Ciência Rural, Santa Maria, v.53:7, e20210871, 2023 http://doi.org/10.1590/0103-8478cr20210871
ISSNe 1678-4596
CROP PRODUCTION

Bradyrhizobium and *Azospirillum* co-inoculation associated with cobalt and molybdenum application in the soybean crop

Henrique Moura Barbosa[1] Rita de Cássia Félix Alvarez[1] Sebastião Ferreira de Lima[1*] Meire Aparecida Silvestrini Cordeiro[1] Mayara Santana Zanella[2] Vitória Fátima Bernardo[1]

[1]Universidade Federal de Mato Grosso do Sul (UFMS), 79560-000, Chapadão do Sul, MS, Brasil. E-mail: sebastiao.lima@ufms.br. [*]Corresponding autor.
[2]Programa de Pós-graduação em Biotecnologia e Biodiversidade, Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, Brasil.

ABSTRACT: Co-inoculation between bacteria of the genera *Bradyrhizobium* and *Azospirillum* can enhance the nodulation and promote the development of the soybean [*Glycine max* (L.) Merrill] root system, contributing to the increase in grain yield, in addition to the reduction in production costs and contamination of natural resources. Cobalt (Co) and molybdenum (Mo) use can also favor biological nitrogen fixation. The research evaluated the co-inoculation effect of bacteria associated with the Co and Mo application in soybean crop. The randomized blocks design was employed, in a 2 x 6 factorial scheme, presence and absence of Co and Mo and five ways of using the products *Bradyrhizobium* and *Azospirillum*, plus control, with four replications. The treatments were formed by the control (not inoculated + 20 kg N ha^{-1}); seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg N ha^{-1}; seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) and three treatments applying *Bradyrhizobium* + *Azospirillum* in furrow, in different doses. Height of insertion of the first pod, total number of pods and grains per plant, weight of 100 grains and grain yield were evaluated. Inoculation of *Bradyrhizobium japonicum* associated with co-inoculation of *Azospirillum brasilense* via foliar and Co and Mo, provided increases in the number of pods per plant, number of grains per pod and weight of 100 grains, reflecting increases in grain yield. The use of Co and Mo, on average, increased soybean yield by 10%, resulting in an average yield of 4,904 kg ha^{-1}.
Key words: diazotrophic bacteria, biological fixation, micronutrients.

Co-inoculação de *Bradyrhizobium* e *Azospirillum* associadas a aplicação de cobalto e molibdênio na cultura da soja

RESUMO: A co-inoculação entre bactérias do gênero *Bradyrhizobium* e *Azospirillum* pode potencializar a nodulação e promover o desenvolvimento do sistema radicular da soja [*Glycine max* (L.) Merrill], contribuindo com o aumento na produtividade de grãos, além da redução nos custos de produção e contaminação dos recursos naturais. A utilização de cobalto (Co) e molibdênio (Mo), também pode favorecer a fixação biológica de nitrogênio. O objetivo do trabalho foi avaliar o efeito da co-inoculação de bactérias associadas a aplicação de Co e Mo na cultura da soja. O delineamento utilizado foi de blocos casualizados, em esquema fatorial 2 x 6, na presença e ausência de Co e Mo e cinco formas de utilização dos produtos *Bradyrhizobium* e *Azospirillum*, mais controle, com quatro repetições. Os tratamentos foram formados pela testemunha (não inoculada + 20 kg de N ha^{-1}); inoculação com *Bradyrhizobium* na semente (100 mL ha^{-1}) + 20 kg de N ha^{-1}; inoculação de sementes com *Bradyrhizobium* (100 mL ha^{-1}) e três tratamentos aplicando *Bradyrhizobium* + *Azospirillum* em sulco em diferentes doses. Foram avaliados altura de inserção da primeira vagem, número total de vagens e grãos por planta, massa de 100 grãos e produtividade de grãos. A inoculação de *Bradyrhizobium japonicum* associado a co-inoculação de *Azospirillum brasilense* via foliar e ao Co e Mo, proporcionou incrementos no número de vagens por planta, número de grãos por vagem e massa de 100 grãos, refletindo acréscimos na produtividade de grãos. O uso de Co e Mo, em média, aumentou o rendimento da soja em 10%, resultando numa produtividade média de 4904 kg ha^{-1}.
Palavras-chave: bactérias diazotróficas, fixação biológica, micronutrientes.

INTRODUCTION

Brazil currently is the largest producer and exporter of soybeans in the world (EMBRAPA SOJA, 2022), reaching production in the 2021/22 harvest of 124,0 million tons (10,2% lower than the previous harvest) (CONAB, 2022). Considering the crop importance and its nutritional and physiological demands, it is essential to search for low-cost products and technologies, capable of improving the sustainability of the production system, without expanding the area (SAATH & FACHIELLO, 2018).

The soybean plant is very demanding in nitrogen (N), as this nutrient is responsible for the increase in the grain's protein content (36 to 42%), production of new cells and tissues and grain productivity (BRANCALIÃO et al., 2015; MARCON et al., 2017). It is estimated that for each

Received 12.11.21 Approved 08.03.21 Returned by the author 07.10.22
CR-2021-0871.R3
Editors: Leandro Souza da Silva Gerson Drescher

ton of grain produced approximately 83 kg of N are needed (HUNGRIA et al., 2013). It is possible that this high demand for N has led to studies with N application, even with *Bradyrhizobium* inoculation, being reported in some situations increase soybean grain yield (BARRANQUEIRO; DALCHIAVON, 2017; MORENO et al., 2018) and in other cases that there is no increase in grain yield (SATURNO et al., 2017).

Biological nitrogen fixation (BNF), promoted by bacteria of the genus, can supply the nutritional requirement of soybean in nitrogen. Technological advances have allowed the crop to express its potential even though it is influenced by edaphoclimatic conditions (CAMPO et al., 2009; ZILLI et al., 2009). Co-inoculation of soybean with *Bradyrhizobium* spp. and *Azospirillum brasilense* results in early nodulation of soybean plants (CHIBEBA et al., 2015), being able to supply all the N needed for plant development (TAIZ et al., 2017). According to HUNGRIA et al. (2013), the co-inoculation management can provide an average increase of 16.1% in soybean yield, in relation to non-inoculated areas.

Bacteria belonging to the genus *Azospirillum*, used worldwide as inoculants in grasses (HUNGRIA et al., 2010), are considered growth-promoting organisms, capable of influencing the formation of phytohormones. In this way, these microorganisms allow anticipate the nodulation of *Bradyrhizobium*, promote high growth of the aerial part and of the roots, increase the absorption capacity of water and nutrients, due to the increase in the volume of exploited soil, provide opportunities for the induction of systemic resistance to diseases and environmental stresses, and also the ability to solubilize phosphate (BRACCINI et al., 2016).

In addition to the proper use of inoculants, cobalt (Co) and molybdenum (Mo) use in soybean crops favors biological nitrogen fixation. Co participates in the structure of vitamin B12, necessary for the synthesis of leghemoglobin, which determines the activity of the nodules, prevents oxidation and gives them their reddish color (MENGEL & KIRKBY, 2001). Mo is a cofactor of the enzymes nitrogenase, nitrate reductase and sulfide oxidase, which is closely related to the transport of electrons during biochemical reactions, influencing its activity and nodulation process (TOLEDO et al., 2010).

Soils with high fertility can meet the need for micronutrients, including Co and Mo. However, in Cerrado soils that have low natural fertility, mineral fertilization with these elements is necessary, in addition, the intensification of the use of areas with agriculture has promoted the export of these soil elements, which has also reduced their availability (DOURADO NETO et al., 2012). It is important to study the application of these micronutrients together with inoculation with microorganisms, to choose the best management.

The inoculation process with *Bradyrhizobium* as well as other bacteria can aid the BNF process. However, its interaction between symbiotics in the case of *Bradyrhizobium* and diazotrophic bacteria belonging to the genus *Azospirillum* has been an object of interest (BÁRBARO et al., 2009).

The use of organic products with or without the addition of minerals has a sustainable and economically viable connotation for the producer. Several studies detail the importance of *Bradyrhizobium* for soybeans (SATURNO et al., 2017), of *A. brasilense* for grasses, and sometimes for soybeans (GALINDO et al., 2017; SOUZA et al., 2019; BOLETA et al., 2020) and the use of Co and Mo for several crops, including soybean (LANA, et al., 2009; GALDINO et al., 2020). However, there are few studies that address the use of this triple association in soybean crop, and the results are not always conclusive (GALINDO et al., 2017; TEIXEIRA et al., 2017). In addition to the divergences on the use of these different products, the research still does not clearly indicate the best way of application. Application via seed is not always indicated due to the chemical treatments or nutrients used, which can be toxic to microorganisms (CAMPO et al., 2009). This opens the application option in furrow and foliar.

The hypothesis of this research considers that the co-inoculation of soybean seeds with *Bradyrhizobium* and *Azospirillum*, when associated with the foliar supply of Co and Mo, can improve the growth characteristics, production components and crop productivity. So, this research evaluated the effect of bacteria associated co-inoculation with the application of Co and Mo in soybean crop.

MATERIALS AND METHODS

The experiment was a single year trial developed under field conditions in the 2018/2019 harvest season. The experiment was established in an area of the Federal University of Mato Grosso do Sul, MS at coordinates 18°46´17.7″ S and 52º37´27.7″ W; with an altitude of 813 m. The soil of the experimental area is a Latossolo Vermelho distrófico (Oxisol) (EMBRAPA, 2018), originally occupied by Cerrado vegetation. The area's history indicated that it was previously explored with grain-producing crops, mainly soybean and corn (*Zea mays* L.).

Before the experiment installation, soil samples were collected from the experimental area, in the 0 – 0.20 m layer, and chemical analysis was carried out, according to the method proposed by RAIJ et al. (2001). The values reported were P (resin): 37.43 ($cmol_c$ dm^{-3}); O. M. (organic matter) = 38.8 g dm^{-3}; K = 209.7 mg dm^{-3}; Ca, Mg and SB (sum of bases) = 2.8, 1.1 and 8.2 $cmol_c$ dm^{-3}, respectively; pH ($CaCl_2$), Al and H+Al = 5.3, 0.05, 3.7 $cmol_c$ dm^{-3}, respectively; S-SO_4 = 3.3 g dm^{-3} and V (base saturation) = 54.2%.

The characteristic climate of the region, according to Köeppen classification, is humid tropical (Aw), with a rainy season in the summer and a dry season in the winter, and average annual precipitation of 1,850 mm, with annual temperatures ranging from 13 to 28ºC. The average rainfall and air temperature data obtaining in the experiment conduct are shown in figure 1.

The experimental design used was a randomized block, in a 2 x 6 factorial scheme, formed by the Co + Mo presence and absence and five ways of using the *Bradyrhizobium* and *Azospirillum* products, plus a control, with four replications, with the treatments described in table 1. For the NICN (Not inoculated + 20 kg of N ha^{-1}) and SICN (Seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg de N ha^{-1}) treatments (Table 1), 20 kg N ha^{-1} were applied at sowing, using urea as a source. The current recommendation for soybean cultivation consists of the exclusive use of inoculant, without the application of N or the use of up to 20 kg ha^{-1} at sowing (EMBRAPA, 2011). This indication of the use of initial N ends up generating confusion in the production process. Thus, it was established in this experiment the use of 20 kg N ha^{-1} at sowing, to verify the soybean response under these conditions.

The plots consisted of five lines of 5.0 m in length spaced 0.45 m apart. The usable area consisted of three central lines of each plot, totaling 6.75 m^2. The cultivar used was M6410 IPRO (medium size, with indeterminate growth and cycle of 123 days). The seeds were treated with the fungicide Carboxina + Tiram, at doses of 200 g of a.i. L^{-1} and 200 g of a.i. L^{-1}, respectively, and the insecticide Imidacloprid, at a dose of 150 g of a.i. L^{-1}.

As a source of *Bradyrhizobium japonicum*, the inoculant Masterfix soybean®, strain SEMIA 5079 (5 x 10^9 viable cells per mL) was used, and the product was applied in the form of inoculation on seeds or sprayed directly into the seeding furrow. As a source of *Azospirillum brasilense*, the inoculant Masterfix Gramíneas® was used, which contains strains Abv5 and Abv6 (2 x 10^8 viable cells per mL), being the product applied as foliar spray. For the directed application in the seeding and furrow, an electric costal spray was used, with constant pressure of 0.4 mpa, a flow of 0.35 L min^{-1}, equipped with a lance containing two nozzles, working at a height of 10 cm from the target and speed of 1.0 m second, reaching an applied strip of 10 cm in width, providing a spray volume of 100 L ha^{-1}. Cobalt and molybdenum were applied via seed and leaf treatment (V6 - six leaf fully developed) at doses of 50 mL ha^{-1} and 100 mL ha^{-1}, respectively, using commercial product with 0.8% Co (10.5 g L^{-1}) and 8.0% Mo (105.6 g L^{-1}).

The soil was prepared conventionally, by means of plowing and two harrowing, one in the incorporation of limestone at a depth of 0.20 m (470 kg ha^{-1} of dolomitic limestone, with Effective Neutralizing Value of 17,84 $mmol_c$ kg^{-1}) 60 days before planting and one harrowing two days before planting the crop. Fertilization was carried out based on the recommendations of SOUSA & LOBATO (2004) and soil analysis of the area. A rate of 80 kg ha^{-1} of P_2O_5 and K_2O was applied, using single superphosphate and potassium chloride as sources, respectively. P_2O_5 was applied totality at sowing, while K_2O was applied

Table 1 - Description of co-inoculation treatments of *Bradyrhizobium* and *Azospirillum* associated with Co and Mo (cobalt and molybdenum) application in soybean crop.

Treatments	--------------------Description of treatments associated with and without Co and Mo--------------------
NICN	Not inoculated + 20 kg of N ha^{-1}
SICN	Seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg de N ha^{-1}
SIC	Seed inoculation with *Bradyrhizobium* (100 mL ha^{-1})
COIN-I	In-furrow inoculation with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* foliar (100 mL ha^{-1})
COIN-II	In-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* foliar (150 mL ha^{-1})
COIN-III	In-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* foliar (200 mL ha^{-1})

twice, 40 kg ha^{-1} at sowing and 40 kg ha^{-1} in stage V3 (third leaf fully developed). Post-emergence weed management was performed with glyphosate (720 g a.e. kg^{-1}) in V5 (fifth fully developed leaf).

At the V5 and R4 (full formation of pods), growth stages, Imidacloprid + beta-cyfluthrin (100 g a.i. L^{-1} + 12.5 g a.i. L^{-1}), and Triflumuron (480 g a.i. L^{-1}) were applied for pestes control and Prothioconazole + Trifloxystrobin (175 g a.i. L^{-1} + 150 g a.i. L^{-1}) was applied for diseases control.

Before harvesting, five plants were sampled in sequence per plot, determining the height of insertion of the first pod, total number of pods, number of grains per plant and weight of 100 grains. The mass of 100 grains was obtained by weighing four samples of 100 grains in each plot. The total mass of grains obtained from the five plants was added to the value obtained at harvesting the plot, so as not to interfere with the result. Soybean harvest was carried out manually at the R8 phenological stage (physiological maturation), followed by the trail of the plots. The mass of 100 grains and grain yield were corrected for 13% moisture on a wet basis and grain yield expressed in kg ha^{-1}.

The assumptions of normality distribution and homogeneity of variances were verified for the data. The comparison means test using Scott-Knot test was applied at $P \leq 0.05$ significance level. ANOVA and comparison between means were performed using the Sisvar software (FERREIRA, 2011).

RESULTS AND DISCUSSION

There was a significant interaction effect between treatments for the variables height of insertion of the first pod, number of pods per plant, number of grains per plant, mass of one hundred grains and grain yield (Table 2).

For the height of insertion of the first pod, a higher value was reported in the SICN treatment in the absence of Co and Mo application (Table 3). According to CARVALHO et al. (2010) the insertion height of the first pod must be greater than at least 0.10 m from the ground. Furthermore, AMORIM et al. (2011) and CRUZ et al. (2016) stated that plants with insertion height of the first pod of less than 0.12 m for flat areas and 0.15 m for sloping areas can cause grain losses.

The association of *Bradyrhizobium* with mineral nitrogen probably favored the vegetative growth of the plants due to the higher initial supply of this element; however, doses above 20 kg of N ha^{-1} may impair nodulation (SILVA et al., 2011). When comparing the application or not of Co and Mo, in addition to the SICN treatment, the COIN-II and COIN-III treatments also showed higher values in the absence of the application of Co and Mo. Studies of GOLO et al. (2009) and MESCHEDE et al. (2004), with different doses in foliar application of Co and Mo, also reported no positive effects of these nutrients in the height of insertion of the first pod.

For the variables number of pods per plant and number of grains per plant, it was observed that the inoculation mode SIC and COIN-III, in the presence of Co and Mo, provided higher values (Tables 4 and 5).

The micronutrients Co and Mo participate, respectively, in the synthesis of leghemoglobin and in the process of nitrate reductase in root nodules, providing efficiency in the process of biological nitrogen fixation (TOLEDO et al., 2010) and success in the use of inoculants in the soybean crop. In a study with foliar application of Mo and Co in soybean, DOURADO

Table 2 - Summary of the analysis of variance for height of first pod insertion (HFP), number of pods per plant (NPP), number of grains per plant (NGP), 100 grain mass (100GM) and grain yield (YIELD), in response to modes of inoculation of *B. japonicum* and *A. brasilense* and application of Co and Mo (cobalt and molybdenum).

SV	DF	---Mean square---				
		HFP	NPP	NGP	100GM	YIELD
Co and Mo	1	14.71**	270.72**	866.45**	1.61**	2298873.20**
INOC	5	12.68**	62.95**	201.47**	0.54*	339869.40**
Co and Mo x INOC	5	5.24**	72.82**	233.06**	4.13**	395951.10**
Residue	-	0.58	4.22	13.51	0.17	30921.10
CV (%)	-	6.70	3.38	3.38	2.43	3.99

SV - Source of variation; DF - Degrees of freedom; CV - Coefficient of variation; *; ** - Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively, by F test.

Table 3 - Height of first pod insertion (HFP), as a function of Co and Mo (cobalt and molybdenum) application and methods of inoculation with bacteria.

Co and Mo	---------------------Inoculation Modes---------------------					
	NICN	SICN	SIC	COIN-I	COIN-II	COIN-III
	---------------------HFP (cm)---------------------					
With	10.58 aB	12.04 bA	12.09 aA	11.46 aA	10.08 bB	8.63 bC
Without	10.39 aB	15.11 aA	11.33 aB	11.86 aB	11.21 aB	11.62 aB

Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ statistically from each other by the Scott-Knott test at 5%. NICN = control (not inoculated + 20 kg of N ha^{-1}); SICN = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg of N ha^{-1}; SIC = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}); COIN-I = inoculation in the furrow with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* (100 mL ha^{-1}); COIN-II = in-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* (150 mL ha^{-1}) and COIN-III = in-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* (200 mL ha^{-1}).

NETO et al. (2012) also verified an increase in the number of pods and in the number of grains per pod.

CHAGAS et al. (2015) reported that the application of different doses of Mo in soybean seeds positively affected the number of pods per plant, demonstrating a linear behavior with increasing dose. PESSOA et al. (2001) also observed this behavior, in foliar fertilization of Mo in common bean (*Phaseolus vulgaris* L.).

Co-inoculation with *Azospirillum* provided higher number of pods per plant and number of grains per plant only at the highest dose of inoculants- COIN III treatment (Tables 4 and 5). According to DIDONET et al. (2000) the efficiency of inoculation with bacteria of the genus *Azospirillum* occurs when they can compete with other microbial groups in the soil, fact that was probably confirmed in this study, due to the use of a greater amount of the inoculant.

For the variable mass of one hundred grains, the highest values were obtained with the COIN-I inoculation mode (lowest dose of *Azospirillum* in co-inoculation), when applied Co and Mo and also in the isolated inoculation mode (SIC), without the application of Co and Mo (Table 6). The use of bacteria, such as those of the genus *Azospirillum*, which establish themselves in the root system, generates varied results, as they suffer the action of external factors of the soil, such as the presence of other microorganisms that compete there for resources and other environmental conditions that limit the plant responses (BULEGON et al., 2016). These bacteria can survive both in endophytic and associative conditions close to the roots (BALDANI et al., 1997), thus STURZ & NOWAK (2000) suggested that, if the bacteria were only endophytic, the inconsistency of the results would be less, since they would not be exposed to soil and environmental conditions.

Yield was influenced by all inoculation modes, except SICN, with the application of Co and Mo (Table 7), with the highest values observed in SIC, COIN-I and COIN-III treatments. The use of Co and Mo on average increased soybean yield

Table 4 - Number of pods per plant (NPP) as a function of Co and Mo (cobalt and molybdenum) application and methods of inoculation with bacteria.

Co and Mo	---------------------Inoculation Modes---------------------					
	NICN	SICN	SIC	COIN-I	COIN-II	COIN-III
	---------------------NPP---------------------					
With	59.32 aC	55.88 bD	70.85 aA	62.49 aB	62.49 aB	68.29 aA
Without	55.96 bA	59.02 aA	57.20 bA	58.98 bA	61.16 aA	58.50 bA

Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ statistically from each other by the Scott-Knott test at 5%. NICN = control (not inoculated + 20 kg of N ha^{-1}); SICN = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg of N ha^{-1}; SIC = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}); COIN-I = inoculation in the furrow with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* (100 mL ha^{-1}); COIN-II = in-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* (150 mL ha^{-1}) and COIN-III = in-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* (200 mL ha^{-1}).

Table 5 - Number of grains per plant (NGP) as a function of Co and Mo (cobalt and molybdenum) application and methods of inoculation with bacteria.

Co and Mo	------------------------------Inoculation Modes------------------------------					
	NICN	SICN	SIC	COIN-I	COIN-II	COIN-III
	------------------------------NGP------------------------------					
With	106.13 aC	99.98 bD	126.75 aA	111.79 aB	111.79 aB	122.18 aA
Without	100.12 bA	105.59 aA	102.33 bA	105.51 bA	109.42 aA	104.65 bA

Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ statistically from each other by the Scott-Knott test at 5%. NICN = control (not inoculated + 20 kg of N ha^{-1}); SICN = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg of N ha^{-1}; SIC = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}); COIN-I = inoculation in the furrow with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* (100 mL ha^{-1}); COIN-II = in-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* (150 mL ha^{-1}) and COIN-III = in-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* (200 mL ha^{-1}).

by 10.46%. Results obtained can be attributed to the foliar application of Co and Mo, because even though these micronutrients were not analyzed, they were not applied in treatment without Co and Mo. The cultivation environment may have favored the effect of Co and Mo application because the area was conventional soil tillage, which exposes organic matter and affects the availability of micronutrients (BALÍK et al., 2006).

It was possible to clearly observe in the research the beneficial effect of the use of Co and Mo for the variables NPP, NGP and YELD (Tables 4, 5 and 7). According to Galindo et al. (2017) the use of Co and Mo reflects in greater grain filling, highlighting the higher mass value of one hundred grains and; consequently, higher grain yield. GOLO et al (2009) highlighted that Co and Mo make an essential contribution to FBN. Conversely, the use of Co and Mo had a negative effect when applied together with *Bradyrhizobium* and nitrogen fertilizer (SICN), resulting in lower values for NPP, NGP, 100GM and YELD (Tables 4, 5, 6 and 7). As this effect was not noticed when only inoculation with *Bradyrhizobium* (SIC) was used, except for the variable 100GM, it seems to be a strong indication that the use of N in sowing inhibits the positive effects of the use of Co and Mo.

Another point that stands out shows that when *Bradyrhizobium* was inoculated with *Azospirillum* (COIN-I, COIN-II and COIN-III), with or without the application of Co and Mo, better results were not obtained than when using the *Bradyrhizobium* alone; however, does not exclude its potential for use. Biological nitrogen fixation promoted by bacteria of the genus *Bradyrhizobium* replaced mineral nitrogen fertilization, enabling high grain yield in soybeans (HUNGRIA et al., 2005), as observed in this study. Co-inoculation with *Azospirillum* promotes greater root growth, through the production of growth promoters by these bacteria, favoring the root system of plants, bringing benefits such as greater tolerance to pathogens and water stress (BASHAN et al., 2004)

Table 6 - Mass of one hundred grains (100GM) as a function of Co and Mo (cobalt and molybdenum) application and methods of inoculation with bacteria.

Co and Mo	------------------------------Inoculation Modes------------------------------					
	NICN	SICN	SIC	COIN-I	COIN-II	COIN-III
	------------------------------100GM (g)------------------------------					
With	17.64 aB	16.41 bC	16.23 bC	18.30 aA	17.25 aB	16.76 aC
Without	16.23 bC	17.21 aB	18.03 aA	16.30 bC	16.15 bC	16.49 aC

Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ statistically from each other by the Scott-Knott test at 5%. NICN = control (not inoculated + 20 kg of N ha^{-1}); SICN = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg of N ha^{-1}; SIC = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}); COIN-I = inoculation in the furrow with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* (100 mL ha^{-1}); COIN-II = in-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* (150 mL ha^{-1}) and COIN-III = in-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* (200 mL ha^{-1}).

Table 7 - Grain yield (YIELD) as a function of Co and Mo (cobalt and molybdenum) application and bacterial inoculation modes.

Co and Mo	---------------------------Inoculation Modes---------------------------					
	NICN	SICN	SIC	COIN-I	COIN-II	COIN-III
	---------------------------YIELD (kg ha^{-1})---------------------------					
With	4478.08 aB	3927.50 bC	4921.73 aA	4892.40 aA	4614.20 aB	4898.53 aA
Without	3885.57 bB	4343.34 aA	4412.82 bA	4111.23 bB	4226.88 bA	4126.45 bB

Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ statistically from each other by the Scott-Knott test at 5%. NICN = control (not inoculated + 20 kg of N ha^{-1}); SICN = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}) + 20 kg of N ha^{-1}; SIC = seed inoculation with *Bradyrhizobium* (100 mL ha^{-1}); COIN-I = inoculation in the furrow with *Bradyrhizobium* (150 mL ha^{-1}) + *Azospirillum* (100 mL ha^{-1}); COIN-II = in-furrow inoculation with *Bradyrhizobium* (200 mL ha^{-1}) + *Azospirillum* (150 mL ha^{-1}) and COIN-III = in-furrow inoculation with *Bradyrhizobium* (300 mL ha^{-1}) + *Azospirillum* (200 mL ha^{-1}).

resulting in more vigorous plants (HUNGRIA, 2011). Thus, in adverse conditions, such as dry spells or greater pressure from pathogens, which expose the plant to a higher level of stress, the use of bacteria could be a favorable factor for the development of the crop. This condition was not encountered during the conduct of the experiment, but they are relatively common.

The applicability of the results of this study must undergo further confirmation since the research was carried out in just one year and one location due to COVID-19 pandemic and subsequent lockdown measures. Despite this restriction, the research showed that soybean producers may have two conditions for the use of these inputs. For years with more stable climate forecasts and less risk of disease pressure, producers could use only inoculation of soybean seeds with *Bradyrhizobium* and application of Co and Mo. But in years of greater risk, soybean producers could enhance this process with co-inoculation.

The use of N together with inoculation with *Bradyrhizobium* had a positive effect only for height of insertion of the first pod. For all other variables it was unfavorable, as was also observed by ZUFFO et al. (2019). In terms of grain yield, it was observed that the use of inoculation without N resulted in 20.0% higher values when compared to the use with N. This fact favors the producer because in addition to the direct savings with the use of the mineral, it also brings environmental advantages, reducing risks of contamination of water bodies and emission of greenhouse gases.

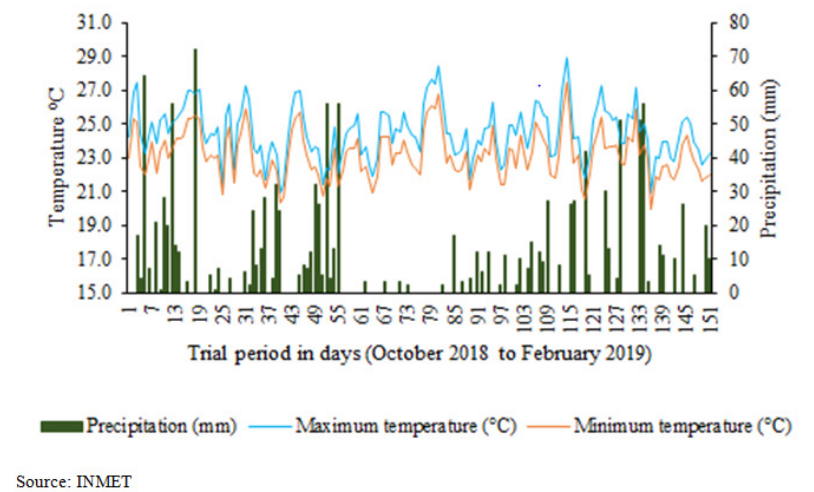

Figure 1 - Rainfall data and maximum and minimum air temperature. Chapadão do Sul, MS, Brazil, 2018/19.

Although, abundant in the literature, the effects of inoculation and co-inoculation of bacteria of the genera *Bradyrhizobium* and *Azospirillum*, and the use of Co and Mo, are still inconsistent, and it is important that future research consolidate the use of this technology, aiming to increase grain yield.

CONCLUSION

With the study carried out in only one place and one year, it was possible to verify that inoculation with *Bradyrhizobium japonicum* associated with *Azospirillum brasilense* co-inoculation via foliar and Co and Mo, provides increments in the number of pods per plant, number of grains per pod and weight of 100 grains, reflecting increases in soybean grain yield.

ACKNOWLEDGEMENTS

Support from the Universidade Federal de Mato Grosso do Sul (UFMS).

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization and Data acquisition: HMB, RCFA, SFL and VFB. Design of methodology and data analysis: HMB, RCFA, SFL, MSZ and VFB. HMB, RCFA, SFL, MSV and MASC prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

AMORIM, F.A. et al. Sowing seasons on soybean yield potential in Uberlândia – MG. **Semina**: Ciências Agrárias, v. 32, n. 4, sup.1, p.1793-1802, set. 2011. Available from: <https://www.uel.br/revistas/uel/index.php/semagrarias/issue/archive?issuesPage=2#issues>. Accessed: Sept. 23, 2020. doi:10.5433/1679-0359.2011.

BALÍK, J. et al. The fluctuation of molybdenum content in oilseed rape plants after the application of nitrogen and sulphur fertilizers. **Plant Soil Environ**, Praha, v. 52, n. 7, p. 301-307, 2006. doi:10.17221/3445-PSE.

BALDANI, J. et al. Recent advances in BNF with non-legume plants. **Soil Biology and Biochemistry**, v. 29, n. 5-6, p 911-922, may – june 1997. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0038071796002180?via%3Dihub>. Accessed: Jan. 15, 2020. doi: 10.1016/S0038-0717(96)00218-0.

BÁRBARO, I. M. et al. Soybean yield in response to standard inoculation and co-inoculation. **Colloquium Agrariae**, v. 5, n. 1, p. 01-07, jan-jun. 2009. Available from: <https://revistas.unoeste.br/index.php/ca/article/view/372>. Accessed: Jan. 17, 2020. doi: 10.5747/ca.2009.v05.n1.a0040.

BARRANQUEIRO, H. R.; DALCHIAVON, F. C. Nitrogen application on soybean. **Revista de Ciências Agrárias**, v. 40, n. 1, p. 196-204, 2017. Available from: <http:// www.scielo.mec.pt/pdf/rca/v40n1/v40n1a22.pdf>. Acesso em: Apr. 02, 2022. doi: 10.19084/RCA16030.

BASHAN, Y. et al. *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances (1997-2003). **Canadian Journal of Microbiology**, v. 50, n. 8, p. 521-577, aug. 2004. Available from: <https://pubmed.ncbi.nlm.nih.gov/15467782>. Accessed: Mar. 15, 2020. doi: 10.1139/w04-035.

BOLETA, E. H. M. et al. Inoculation With Growth-Promoting Bacteria *Azospirillum brasilense* and Its Effects on Productivity and Nutritional Accumulation of Wheat Cultivars. **Frontiers in Sustainable Food Systems**, v.4, p. 1-10, 2020. Available from: <https://www.frontiersin.org/articles/10.3389/fsufs.2020.607262/full>. Acessed: Jun. 06, 2022. doi: 10.3389/fsufs.2020.607262.

BRACCINI, A. L. et al. Soybean co-inoculation with *Bradyrhizobium japonicum* e *Azospirillum brasilense* and nitrogen fertilization on plant nodulation and crop yield. **Scientia Agraria Paranaensis**, v. 15, n. 1, p. 27-35, mar. 17, 2016. Available from: <https://e-revista.unioeste.br/index.php/scientiaagraria/article/view/10565>. Accessed: Nov. 12, 2021. doi: 10.18188/sap.v15i1.10565.

BRANCALIÃO, S. R. et al. Grain yield and composition of soybean crop after the supply of nitrogen with the use of cover crops at direct sowing. **Nucleus**, v. 12, n. 1, p.1-8, apr. 2015. Available from: <http://www.nucleus.feituverava.com.br/index.php/nucleus/article/view/1001>. Accessed: Sept. 23, 2021. doi: 10.3738/1982.2278.1001.

BULEGON, L. G. et al. Components of production and yield of soybean inoculated with *Bradyrhizobium* and *Azospirillum*. **Terra Latinoamericana**, v. 34, n. 2, p 196 – 176, abr-jun. 2016. Available from: <http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-57792016000200169&lng=es&nrm=iso&tlng=pt>. Accessed: Oct. 06, 2021.

CAMPO R. J. et al. Molybdenum-enriched soybean seeds enhance N accumulation, seed yield, and seed protein content in Brazil. **Field Crops Research**, v. 110, n. 3, p. 219–224. 2009. Available from: <http://www.bashanfoundation.org/contributions/Hungria-M/2009.-Hungria-FCR.pdf>. Accessed: Jun. 06, 2022. doi: 10.1016/j.fcr.2008.09.001.

CARVALHO, E. R. et al. Performance of soybean [*Glycine max* (L.) Merrill] cultivars in the summer cropping in the south of Minas Gerais. **Ciência e Agrotecnologia**, v. 34, n. 4, p. 892 – 899, Ago. 2010. Available from: <https://www.scielo.br/j/cagro/a/MCHfTYsKqNvM5RBVybTtvDd/abstract/?lang=pt>. Accessed: Ago, 13, 2020. doi: 10.1590/S1413-70542010000400014.

CHAGAS, H. et al. Application of molybdenum via sedes and its effect on nodulation, nitrate reductase activity and physiological soybeans seed quality. **Enciclopédia Biosfera**, v. 11, n. 21, p. 190-202, Jun. 2015. Available from: <https://conhecer.org.br/ojs/index.php/biosfera/article/view/1739>. Accessed: Jul. 11, 2020.

CHIBEBA, A. M. et al. Co-inoculation of soybean with *Bradyrhizobium* and *Azospirillum* Promotes early nodulation. **American Journal of Plant Sciences**, v. 6, n. p. 1641-1649, Jun. 2015. Available from: <https://ainfo.cnptia.embrapa.br/

digital/bitstream/item/126153/1/AJPS-2015063013320647.pdf>. Accessed: Sept. 13, 2020. doi:10.4236/ajps.2015.610164.

Companhia Nacional de Abastecimento. **Acompanhamento da safra brasileira de grãos. 10º Levantamento**. Brasília: Conab, 2022. 88p.

CRUZ, S. C. S. et al. Soybean cropping under different plant densities and spatial arrangements. **Revista de Agricultura Neotropical**, v. 3, n.1, p. 1 – 6, Feb. 2016. Available from: <https://periodicosonline.uems.br/index.php/agrineo/article/view/431>. Accessed: Apr. 18, 2020. doi: 10.32404/rean.v3i1.431.

DIDONET, A. D. et al. Reallocation of nitrogen and biomass to the sedes in wheat inoculated with *Azospirillum* bacteria. **Pesquisa Agropecuária Brasileira**, v. 35, n. 2, p. 401- 411, 2000. doi:10.1590/S0100-204X2000000200019.

DOURADO NETO, D. et al. Adubação mineral com cobalto e molibdênio na cultura da soja. **Semina:** Ciências Agrárias, v. 33, n. 1, p. 2741 – 2752, dec. 2012. Available from: <https://repositorio.usp.br/bitstream/handle/BDPI/34096/Douradoetal-Semina2012.pdf;sequence=1>. Accessed: Feb. 14, 2019. doi: 10.5433/1679-0359.2012v33Supl1p2741.

EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária. **Tecnologias de produção de soja:** Região Central do Brasil. Londrina: Embrapa Soja, 2011.

EMBRAPA- Empresa Brasileira de Pesquisa Agropecuária**. Sistema Brasileiro de Classificação de Solos.** Brasília: Embrapa, 2018. 355p.

EMBRAPA SOJA. **Dados econômicos**. Available from: <https://www.embrapa.br/soja/cultivos/soja1/dados-economicos>. Accessed: Apr. 01, 2022.

FERREIRA, D. F. 2011. Sisvar: A computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011. doi: 10.1590/S1413-70542011000600001.

GALDINO, P. L. G. et al. Cobalt and Molybdenum applicated via leaf in the growth vegetative and yield of soybean.**Scientific Electronic Archives**,v. 13, n. 4, p. 51–59. Available from: <https://sea.ufr.edu.br/SEA/article/view/846?articlesBySameAuthorPage=3>. Accessed: Jun. 06, 2022. doi.org/10.36560/1342020846.

GALINDO, F. S. et al. Modes of application of cobalt, molybdenum and *Azospirillum brasilense* on soybean yield and profitability**. Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 21, n. 3, p. 180–185, 2017. Available from: <https://www.scielo.br/j/rbeaa/a/MHsnnrncNJBdThXDRZzYf4D/?lang=en>. Accessed: Jun. 06, 2022. doi: 10.1590/1807-1929/agriambi.v21n3p180-185.

GOLO, A. L. et al. Quality of soybean sedes produced with different doses of molybdenum and cobalt. **Revista Brasileira de Sementes**, v. 31, n. 1, p. 40-49, 2009. Available from: <https://www.scielo.br/j/rbs/a/cLjdCC44P5LLzXsmSXVvdNk/?lang=pt>. Accessed: Jun. 06, 2022. doi: 10.1590/S0101-31222009000100005.

HUNGRIA M. et al. The Importance of Nitrogen Fixation to Soybean Cropping in South America. In: WERNER D., NEWTON W.E. (eds) **Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment. Nitrogen Fixation:** Origins, Applications, and Research Progress, vol 4. Springer, Dordrecht, 2005. Available from: <https://link.springer.com/chapter/10.1007/1-4020-3544-6_3>. Accessed: Mar. 17, 2020. doi:10.1007/1-4020-3544-6_3.

HUNGRIA, M. **Inoculação com** *Azospirillum brasilense*: **inovação em rendimento a baixo custo.** Embrapa soja, 2011. 36p. (Documentos Embrapa Soja, 325).

HUNGRIA, M. et al. **Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. Plant Soil**, v. 331, p. 413–425, jan. 2010. Available from: <https://link.springer.com/article/10.1007/s11104-009-0262-0>. Accessed: Jun. 12, 2020. doi: 10.1007/s11104-009-0262-0.

HUNGRIA, M. et al. Co-inoculation of soybeans and common beans with *rhizobia* and *azospirilla*: strategies to improve sustainability. **Biology and Fertility Soils**, v. 49, p. 791–801, Jan. 2013. Available from: <https://link.springer.com/article/10.1007/s00374-012-0771-5#citeas>. Accessed: Jan. 19, 2020. doi: 10.1007/s00374-012-0771-5.

LANA, R. M. Q. Cobalt and molybdenum concentrated suspension for soybean seed treatment. **Revista Brasileira de Ciência do Solo**, v. 33, n. 6, p. 1715-1720, 2009. Available from: < https://www.scielo.br/j/rbcs/a/V9z6YVxh745dGSVygM6XTKD/?lang=en>. Accessed: Jun. 06, 2022. doi: 10.1590/S0100-06832009000600020.

MARCON, E.C. et al. Use of different sources of nitrogen in soybean crop. **Revista Thema**, v. 14, n. 2, p. 298 – 308, 2017. Available from: <https://periodicos.ifsul.edu.br/index.php/thema/article/view/427>. Accessed: Mar. 18, 2020. doi:.10.15536/thema.14.2017.298-308.427.

MESCHEDE D. K. et al. Grain yield, seeds protein content and plant agronomic traits of soybean in response to foliar fertilization and molybdenum and cobalt seed treatment. **Acta Scientiarum Agronomy**, v. 26, n. 2, p. 139-145, 2004. Available from: <https://www.scielo.br/j/rbcs/a/V9z6YVxh745dGSVygM6XTKD/?lang=en>. Accessed: Jun. 06, 2022. doi: 10.4025/actasciagron.v26i2.1874.

MENGEL, K.; KIRKBY, E. A. **Principles of plant nutrition**. Dordrecht: Kluwer Academic Publishers, 2001. 849p.

MORENO, G. et al. Application of nitrogen fertilizer in high-demand stages of soybean and its effects on yield performance. **Australian Journal of Crop Science**, v. 12, n. 1, p. 16-21, 2018. Available from: <http://www.cropj.com/moreno_12_1_2018_16_21.pdf>. Accessed: Apr. 02, 2022. doi:10.21475/ajcs.18.12.01.pne507.

PESSOA, A. et al. Nitrogenase and nitrate reductase activities and productivity of common beans in response to foliar application of molibdenum. **Revista Brasileira de Ciências do Solo**, v.25, n. 1, p.217- 224, mar. 2001. Available from: <https://www.researchgate.net/publication/237026739_Atividades_de_nitrogenase_e_redutase_de_nitrato_e_produtividade_do_feijoeiro_Ouro_Negro_em_resposta_a_adubacao_foliar_com_molibdenio>. Accessed: Mar. 18, 2020. doi: 10.1590/S0100-06832001000100023.

RAIJ, B. VAN; ANDRADE, J.C. DE; CANTARELLA, H.; QUAGGIO, J.A. **Análise Química para Avaliação da Fertilidade de Solos Tropicais**. Campinas, Instituto Agronômico, 2001, 285p.

SAATH, K. C. O.; FACHINELLO, A. L. Crescimento da demanda mundial de alimentos e restrições do fator terra no Brasil. **Revista de Economia e Sociologia Rural**, v. 56, n. 2, p. 195 – 212, apr – jun. 2018. Available from: <https://www.scielo.br/j/resr/a/DdPXZbMzxby89xBDg3XCTgr/?lang=pt>. Accessed: Mar. 18, 2020. doi: 10.1590/1234-56781806-94790560201.

SATURNO, D. F. et al. Mineral nitrogen impairs the biological nitrogen fixation in soybean of determinate and indeterminate growth types. **Journal of Plant Nutrition**, v. 40, n. 12, p. 1690–1701, 2017. Available from: <https://www.tandfonline.com/doi/full/10.1080/01904167.2017.1310890>. Accessed: Jun. 06, 2022. doi: 10.1080/01904167.2017.1310890.

SOUSA, D. M. G.; LOBATO, E. Correção da acidez do solo. In: SOUSA, D. M. G.; LOBATO, E. **Cerrado**: Correção do solo e adubação. Brasília: Embrapa Informação Tecnológica, 2004. Cap. 3. p. 81-96.

SILVA, A. F. et al. Inoculation with Bradyrhizobium and application forms of Cobalt and Molybdenum in soybean crop. **Revista Agrarian**, v. 4, n. 12, p. 98–104. 2011. Available from: <https://agris.fao.org/agris-search/search.do?recordID=BR2012403842>. Accessed: Jun. 06, 2022.

SOUZA, E. M. Does the nitrogen application associated with *Azospirillum brasilense* inoculation influence corn nutrition and yield? **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 23, n. 1, p. 53–59, 2019. Available from: <https://www.scielo.br/j/rbeaa/a/hQsWS58c43FB8DKzbn8zwbs/abstract/?lang=en>. Accessed: Jun. 06, 2022. doi: 10.1590/1807-1929/agriambi.v23n1p53-59.

STURZ, A. V.; J. NOWAK. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. **Applied Soil Ecology**, v. 15, n. 2, p. 183-190, oct. 2000. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0929139300000949>. Accessed: May, 16, 2020. doi: 10.1016/S0929-1393(00)00094-9.

TAIZ, L. et al. **Fisiologia vegetal e desenvolvimento vegetal**. 6ªed. Porto Alegre: Editora Artmed, 2017.

TEIXEIRA FILHO, M. C. M. et al. Inoculation with *Bradyrhizobium* sp. and *Azospirillum brasilense* Associated with Application of Cobalt and Molybdenum on Nutrition and Soybean Yield. **In Soybean-The Basis of Yield, Biomass and Productivity.** In:. KASAI M. (Ed.), IntechOpen, London, United Kingdom. pp. 63–73, 2017 Available from: <https://www.intechopen.com/chapters/53241>. Accessed: Jun. 06, 2022. doi: 10.5772/63118.

TOLEDO, M. Z. et al. Nodulation and nitrate reductase activity affected by molybdenum application in soybean. **Bioscience Journal**, v. 26, n. 6, p. 858-864, nov/dec 2010. Available from: <https://repositorio.unesp.br/bitstream/handle/11449/17320/WOS000285687300004.pdf?sequence=3&isAllowed=y>. Accessed: Jan. 22, 2020.

ZILLI, J. E.et al. Influence of fungicide seed treatment on soybean nodulation and grain yield. **Revista Brasileira de Ciência de Solo**, v. 33, n.4, p. 917-923, aug. 2009. Available from: <https://www.scielo.br/j/rbcs/a/fTRTNjrCfTtSjR54W7vmfyk/abstract/?lang=en&format=html>. Accessed: Mar. 17, 2020. doi: 10.1590/S0100-06832009000400016.

ZUFFO, A. M. et al. Nitrogen fertilization in soybean inhibits nodulation and fails to improve plants initial growth. **Revista em Agronegócio e Meio Ambiente**, v. 12, n. 2, p. 333-349, 2019. Available from: <https://periodicos.unicesumar.edu.br/index.php/rama/article/view/5959>. Accessed: Mar. 15, 2022. doi:10.17765/2176-9168.2019v12n2p333-349.