculant plus application of CoMo for grain mass and yield.

Golo *et al.* (2009) reported that when working with inoculant plus application of CoMo, there was no significant interaction for plant height, insertion height of the first pod, number of pods per plant, number of seeds per plant, number of seeds per pod, the mass of 1000 grains and productivity. Nevertheless, according to the authors, the doses of CoMo significantly influenced the mass of 1000 seeds and productivity. For the root length, the results were similar for all treatments, showing no significant difference; however, the treatment that stood out in root length was T6 (*Bradyrhizobium* + Treatment + CoMo Foliar application) with 32.50 cm.

CONCLUSION

Combinations of inoculant, CoMo, seed treatment with insecticide, and nitrogen application did not influence nodulation, stem diameter, plant height, root length, a mass of 1000 grain, and productivity. Application of nitrogen at R2 and seed treatment can provide a greater number of nodes, pods, and grains per plant.

It is worth mentioning that the use of chemical seed treatment together with inoculation with *Bradyrhizobium* and foliar application of CoMo can result in greater root development of the crop, which can allow productivity increases when not subjected to stress.

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