Fertilization with nickel and molybdenum in soybean: effect on agronomic characteristics and grain quality
Adubação com níquel e molibdênio na soja: efeito sobre características agronômicas e qualidade de grãos

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SUMMARY

Soybean is one of the most valuable oilseeds in the world, an important source of protein for animal and human food. Correct management of fertilization is essential, especially nitrogen (N), which is demanded in greater quantity. Molybdenum (Mo) and nickel (Ni) are essential micronutrients for the plant and are directly involved in the N cycle and production of amino acids. The objective of this work was to evaluate the effect of applying Mo and Ni on soybean yield components, crop yield, N assimilation and protein yield. Applying Ni and Mo, individually reduced the insertion height of the first pod, and Ni and Ni + Mo significantly increased stem diameter. Application of Ni associated with Mo resulted in a 12% crop yield increase, as well as a 6% higher protein content, relative to the control. The highest protein content was obtained by combining the two nutrients. Thus, application of Ni in association with Mo is a viable alternative in the production of soybean.

Index words: *biological nitrogen fixation, micronutrient, production, urease.*

RESUMO

A soja é uma das oleaginosas mais valiosas do mundo, importante fonte de proteína para alimentação animal e humana. O correto manejo da adubação é essencial, principalmente o nitrogênio (N), já que ele é demandado em maior quantidade. O molibdênio (Mo) e o níquel (Ni) são micronutrientes essenciais para a planta e estão envolvidos diretamente no ciclo de nitrogênio e produção de aminoácidos. O objetivo do trabalho foi avaliar o efeito da aplicação de molibdênio e níquel sobre os componentes de rendimento, produtividade, assimilação de nitrogênio e produção de proteína na cultura da soja. A utilização de Ni e Mo, aplicados individualmente reduziram a altura de inserção da primeira vagem, Ni e Ni + Mo aumentou significativamente o diâmetro do colmo. A aplicação de Ni associada a Mo resultou num incremento de produtividade de 12% em relação à testemunha, bem como um teor de proteína 6% maior. Assim a aplicação de Ni associado a Mo mostrou-se uma alternativa viável na produção de soja. O maior teor de proteína foi obtido com a combinação dos dois nutrientes.

Palavras chave: *fixação biológica do nitrogênio, micronutriente, produção, urease.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most valuable oilseeds in the world. It is an important source of protein for animal and human food and recently has been used as raw material for biodiesel production. It thus has an important socioeconomic role.

In the agricultural year of 2016/2017 Brazil produced approximately 114 million Mg on 33.92 million hectares, achieving a crop yield of 3.4 Mg per hectare. Mato Grosso, which produces 26.7%, is the main producer state (CONAB, 2017).

With the evolution of agriculture, it is increasingly

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necessary to use nutrients to maintain and conserve soil fertility, and thus maintain or increase crop yield. In addition, the constant increase in production costs makes it necessary to achieve maximum economic efficiency, especially for fertilizers, which are the most expensive input of the soybean production system. (Castro *et al*., 2006)

Soybeans produced in Brazil contain about 40% protein and 20% oil (Moraes *et al*., 2006). The amount of protein in the grain is determined by genetic factors; however, environmental factors and nutrient availability also influence protein content (Souza *et al*., 2009; Veiga *et al*., 2010; Barbosa *et al*., 2011).

Obtaining high soybean yields depends on appropriate edaphoclimatic factors. In this context, correct management of fertilization is essential, especially of nitrogen (N), since it is the nutrient required in higher quantity by the crop. Its function in plants is related to the formation of chlorophyll, amino acids and, consequently, proteins, besides influencing crop growth and development (Marschner, 2011; Taiz and Zeiger, 2013; Buchanan *et al.*, 2015).

For the plant to use the available N through the decomposition of organic matter by the process of mineralization and consequent formation of nitrates, activity of the enzymes nitrate and nitrite reductase is essential for assimilation of ammonium by route GS/GOGAT or enzyme GDH in some cases. When nitrogen is supplied in the form of urea, the presence of nickel is fundamental, as it is part of the enzyme urease, which breaks down the urea into two molecules of ammonia and carbon dioxide. (Dixon *et al*., 1975; Marschner, 2011; Taiz and Zeiger, 2013; Buchanan *et al.*, 2015).

Soybean assimilates most of the N through biological nitrogen fixation by the nitrogenase enzyme. Atmospheric N_2 is broken down into ammonia, which is assimilated and transported to the plant shoot in the form of ureides: allantoic acid and allantoin. Ureides are catabolized by the enzyme allantois amidohydrolase to produce ammonia, or allantoin can undergo hydrolysis for the production of urea, which in turn requires urease for release of ammonia and its subsequent assimilation into amino acids (Buchanan *et al.*, 2015).

Application of Mo can benefit the soybean crop as a constituent element of the plant nitrate reductase enzyme and nitrogenase of the symbionts (*Bradyrhizobium japonicum*), increasing the number of nodules and nitrate reductase activity (Toledo *et al*., 2010). Application of Ni increases urease activity and prolongs enzyme activity time. According to Almeida *et al*. (2013), the enzymes decrease in activity after the plants flower.

In this context, the objective of this work was to evaluate the effect of the application of molybdenum and nickel on yield components, nutrient removal and protein yield in soybean crop.

MATERIALS AND METHODS

The experiment was carried out in the experimental farm of the Integrated College, Campo Mourão in the state of Paraná (23º 59' 24" S and 52º 21' 38" W). The soil is classified as Typic Hapludox (Soil Survey Staff, 2014). The chemical characteristics of the 0-20 cm layer are pH $CaCl_2$ =5.3; H + Al (SMP) = 3.3 $cmol_c$ dm^{-3}; organic matter = 2.8%; P (Mehlich 1) = 6.4 mg dm^{-3}; Ca = 2.9 $cmol_c$ dm^{-3}; Mg = 0.95 $cmol_c$ dm^{-3}; K = 0.3 $cmol_c$ dm^{-3}; Mo = 129 mg dm^{-3}; Ni = 25.8 mg dm^{-3} and soil base saturation (V%) = 54. The site has been under no-till for about 20 years, with soybean or corn in the summer and oat in the winter.

The experiment was organized in a randomized block design with four treatments: i) Control, ii) Ni, iii) Mo and iv) Ni+Mo and five replicates. Each plot was two meters wide and five meters long. The sources used were nickel chloride with 29% nickel and sodium molybdate having 39% molybdenum.

Sowing was performed on 10/18/2016, using cultivar NA 5909 RR at a density of 290,000 plants ha^{-1}. The seeds were inoculated with *Bradyrhizobium japonicum* and fertilization was based on soil analysis and as recommended by Embrapa (2013) with 250 kg ha^{-1} of the 03-30-10 NPK formulation. The treatments (23.2 g ha^{-1} Ni, 31.2 g ha^{-1} Mo, 23.2 g ha^{-1} Ni + 31.2 g ha^{-1} Mo) were applied 25 days after emergence using a CO_2 pressurized backpack sprayer, equipped with a spray bar with flat spray tips, type 110 02, spaced 50 cm apart and volume of 150 L ha^{-1}. Throughout the cropping season applications of insecticides, fungicides and herbicides were carried out according to the technical recommendations for the crop. Precipitation and minimum and maximum temperatures during the experiment conduction are presented in (Figure 1).

The variable of interest evaluated before harvest were plant height, first pod insertion height and stem diameter. All measurements were made in 10 plants of the central area of each plot. After physiological maturation, 3 m^2 of each plot were harvested and the

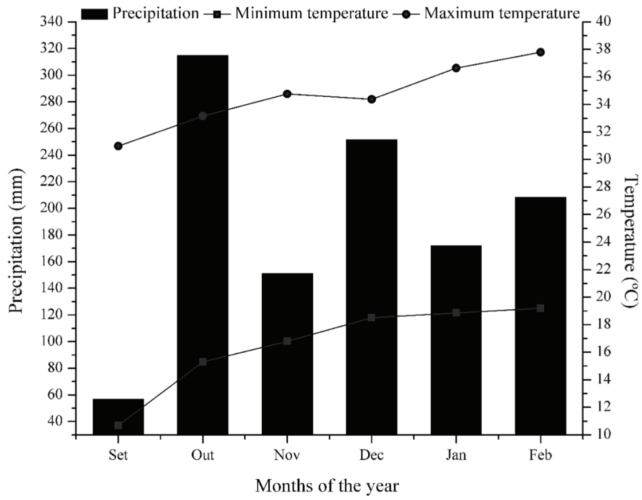

Figure 1. Precipitation data (mm), minimum and maximum temperature (ºC) recorded in the municipality of Campo Mourão - PR between September 2016 and February 2017.
Figura 1. Dados de precipitação (mm), temperatura mínima e máxima (ºC) registrados no município de Campo Mourão - PR entre setembro de 2016 e fevereiro de 2017.

number of pods per plant (NPP), number of grains per pod (NGP), mass of one thousand grains (MTG) (Brazil, 2009) and crop yield (CY) were evaluated. The nitrogen and protein content in the grains was determined following the Kjeldahl methodology (AOAC, 2016). To obtain nitrogen extraction and protein yield, values obtained in the analyses were multiplied by the productivities.

After verifying the normality and homogeneity of variance assumptions, analysis of variance (ANOVA) was applied according to the experimental design of randomized blocks. In the case of a significant effect of the qualitative treatments, means were compared with the Tukey test at $P \leq 0.05$ with the aid of the statistical package Sisvar (Ferreira, 2014).

RESULTS AND DISCUSSION

Table 1 shows the data of plant height, height of insertion of the first pod and stem diameter. The highest first pod insertion height was found in the control treatment (without application), this could have occurred possibly because of the decrease in emission of reproductive stems due to lower availability of N, which may also have determined the smaller stem diameter.

Table 1. Plant height (PH), height of insertion of the first pod (HIFP) and stem diameter (SD) of soybean, as a function of leaf spray application of Mo and Ni.
Tabela 1. Altura da planta (PH), altura de inserção da primeira vagem (HIFP) e diâmetro do caule (SD) da soja, em função da pulverização foliar de Mo e Ni.

Treatments	PH	HIFP	SD
	- - - - - cm	- - - - -	mm
Control	91 a†	9.2 b	8.5 b
Molybdenum (Mo)	88 a	7.7 a	9.0 ab
Nickel (Ni)	87 a	7.7 a	9.7 a
Mo + Ni	89 a	8.1 ab	9.6 a
LSD	5.70	1.40	0.84
VC	3.42	9.11	4.85

† Averages followed by the same letter in a column do not differ (Tukey, $P \leq 0.05$).
† As médias seguidas pela mesma letra na coluna não diferem pelo teste de Tukey em $P \leq 0.05$.

Nakao *et al*. (2014) working at rates of up to 800 g ha^{-1} Mo applied in two seasons (R3 and R4.3) did not find any effect on PH or HIFP. The authors attributed the result to the time of Mo application because they used a cultivar of determined growth, which in its reproductive phase is already at its maximum size. In the same line, Rossi *et al*. (2012), working with doses of up to 120 mg ha^{-1} of Mo applied to foliage 34 days after plant emergence, also found no effect on plant height. The cultivar used for the work was of determined cycle and the conventional system, not being presented the amount of Mo in soil. Toledo *et al*. (2010) did not observe increased protein content with applications of 30 and 60 g ha^{-1} Mo applied by leaf spray or with 24 g ha^{-1} per seed treatment.

This result can be attributed to the greater availability of nitrogen to the plants. Since N acts in the synthesis of chlorophyll and protein compounds, it can increase plant capacity to produce reproductive buds (Malavolta, 2006).

Rossi *et al*. (2012) obtained 96.94 NPP with a dose of 48.6 g ha^{-1} Mo, differing from our study which found no effect of the application of 31.2 g ha^{-1} Mo. It should be noted that in the work of Rossi *et al*. (2012) the source of Mo was ammonium molybdate, which has N in its composition; in addition, the Mo dose was higher than in our work. The different the response in the two studies may possibly be attributed to these factors.

Heidarzade *et al*. (2016) verified a 19% increase in the number of pods per plant compared to the control when they applied 4 mg ha^{-1} Mo via leaf spray in three stages of soybean development (stem elongation, floral bud formation and pod formation). Toledo *et al*. (2010) observed a significant increase in the activity of nitrate reductase only with application of 60g ha^{-1} Mo to foliage. Although they did not have significant results with seed application, they observed an increase in number of nodules and nodule dry mass with both seed and foliar applications.

In the literature, Ni research on soybean crop is scarce, but Lopes *et al*. (2016) working with common bean observed a linear increase in the NGP with rates of up to 60 g ha^{-1} Ni but found no effect on NPP.

For NGP and MTG, no effects of treatments were observed. These results are in agreement with those of Heidarzade *et al.* (2016), who found no effect of Mo on mass of a thousand grains.

The crop yield obtained in the experiment was higher than the average of the municipality of Campo Mourão in the agricultural year of 17/18 (3492 kg ha^{-1}) (SEAB, 2018), due to the good climatic conditions during the conduction of the study (Figure 1) and also because the phytosanitary management was adequate. The yield was 9% higher when only Ni was applied relative to the control, and the treatment with Mo was 12% higher when the two micronutrients were combined (Table 2). The higher crop yield in these treatments was due to the increase in the number of pods per plant that caused the plants to produce more grains per unit area.

Rossi *et al.* (2012) obtained a yield of 3423 kg ha^{-1} with application of 55.7 g ha^{-1} Mo, obtaining a crop yield gain of 37% over the control treatment. Oliveira *et al.* (2017), working at rates of 400 and 800 g ha^{-1} applied via leaf spray at stages R3 and R6, found no effect on crop yield.

Table 3 presents the data on calcium, magnesium, potassium and nitrogen removal. It can be observed that only nitrogen was influenced by the treatments, where the application of nickel alone increased export by 7%, and the association of molybdenum and nickel did so by 14%, relative to the other two treatments. These results indicate that application of nickel increases urease activity, which causes the plant to assimilate and export more nitrogen (Kutman *et al*., 2013).

The higher N input promoted differences in protein production as can be observed in Figure 2. The treatment that combined the two nutrients stood out over the others, producing approximately 1613 kg ha^{-1} crude protein, 18% more protein than the control treatment. However, in all treatments the amount

Table 2. Number of pods per plant (NPP), number of grains per pod (NGP), mass of one thousand grains (MTG) and crop yield (CY) of soybean, as a function of leaf spray application of Mo and Ni.
Tabela 2. Número de vagens por planta (NPP), número de grãos por vagem (NGP), massa de mil grãos (MTG) e produtividade da cultura (CY) da soja, em função da pulverização foliar de Mo e Ni.

Treatments	NPP	NGP	MTG	CY
			g	kg ha^{-1}
Control	96.75 b†	2.40 a	153.2 a	4625 c
Molybdenum (Mo)	98.35 b	2.42 a	160.9 a	4660 c
Nickel (Ni)	106.37 a	2.38 a	157.1 a	5055 b
Mo + Ni	112.68 a	2.34 a	163.4 a	5270 a
LSD	3.6	0.12	11.44	163.03
VC	6	2.4	10.3	8.2

† Means followed by the same letter in a column do not differ (Tukey, $P \leq 0.05$).
† As médias seguidas pela mesma letra na coluna não diferem pelo teste de Tukey em $P \leq 0.05$.

Table 3. Nutrient removal of calcium (kg ha^{-1}), magnesium (kg ha^{-1}), potassium (kg ha^{-1}) and nitrogen (kg ha^{-1}) by soybean grains, as a function of leaf spray application of Mo and Ni.
Tabela 3. Extração de cálcio (kg ha^{-1}), magnésio (kg ha^{-1}), potássio (kg ha^{-1}) e nitrogênio (kg ha^{-1}) por grãos de soja, em função da pulverização foliar de Mo e Ni.

Treatments	Ca	Mg	K	N
	- - - - - - - - kg ha^{-1} - - - - - - - -			
Control	13.40 a†	11.68 a	44.74 a	210.36 c
Molybdenum (Mo)	13.80 a	11.69 a	45.88 a	220.18 c
Nickel (Ni)	14.88 a	12.61 a	59.12 a	230.96 b
Mo + Ni	13.97 a	13.10 a	52.59 a	250.82 a
LSD	5.38	4.01	19.85	10.09
VC	17.39	14.83	17.78	3.0

† Means followed by the same letter in a column do not differ (Tukey, $P \leq 0.05$).
† As médias seguidas pela mesma letra na coluna não diferem pelo teste de Tukey em $P \leq 0.05$.

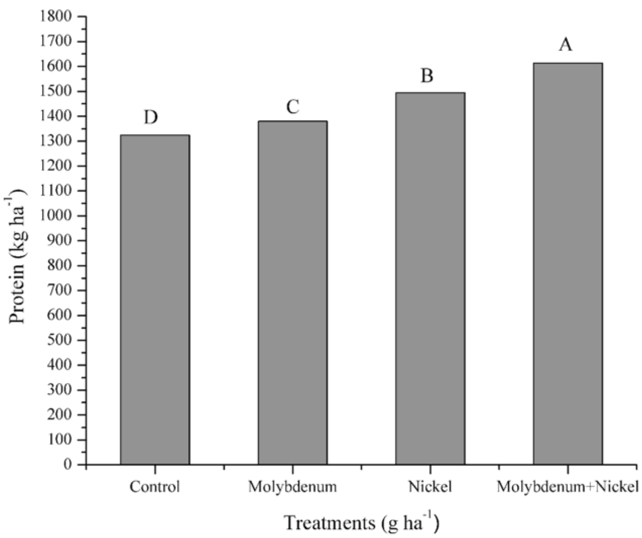

Figure 2. Protein yield for different combinations of Mo and Ni fertilization. Means followed by the same letter do not differ (Tukey, $P \leq 0.05$).

Figura 2. Rendimento de proteína para diferentes combinações de fertilização Mo e Ni. Médias seguidas pela mesma letra não diferem pelo teste de Tukey em $P \leq 0.05$.

of protein was below the Brazilian average, which is 40% (Moraes *et al*., 2006).

Lopes *et al*. (2016) found that the application of 80 g ha^{-1} Mo increased the amount of N and protein content in bean grains, differing from our study which found no effect on these variables by Mo applied individually. Oliveira *et al.* (2017) found a linear increase in the protein content of soybean when they applied doses of 0 to 800 g ha^{-1} molybdenum via leaf spray.

These results could be attributed to the higher assimilation of N by the plants. Plants that received the application of Ni or Mo + Ni translocated a larger amount of N to the grain, evidencing that there was more availability of amino acids, probably due to the greater biological fixation of N, enhanced by Mo for the enzyme nitrogenase, producing a greater quantity of ureides. Ureides are transported from the nodules to the aerial part and later converted into amino acids, where the enzyme urease containing Ni plays an important role in the breaking down urea to release ammonia that will be assimilated through the GS/GOGAT route. This process results in greater availability of amino acids and, consequently, higher protein content of the grains (Dixon *et al*., 1975; Marschner, 2011; Taiz and Zeiger, 2013; Buchanan *et al*., 2015).

CONCLUSION

The application of Ni alone or in combination with Mo to soybean foliage increases the number of pods per plant, number of grains per pod, yield and protein yield in the beans. Application of Mo alone increases protein yield in soybean grains.

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