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Modes of application of cobalt, molybdenum and *Azospirillum brasilense* on soybean yield and profitability

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A B S T R A C T

The use of cobalt (Co) and molybdenum (Mo) along with the co-inoculation with *Azospirillum brasilense* on soybean can contribute to biological nitrogen fixation (BNF), allowing higher yields. Thus, this work aimed to study the technical and economic viability of soybean in the Cerrado, according to the mode of application of Co + Mo, and seed or leaf co-inoculation with *Azospirillum brasilense*. The experiment was conducted in Selvíria-MS, in a no-tillage system in an Oxisol, arranged in a randomized block design with six treatments (seed application of two treatments with Co + Mo, with or without inoculation with *A. brasilense*, respectively; Foliar application of Co + Mo at soybean V3 stage; Foliar application of Co + Mo together with *A. brasilense* at V3 growth stage; Foliar application of *A. brasilense* at V3 growth stage; and the Control, consisting of only inoculation with Rhizobium), and four replicates. Seed application of Co + Mo associated with seed inoculation with *A. brasilense* promoted the highest leaf N content, hundred-grain weight, yield and profitability with the soybean crop.

Palavras-chave:
coinoculação com *Azospirillum brasilense*
custo operacional total
viabilidade econômica
viabilidade técnica
concentração de nitrogênio foliar

Modos de aplicação de cobalto, molibdênio e *Azospirillum brasilense* na produtividade e lucratividade da soja

R E S U M O

A utilização de cobalto (Co) e molibdênio (Mo) pode, juntamente com a coinoculação com *Azospirillum brasilense* na cultura da soja, contribuir na fixação biológica de nitrogênio (FBN), possibilitando maiores produtividades. Por esta razão, se objetivou estudar a viabilidade técnica e econômica da cultura da soja no Cerrado em função do modo de aplicação de cobalto e molibdênio e da coinoculação de sementes ou via foliar com *Azospirillum brasilense*. O experimento foi desenvolvido em Selvíria, MS, em sistema de semeadura direta, em Latossolo Vermelho distrófico, dispostos em delineamento experimental de blocos casualizados com seis tratamentos (aplicação via semente, em dois tratamentos de Co e Mo, com ou sem a inoculação com *A. brasilense*, respectivamente; aplicação via foliar de Co e Mo no estádio V3 da soja; aplicação via foliar de Co e Mo juntamente com *A. brasilense* no estádio V3; aplicação via foliar de *A. brasilense* no estádio V3 e a Testemunha, constando apenas da inoculação com bradirrizóbio) e quatro repetições. A aplicação de Co e Mo via semente associados à inoculação com *A. brasilense* da semente proporcionou a maior concentração de N foliar, massa de 100 grãos, produtividade e lucratividade com a cultura da soja.

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Introduction

Due to the large nitrogen (N) requirement by the crops to achieve high yields, N_2 fixation must function with maximum efficiency (Figueiredo et al., 2008; Vieira Neto et al., 2008; Zilli et al., 2008; Hungria et al., 2010; Rodrigues et al., 2012; Ignácio et al., 2015; Bulegon et al., 2016). Besides using inoculant with *Bradyrhizobium* selected and efficient, cobalt (Co) and molybdenum (Mo) decisively contribute to the biological N_2 fixation (BNF) (Dourado Neto et al., 2012), influencing the synthesis of leghemoglobin, which determines the activity of the nodules and, being a structural component of the nitrogenase, catalyzes the reduction of atmospheric N_2 to NH_3 (Bárbaro et al., 2009a; Golo et al., 2009).

Considering the main current and potential limitations of BNF in the soybean crop and the benefits attributed to the various crops by the inoculation with *Azospirillum* (free- living diazotrophic bacteria), especially the greater development of the root system and, consequently, also higher absorption of water and nutrients (Galindo et al., 2016), it is deduced that, with the co-inoculation in microorganism, there may be improvements in the performance of the crops in an approach that respects the current demands for agricultural, economic, social and environmental sustainability (Hungria et al., 2013).

It becomes necessary, in this context, to conduct studies that aim to increase BNF efficiency associating the mode of application of Co + Mo with the co-inoculation of *Bradyrhizobium* and *Azospirillum brasilense*, allowing better use of water and nutrients, without causing reduction in yield or quality of agricultural products. However, there are not many studies in the literature that economically evidence the effect of co-inoculation with *Azospirillum brasilense* associated with the application of Co + Mo in the soybean crop.

Based on the above, this study aimed to evaluate the technical and economic viability of the soybean crop in the Cerrado, as a function of the mode of application of Co + Mo and its co-inoculation in seeds or leaves with *Azospirillum brasilense*.

Material and Methods

The research was carried out at the experimental area of the Faculty of Engineering - UNESP, located in Selvíria, MS, Brazil, at altitude of 335 m. The soil of the experimental area was classified as dystrophic Red Latosol with clayey texture, according to the classification of EMBRAPA (2013). This soil has been cultivated by annual crops for more than 27 years and, in the last 10 years, has been under no - tillage system, with maize as the previous crop. In this area, there is no history of artificial introduction of *Azospirillum brasilense*. The climatic type in the region is Aw, according to Köppen's classification, characterized as humid tropical with rainy season in the summer and dry season in the winter. The climate data recorded during the experimental period are shown in Figure 1.

The soil chemical attributes in the arable layer were determined before installing the experiment, according to the methodology proposed by Raij et al. (2001), showing the following results: 10 mg dm^{-3} of P (resin); 5 mg dm^{-3} of S-SO_4; 22 g dm^{-3} of OM; 5.3 of pH ($CaCl_2$); K, Ca, Mg, H + Al = 2.4; 21.0; 18.0 and 28.0 $mmol_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 3.2; 22.0; 24.2 and 1.2 mg dm^{-3}, respectively; 0.16 mg dm^{-3} of B (hot water) and 60% of base saturation. Based on the soil analysis and on the recommendation of fertilization for the soybean crop (Ambrosano et al., 1997), the fertilization at sowing was applied in the sowing furrow with 80 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O.

The seeds were treated with the fungicides Carbendazim + Thiram at the dose of 30 + 70 g of a.i. for every 100 kg of seeds, respectively. After drying the seeds, they were inoculated with rhizobium at the dose of 200 mL ha^{-1} (strains: SEMIA 5019 (*Bradyrhizobium elkanii*) and SEMIA 5079 (*Bradyrhizobium japonicum*), with guarantee of 5 x 10^9 of viable cells per mL), inside a clean concrete mixer to incorporate them into the seeds. This procedure was performed one hour before soybean sowing.

The experiment was installed in December 2014, with mechanical sowing of the soybean cultivar 'BMX Potência RR', at spacing of 0.45 m between rows, by planting 17 seeds per meter.

The experimental design was randomized blocks with six treatments and four replicates. The treatments were: 1) Control (soybean without inoculation with *Azospirillum brasilense* and without Co + Mo application); 2) Seed with Co + Mo application at the dose of 150 mL ha^{-1} of the commercial product (15% of Mo and 1.5% of Co), which

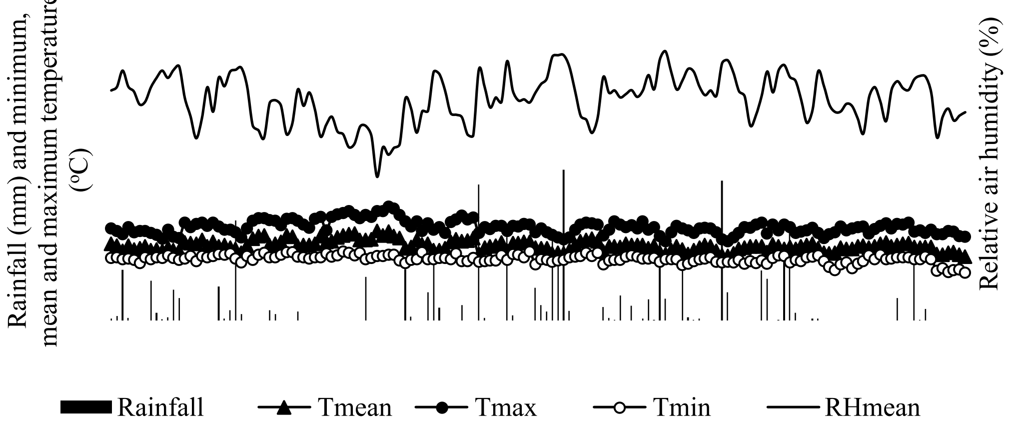

Figure 1. Rainfall, relative air humidity (RHmean) and maximum, mean and minimum temperatures (Tmean, Tmax and Tmin) recorded in the meteorological station situated at the Teaching, Research and Extension Farm of the FE/UNESP during the soybean cultivation from December 2014 to April 2015

is the recommendation of the Embrapa Soybean; 3) Seed inoculated with *Azospirillum brasilense* at the dose of 200 mL ha^{-1} (strains Abv5 and Abv6, with guarantee of 2 x 10^8 CFU per mL) and with Co + Mo application at the above-mentioned dose; 4) Foliar application of *Azospirillum brasilense* in the V3 stage of soybean, at the previously mentioned dose; 5) Foliar application of Co + Mo in the V3 stage, at the previously mentioned dose; 6) Foliar application of Co + Mo along with foliar inoculation of *Azospirillum brasilense* in the V3 stage, at the above-mentioned doses.

Each plot was composed of seven 5-m-long soybean rows, spaced by 0.45 m, totaling 15.75 m^2. The three central rows of each plot, disregarding the single border, were used for evaluations, totaling an effective sampling area of 6.75 m^2 plot^{-1}.

The following variables were evaluated: a) leaf N content, determined using the methodology described by Malavolta et al. (1997), by collecting the upper third trifoliate of 30 soybean plants, at full flowering, based on the methodology described in Ambrosano et al. (1997); b) hundred-grain weight, determined on a precision scale (0.01 g), at 13% (wet basis); c) grain yield, determined through the harvest of plants in the three central rows of each plot. After mechanical threshing, the grains were quantified and the data were transformed to kg ha^{-1} at 13% (wet basis). These results were subjected to analysis of variance (F test) using the Tukey test at 0.05 probability level for the comparison of means.

The economic analysis was made using the structure based on the total operating cost (TOC) of production used by the Institute of Agricultural Economy (IEA), according to Matsunaga et al. (1976), which consists in the sum of all costs: operations, inputs (fertilizers, seeds, pesticides, etc.), labor, machinery and irrigation, referred to as effective operating cost (EOC). Besides the TOC, this study considered other expenses and interest of costs, corresponding to 5% of the EOC (Matsunaga et al., 1976), thus resulting in the total operating cost (TOC), which was extrapolated to one hectare.

The profitability of the treatments was determined according to Martin et al. (1998), as follows: gross revenue (GR) (in R$), as the product of the produced amount (number of 60 kg sacks) and the mean sale price (in R$), and the operating profit (OP), as the difference between the gross revenue and the total operating cost; profitability index (PI), considered as the relationship between the operating profit (OP) and the gross revenue (GR) in percentage.

The analyses considered the prices paid in 2016 (Agrianual, 2015) adjusted to those practiced in commercial plantations in the region of Selvíria, MS, based on the mean of the last 3 agricultural years. In this study, simulations were performed as if each treatment of the experiment represented commercial plantations. To facilitate the discussion, values referring to the yields were transformed to 60 kg sacks, since this is the basic unit of marketing by the local producers.

Results and Discussion

The N content was influenced by the treatments whose Co + Mo application associated with *Azospirillum brasilense* inoculation in the seeds and the foliar application of Co + Mo promoted higher content of the nutrient in comparison to the foliar application of *A. brasilense* and the non-application of Co + Mo and *A. brasilense* (Table 1).

This greater N absorption promoted by Co + Mo application associated with the inoculation of *A. brasilense* in the seed reflected in greater plant development (data not shown) and grain filling, which is evidenced by the higher value of hundred-grain weight and, consequently, increase in soybean grain yield in this treatment, in comparison to the foliar application of *A. brasilense* and the control without Co + Mo application and inoculation of *A. brasilense* (Table 1). Considering the best treatment cited above, there were increments of 1,007 and 752 kg ha^{-1} of grains, equivalent to 18.4 and 13.0% in relation to the control and the mean of the other treatments, respectively. These results corroborate those of Golo et al. (2009), who report that Co and Mo contribute decisively to the biological N_2 fixation (BNF) and those of Hungria et al. (2013), who studied co-inoculation with *A. brasilense* and obtained increment of 16.1% in soybean grain yield, in comparison to the isolated use of *Bradyrhizobium* strains.

According to Bárbaro et al. (2009b), the literature reports that bacteria called PGPB (plant growth-promoting bacteria), such as *A. brasilense*, can act in the relationships between rhizobia and leguminous crops, promoting increments in plant growth and grain yield, in the total biologically fixed N, besides improvements in the use of N obtained by the plant through the symbiosis with rhizobium, corroborating the results found in the present study.

These effects can be due to various mechanisms, which include an anticipation in the BNF of the nodules, increment in the dry matter of the nodules, promotion of occurrence of heterologous nodulation through the increase in the formation of root hairs and secondary roots, with increment in the infection sites, inhibition of phytopathogens and production of phytohormones, besides the influences in the partition of dry matter between roots and shoots (Bárbaro et al., 2009b). In addition, as pondered by Hungria et al. (2013), these effects promoted by the co-inoculation of PGPB and

Table 1. Leaf N content, hundred-grain weight and grain yield of soybean as a function of the treatments

Treatment#	N content (g kg^{-1} of DM)	Hundred-grain weight (g)	Grain yield (kg ha^{-1})
(1) Control	48.65 b	14.68 b	5,550 b
(2) CoMo seed	50.91 ab	15.88 ab	6,083 ab
(3) CoMo + Azos seed	56.21 a	16.10 a	6,557 a
(4) Azos via foliar	49.00 b	14.80 ab	5,355 b
(5) CoMo via foliar	55.95 a	14.60 b	5,685 ab
(6) CoMo + Azos foliar	54.30 ab	14.88 ab	5,602 ab
Overall Mean	52.50	15.15	5,805
CV (%)	4.34	3.76	7.27
LSD (5%)	6.46	1.31	970

Means followed by the same letters in the column do not differ by Tukey at 0.05 probability level.
The numbers 1, 2, 3, 4, 5 and 6 refer to the treatments: 1) Control (soybean without inoculation of *Azospirillum brasilense* and without Co + Mo application); 2) Soybean seed without inoculation of *Azospirillum brasilense* and with Co + Mo application; 3) Soybean seed inoculated with *Azospirillum brasilense* and without Co + Mo application; 4) Soybean seed without inoculation of *Azospirillum brasilense* and without Co + Mo application, but with foliar application of *Azospirillum brasilense* at the soybean V3 stage; 5) Soybean seed without inoculation of *Azospirillum brasilense* and without Co + Mo application, but with foliar application of Co + Mo at the V3 stage; 6) Soybean seed without inoculation of *Azospirillum brasilense*, but with foliar application of Co + Mo along with foliar inoculation of *Azospirillum brasilense*, at the soybean V3 stage

rhizobium seem to be under the influence of specific signals between the involved bacterial genotypes and the host plant genotype, which requires more studies on the response of the co-inoculation as a function of the genotypes, in order to obtain more-responsive genotypes.

Table 2 shows the structure of the total operating cost (TOC) of the soybean crop, describing the control treatment, in one hectare. This model of TOC structure was used in all treatments.

The initial investments with soil tillage and liming were not considered in this study, since these practices were not performed, especially because the area had been under no - tillage system for more than ten years when the experiment was installed, thus contributing to the reduction of the initial costs with the planting of the soybean crop.

The expenses with mechanized operations and inputs, which constitute the effective operating cost (EOC), were 1,668.35 ha^{-1} and the total operating cost (TOC) was R$ 1,805.99 ha^{-1} (Table 1).

In the operations that constitute the EOC, the costs with irrigation stand out, totaling 21% of the expenses, because, to obtain the high grain yields observed in the cultivation period, besides the characteristics of the studied region (Cerrado), it was necessary to adopt this technology (irrigation). However, for the expenses with inputs, fertilizers and pesticides stand out, being responsible for 36.22 and 10.51% of the EOC, respectively (Table 2). Probably, this occurred due to the large requirement for nutrients by the soybean crop, which demands a considerable amount of fertilizer for high grain yields, besides the need of desiccation and control of weeds and pathogens.

In general, the expenses with mechanized operations, followed by fertilizers, were the highest ones, corresponding to 42.6 and 33.5% of the TOC, respectively (Table 2). It should be pointed out that, with the application of Co + Mo and with the inoculation of *A. brasilense*, there is an increase in the percentage of expenses in relation to the TOC. The costs with the use of Co + Mo and with inoculation of *A. brasilense*, individually or combined, corresponded to 0.6, 1.1 and 1.7% of the TOC, respectively.

As to the TOC and the grain yield of the treatments (Table 3), the highest value of TOC refers to the treatment with inoculation of *A. brasilense* and foliar application of Co + Mo, whereas the lowest TOC value corresponds to the control treatment, i.e., only with the inoculation of *Bradyrhizobium* strains. However, it is important to highlight that, to obtain the highest soybean grain yields (Table 1), both the Co + Mo application and the inoculation of *A. brasilense* were important to increase BNF efficiency, since there was an increment in the leaf N content.

As to grain yield, the application of Co + Mo and the co-inoculation of the soybean crop with *A. brasilense* via seeds stand out, which led to mean yield of 109 sacks of 60 kg, approximately 17 sacks of grains more than the control (Table 3).

Regarding the gross revenues per hectare (Table 3) obtained in the combinations of the treatments for the soybean crop,

Table 3. Total operating cost (TOC), grain yield (GY) and gross revenue (GR) of the soybean crop as a function of combination of treatments

Treatment	TOC R$	GY sc 60 kg ha^{-1}	GR R$
Control	1,805.99	92.50	5,735.00
CoMo seed	1,817.87	101.38	6,285.77
A. brasilense + CoMo seed	1,839.52	109.28	6,775.57
A. brasilense foliar	1,882.84	89.25	5,533.50
CoMo foliar	1,817.87	94.75	5,874.50
A. brasilense + CoMo foliar	1,894.73	93.37	5,788.73
Mean	1,843.14	96.76	5,998.85

**Soybean marketing price - R$ 62.00 sc

Table 2. Estimate of total operating cost of soybean for the control treatment calculated for one hectare (ha)

Description	Production cost				
	Specification[1]	Nº times	Coefficient	Unitary value (R$)	Total value (R$)
A - Mechanized operations					
Desiccation	HM	1.00	0.50	85.00	42.50
Mowing (triton)	HM	1.00	0.50	85.00	42.50
Sowing	HM	1.00	1.00	110.00	110.00
Spraying	HM	3.00	0.60	85.00	153.00
Harvest	HM	1.00	0.60	118.00	70.80
Irrigation (pivot)	mm	1.00	140.00	2.50	350.00
Subtotal A					768.80
B - Inputs					
Fertilizer 04-20-20	t	1.00	0.40	1,510.71	604.28
Inoculant (*B. elkanii* and *B. japonicum*)	100 mL	1.00	2.00	10.00	20.00
Soybean seeds	sc 50 kg	1.00	1.00	100.00	100.00
Herbicide Glyphosate	L	1.00	4.00	14.51	58.04
Herbicide 2,4-D	L	1.00	1.00	13.24	13.24
Herbicide chlorimuron	kg	1.00	0.03	146.68	4.40
Fungicide seed treatment carbendazim	L	1.00	0.05	45.57	2.28
Insecticide seed treatment thiamethoxam	L	1.00	0.10	407.68	40.77
Fungicide azoxystrobin + cyproconazole	L	1.00	0.30	150.89	45.27
Insecticide methomyl	L	1.00	0.50	22.54	11.27
Subtotal B					899.55
Effective operating cost (EOC)				1,668.35	
Other expenses				83.42	
Interest of costs				54.22	
Total operating cost (TOC)				1,805.99	

it was observed that, for a constant price of the soybean, the gross revenues of the treatments followed the same trend of the yields (Table 3), i.e., the increments in the revenue occur through the increments in grain yield. This result in agreement with Silva et al. (2007) and Duete et al. (2009), who claim that yield is an essential factor to guarantee good profitability to the producer. According to Duete et al. (2009), even in regions in which the producer obtains good prices in the marketing of grains, if the yield is low, the profitability is compromised. Thus, the investment in management practices, such as balanced fertilization and inoculation with PGPB, increase grain yield and the gross margin of the crops, regardless of the site.

The operating profit (OP) was positive for all treatments (Table 4), regardless of the application of Co + Mo and inoculation with *Azospirillum brasilense*. The highest OP was obtained with the application of Co + Mo with inoculation of *A. brasilense* via seeds (R$ 4,936.04). In the absence of Co + Mo application and inoculation with *A. brasilense*, which would cause reduction in the costs, with possibility of increase in OP, if good yields were obtained, soybean cultivation would be viable, although with a decrease of R$1,007.03 ha^{-1} in the profit, corresponding to a reduction of 20.4% in the profitability.

As observed for OP, the treatment that led to the highest PI was the application of Co + Mo associated with the inoculation of *A. brasilense* (72.85%), being 4.34% higher in comparison to the control (Table 4), reinforcing the importance of using Co + Mo and co-inoculation of *A. brasilense* to obtain high grain yields with financial return.

The obtained economic results validate what is pondered by Hungria et al. (2013). These authors claim that microbial inoculants are very inexpensive and considering only the soybean crop, it is estimated that Brazil saves, per year, about US$ 7 billion with BNF. Therefore, due to the better nutrition of the plant not only with N and the increase in soybean yield, the tendency is that the co-inoculation with *A. brasilense* will increase in Brazil, especially because it is a low-cost and low-investment technique, with easy application and use, non-polluting and also it fits in the currently aimed sustainable concept.

Table 4. Operating profit (OP) and profitability index (PI) of the soybean crop as a function of the treatments

Treatment	OP R$	PI (%)
Control	3,929.01	68.51
CoMo seed	4,467.89	71.08
A. brasilense + CoMo seed	4,936.04	72.85
A. brasilense foliar	3,650.66	65.97
CoMo foliar	4,056.63	69.05
A. brasilense + CoMo foliar	3,894.00	67.27
Mean	4,155.71	69.12

Conclusions

1. Co + Mo application via seeds, associated with the inoculation of *A. brasilense* in the seeds, promoted the highest leaf N content, hundred-grain weight and grain yield in the soybean crop.

2. The co-inoculation with *A. brasilense* and Co + Mo application via seeds promote higher profitability with the soybean crop, being technically and economically viable.

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