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Enrichment, quality, and productivity of soybean seeds with cobalt and molybdenum applications[1]

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ABSTRACT – Cobalt (Co) and molybdenum (Mo) are vital nutrients for biological fixation and nitrogen assimilation by soybean. This study aimed to evaluate the increase of Co and Mo contents in soybean seeds by leaf applications. The experiment was carried out in a seed production field in a 3 × 3 factorial scheme with three Co and Mo doses and three treatments (times). Each treatment consisted of two application times: R3+R5.1 (T1), R3+R5.4 (T2), and R5.1+R5.4 (T3). Doses were 0, 400, and 600 g.ha^{-1} of Mo and 0, 40, and 60 g.ha^{-1} of Co. No difference was observed for productivity, one thousand-seed weight, and germination. An interaction was observed between dose and application time in the Mo content. The application of 400 g ha^{-1} of Mo in T1 (R3+R5.1) provided a higher Mo content in the seeds (13.5 g.kg^{-1}), which is 1350% higher when compared to the control. For Co, there was a difference only for doses, and the application of 40 or 60 g.ha^{-1} of Co increased seed nutrient content by 30%. Therefore, a higher enrichment of Co and Mo was observed in seeds with applications of 40 and 400 g.ha^{-1}, respectively, applied in R3+R5.1.

Index terms: doses, times, leaf application, micronutrients.

Enriquecimento, qualidade e produtividade de sementes de soja com aplicações de cobalto e molibdênio

RESUMO – Cobalto (Co) e molibdênio (Mo) são nutrientes indispensáveis para a fixação biológica e assimilação de nitrogênio pela soja. Neste trabalho teve-se como objetivo avaliar o aumento do Co e Mo nas sementes de soja pela aplicação via foliar desses nutrientes na planta mãe. O experimento foi realizado em lavoura de produção de sementes, em esquema fatorial 3x3 com três doses de Co e Mo e três tratamentos (épocas). Cada tratamento foi constituído de duas épocas de aplicação R3+R5.1 (T1); R3+R5.4 (T2); R5.1+R5.4 (T3). As doses foram 0, 400 e 600 g.ha^{-1} de Mo e 0, 40 e 60 g.ha^{-1} de Co. Não houve diferença na produtividade, peso de mil sementes e germinação. Houve interação entre dose e época no teor de Mo. A aplicação de 400 g.ha^{-1} de Mo no tratamento T1 (R3+R5.1) proporcionou maior teor de Mo nas sementes (13,5 g.kg^{-1}), 1350% maior do que a testemunha. Para o Co houve diferença apenas para as doses, sendo que a aplicação de 40 ou 60 g.ha^{-1} de Co elevou em 30% o teor do nutriente nas sementes. Conclui-se que há maior enriquecimento de Co e Mo nas sementes com a aplicação de, respectivamente, 40 e 400 g.ha^{-1} aplicados em R3+R5.1.

Termos para indexação: doses, épocas, aplicação foliar, micronutrientes.

Introduction

The micronutrients cobalt (Co) and molybdenum (Mo) are indispensable for soybean (*Glycine max*) because they are closely related to the biochemical process of biological nitrogen fixation (BNF) (Bergersen and Turner, 1967), and for all plants in the process of nitrogen assimilation.

Mo is related to the cofactor in the enzymes nitrogenase, essential to BNF (Teixeira et al., 1998; Reis and Teixeira, 2006), nitrate reductase, indispensable for N assimilation by plants (Sfredo and Oliveira, 2010; Possenti and Villela, 2010), and sulfide oxidase, which is related to electron transport

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during biochemical reactions (Sfredo and Oliveira, 2010).

Co is not an essential element for all plants, but soybean (Fabaceae family) requires it to allow that nodules of the symbiosis with bacteria of the genus *Bradyrhizobium* to be functional and BNF occurs (Mulder, 1954; Burris, 1966), being then classified as an essential element for this family (Kerbany, 2004). This element participates in the cobamide synthesis (vitamin B12) and subsequently of leghemoglobin in nodules, responsible for transporting oxygen to bacterioid respiration (Scholander, 1960; Dourado Neto et al., 2012).

Applications of Co and Mo in soybean has been little studied, but seed treatment (ST) is the main mode of application of these nutrients (Santos, 1999). However, several studies have shown that Co and Mo application via ST reduces nodulation and BNF efficiency (Hungria et al., 2001; Moreira and Siqueira, 2002).

Mo concentration near seeds in contact with the inoculant, as in ST, affects *Bradyrhizobium* because it decreases the number of viable cells of the bacterium (Albino and Campo, 2001), impairing nodulation and BNF (Tong and Sadowsky, 1994). When supplied via ST, Co may cause phytotoxicity in plants, as well as hinder iron absorption by plants, which is another essential element for FBN (Sfredo and Oliveira, 2010).

An alternative for supplying these nutrients would be their leaf application. However, Co and Mo spraying at high concentrations may cause the burn of soybean leaves (Borkert, 1987).

Another alternative would be the use of Co- and Mo-enriched seeds, which can provide the necessary amount of these elements for the early development of soybean seedlings (Milani, 2010).

The enrichment of soybean seeds with Mo can be carried out by its leaf application (Jacob-Neto and Franco, 1995). Leaf application of Mo for seed enrichment does not interfere with the nodulation and plant development, and plants from seeds enriched or treated with Mo have a higher N accumulation in the leaves (Milani, 2008).

This study aimed to evaluate the influence of leaf application of Co and Mo in soybean seeds on germination, productivity, one thousand-seed weight, and Co and Mo contents of soybean seeds.

Material and Methods

The experiment was carried out in a seed production field in *Mangueirinha*, PR, Brazil. This area is located at the geographical coordinates 25°56′42″ S and 52°11′16″ W, with an altitude of 849 meters above sea level and a flat relief. The mean annual precipitation of this municipality is 1897 mm, and the climate is classified as Cfb (mesothermal humid subtropical) according to Köppen and Geiger classification.

The experimental area has been cultivated with annual crops under the no-tillage system for about 30 years. Before the implantation of the soybean crop, the area was cultivated with an intercropping of oat and ryegrass. The soil in the 0–20 cm layer is composed of 22% of sand, 24% of silt, and 54% of clay. According to the chemical analysis, soil conditions were as follows: pH ($CaCl_2$): 4.4; H+Al: 6.76 $cmol_c.dm^{-3}$; Al: 0.6 $cmol_c.dm^{-3}$; Ca: 4.3 $cmol_c.dm^{-3}$; Mg: 2.0 $cmol_c.dm^{-3}$; K: 0.28 $cmol_c.dm^{-3}$; P (Mehlich): 3.7 $mg.dm^{-3}$; and OM: 39.6 $g.dm^{-3}$.

Seeds were treated with fungicide + insecticide + plant growth regulator, inoculated with selected strains of *Bradyrhizobium japonicum* (100 mL of liquid inoculant per 50 kg of seeds).

Sowing was carried out on November 7, 2015, with the soybean variety Pioneer 95Y21, which has an indeterminate growth habit, maturation group 5.2, super-early, and total cycle between 105 and 115 days. The interrow spacing was 0.45 m, with a population of 222,222 plants ha^{-1}. Base fertilization consisted of 350 $kg.ha^{-1}$ of the fertilizer formulated with 2–28–20 of N–P–K + 38 $kg.ha^{-1}$ of K_2O as topdressing fertilization at 15 days after soybean sowing.

The experimental design was a randomized block design with four replications arranged in a 3 × 3 factorial scheme (three doses of Co and Mo and three application times). Each treatment consisted of two phenological stages, with applications at the following phenological stages: T1 – R3 and R5.1; T2 – R3 and R5.4; and T3 – R5.1 and R5.4. Doses were 0, 20, and 200 $g.ha^{-1}$ of Co and 0, 30, and 300 $g.ha^{-1}$ of Mo applied at each vegetative stage of treatments, totaling 0, 40, and 400 $g.ha^{-1}$ of Co and 0, 60, and 600 $g.ha^{-1}$ of Mo. A commercial product (Molybdenum 15% and Cobalt 1.5%, density of 1.61 $g.mL^{-1}$) was used for Co and Mo applications so that each treatment received a dose of 0.918 $mL.ha^{-1}$ of Co and Mo to reach a dose of 20 $g.ha^{-1}$ of Co and 200 $g.ha^{-1}$ of Mo.

The applications were carried out on January 7, 2016 (phenological stage R3) at 16:00 h, January 19, 2016 (phenological stage R5.1) at 17:00 h, and January 27, 2016 (phenological stage R5.4) at 11:00 h, with no precipitations registered after applications. A backpack sprayer, regulated for a flow of 333 $L.ha^{-1}$ of spray solution, was used in the applications.

The area of the experimental unit was 10.8 m^2 (1.8 × 6 m), with four sowing rows of 6 m long spaced at 0.45 m, totaling 388.8 m^2. The useful area consisted of the two central rows of each plot without 1.75 m of each end, leaving two rows of 2.5 m each (2.25 m^2 of useful area).

On March 8, 2016, soybean plants at the R8 stage were harvested from the useful area of the plot. Moisture, germination, one thousand-seed weight (TSW), productivity

(13% of moisture), and Co and Mo content were evaluated.

For the germination test, 400 seeds from each treatment and replication were used to obtain the percentage of germination. Seeds from each treatment were sown on paper towels moistened with distilled water using an amount of water equivalent to three times the dry paper weight. Rolls were made and taken to a BOD incubator previously regulated at 25 °C. Evaluations of the number of normal seedlings were carried out at 7 days after sowing, and the results were expressed as the percentage of normal germinated seedlings, according to the Rules for Seed Testing (Brasil, 2009).

The analysis of Co and Mo contents in soybean seeds was carried out at the laboratory of the *Instituto Brasileiro de Análises* (IBRA), and readings were carried out in an atomic absorption spectrophotometer (Malavolta et al., 1997).

The results were submitted to the tests of normality and homogeneity of variance and then to the analysis of variance by the F test at 5% probability using the software Statistical Analysis System version Windows 9.2. When the null hypothesis was rejected, the tests of means comparison of Fisher (LSD) $p \leq 0.05$ were performed for times and regression analysis for doses.

Results and Discussion

No significant difference was observed between the values of the percentage of seed germination (Table 1) with Co and Mo application at different times and doses, nor was there an interaction between these two control variables. The mean of treatments in the germination test was 97.3% (Table 2). Similar results have been observed by Vazquez et al. (2005), Vieira et al. (2011), Milani (2010), Silva et al. (2012), Chagas et al. (2010), and Possenti and Villela (2010), who observed that Co and Mo applications did not influence the physiological quality of seeds according to germination tests. However, Guerra et al. (2006), working with Co and Mo applications via seed in a dystroferric Red Latosol in the Cerrado region, verified an increase in germination and seedling emergence in the field; Co application resulted in a 2.9% increase of germination when compared to the control.

In this study, we observed that leaf applications of Co and Mo at reproductive stages did not interfere with the germination. In all treatments and doses, more than 90% of the seeds germinated normally, indicating their high percentage of germination, even the control.

Table 1. Analysis of variance for germination test, one thousand-seed weight (TSW), productivity, and cobalt and molybdenum contents of soybean submitted to doses and times of cobalt and molybdenum.

Response variable/ Treatment	Germination	TSW	Productivity	Co content	Mo content
			Pr>F		
Block	0.5769^{ns}	0.0017*	0.7658^{ns}	0.9356^{ns}	0.4796^{ns}
Dose	0.0552^{ns}	0.6629^{ns}	0.0796^{ns}	0.016**	0.0001**
Treatment	0.9562^{ns}	0.2182^{ns}	0.5155^{ns}	0.1074^{ns}	0.0002**
Treatment x Dose	0.5287^{ns}	0.3993^{ns}	0.8956^{ns}	0.5913^{ns}	0.0012**
CV %	2.13	1.52	6.19	30.52	6.32

*Significant at 5% probability by the F test; **Significant at 1% probability by the F test; ^{ns}Not significant.

Table 2. Percentage of germination, one thousand-seed weight (TSW), and productivity as a function of treatments for different times and application doses of Co and Mo.

Tratamento	Dose Co + Mo (g.ha^{-1})	Germination (%)	PMS (g)	Productivity (kg.ha^{-1})
T1 (R3 + R5.1)	0 + 0	95.5 a	197.6 a	5412.5 a
	40 + 400	98.5 a	193.3 a	5103.7 a
	60 + 600	97.5 a	195.1 a	5226.9 a
T2 (R3 + R5.4)	0 + 0	95.7 a	193.5 a	5488.0 a
	40 + 400	99.0 a	193.0 a	5204.5 a
	60 + 600	97.5 a	193.3 a	5384.7 a
T3 (R5.1 + R5.4)	0 + 0	97.0 a	194.1 a	5281.3 a
	40 + 400	97.0 a	195.7 a	4970.9 a
	60 + 600	98.0 a	195.1 a	5375.2 a
Mean		97.3	195.5	5271.2

Means followed by the same letter do not differ from each other by the Tukey's test at 5% probability.

No significant difference was observed TSW as a function of doses, times or interaction between variables (Table 1). The mean TSW was 194.5 g (Table 2). Similar results were observed by Possenti and Villela (2010), Zancanaro et al. (2003, 2004), Milani (2008, 2010), and Vieira et al. (2011), who applied Mo and Co in soybean via leaf and also did not observe a difference in TSW. However, Barbosa et al. (2005) verified an increase in TSW of bean with leaf application of Mo. Silva et al. (2012) also observed that Co and Mo application significantly increased the grain weight regardless of the mode of application (via seed or leaf) when compared to the control. These authors verified that the difference was due to Mo availability, which participates as a cofactor in the nitrate reductase enzyme, assisting in N assimilation and acting on nitrogenase, which is responsible for BNF, allowing a better fixation and nutrition with N. In our study, we observed for soybean that the amount of Co and Mo provided by the soil was already sufficient for a good seed filling and hence the treatments did not interfere with this variable response.

Regarding soybean productivity, no significant difference was observed with the variation of doses, times, and interactions between variables (Table 1). The mean of treatments for productivity was 5271.2 $kg.ha^{-1}$ (Table 2). Similar results were found by Diesel et al. (2010), Possenti and Villela (2010), Zancanaro et al. (2003, 2004), and Milani (2008, 2010) in soybean and Vieira et al. (2011) and Ferreira et al. (2003) in bean after applying Co and Mo via leaf and did not find significant effects on productivity. Different results were found by Pöttker and Jacobsen (1997), Campo et al. (2001), Dourado Neto et al. (2012), Lana et al. (2009), Broch (2003, 2004), Broch and Ranno (2005), Ceretta et al. (2005), and Oliveira et al. (2015), who obtained increases in productivity. The increase in productivity observed by these authors is explained by the deficiency of Co and Mo in soils where the experiments were performed. These authors emphasize that, regardless of the mode of application (via seed or leaf), an increase was observed in productivity by Co and Mo addition to soybean, being linear to the increased application doses in some cases.

Co and Mo deficiency impairs BNF since these nutrients are indispensable for it and their application to deficient soils may have improved BNF process in the studied legumes and contributed to increasing productivity. In the present study, the absence of difference in productivity with Co and Mo applications may be due to the soil of the site, which has a high organic matter content and fertility maintained at appropriate levels. Sfredo and Oliveira (2010) pointed out that Mo absorption by soybean plants occurs predominantly as MoO_4^{2-} when soil pH is equal to or higher than 5.0. It was confirmed by Marcondes and Caires (2005), who observed no influence of Mo applied to seeds on nodulation, N content in leaves and grains, and productivity in a soil with a pH of 5.2. Therefore, this result is in accordance with these studies, which showed that an increased soybean productivity was observed when soils had a pH lower than 5.0, proving that Mo application via seed or leaf supply soybean plants when there is no availability of soil Mo, which did not occur in the present study.

A difference was observed in the Mo content of seeds, which showed an interaction between doses and application times (Table 1). It means that, at each application time, doses exhibit a distinct behavior in the enrichment of Mo in the seeds (Figure 1).

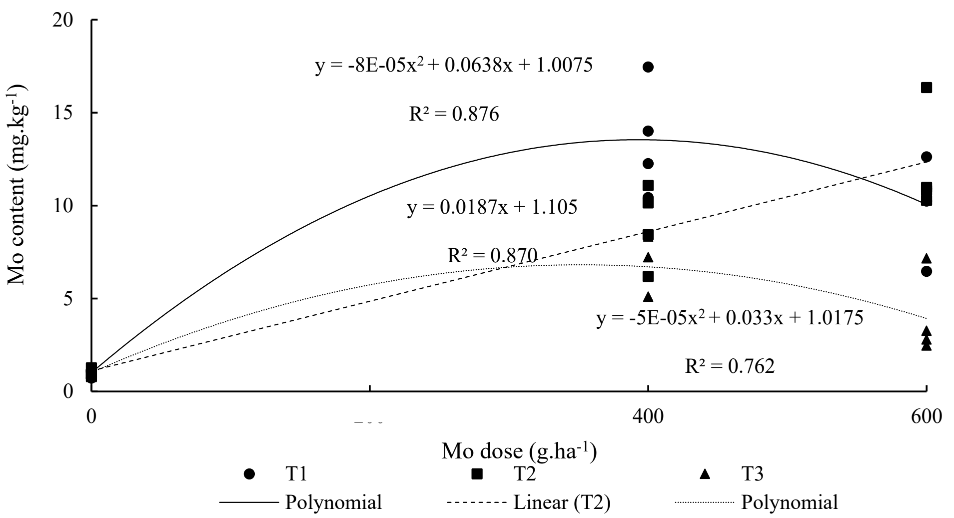

Figure 1. Mo content in enriched seeds as a function of application times and doses.

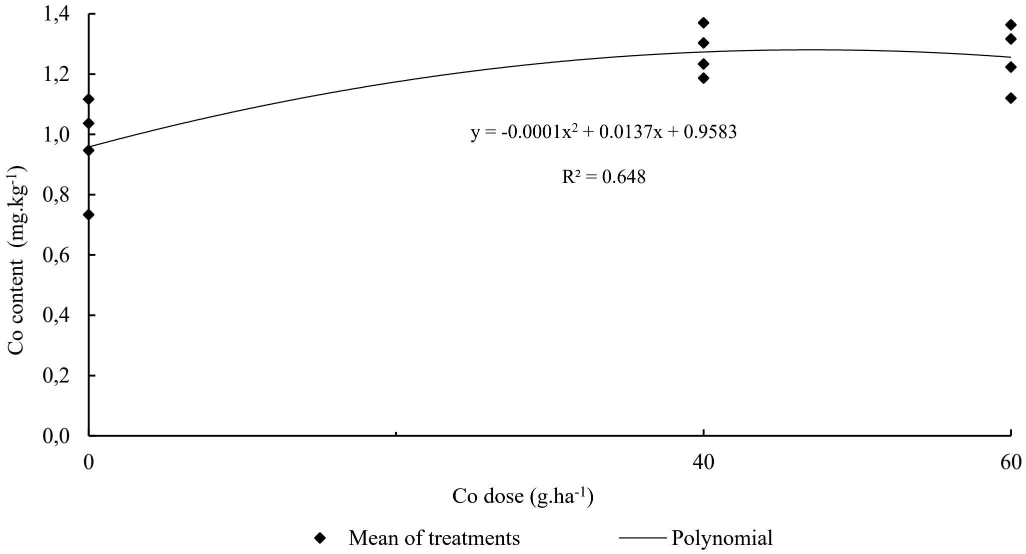

Figure 2. Co content in soybean seeds as a function of the applied doses of Co.

In treatment T2 (R3+R5.4), the means of the doses 0, 400, and 600 g. ha^{-1} were 1.0, 9.0, and 12.1 mg Mo kg^{-1} of grains, respectively. It represents a linear increase in Mo content in the seeds. For the other treatments (T1 and T3), the increase in Mo content was quadratic. In T1 (R3+R5.1), the means of the doses 0, 400, and 600 g. ha^{-1} were 1.0, 13.5, and 10.1 mg of Mo. kg^{-1} of grains, while for T3 (R5.1+R5.4), it was 1.0, 6.7, and 3.9 mg Mo. kg^{-1} of grains, respectively.

Applications of the dose 600 g. ha^{-1} in T2 (R3+R5.4) showed a higher Mo enrichment in the seeds when compared to the other treatments at the same dose (12.1 mg Mo kg^{-1} of seeds), being 16.5 and 67.7% higher for T1 and T3, respectively. It demonstrates that late Mo applications enrich less the seeds.

The treatments T1 (R3+R5.1) and T3 (R5.1+R5.4), treatments with quadratic behavior for the applied doses, showed that the dose of 400 g.ha^{-1} provided a higher Mo enrichment in the seed, while the application of 600 g.ha^{-1} of Mo led to a reduction in this enrichment. This quadratic behavior may be due to the interval between applications, which were lower than 10 days and necessary for plants to have better absorption of the applied Mo. The split application of 400 g.ha^{-1} of Mo between the reproductive stages R3 and R5.4, with a minimum interval between applications of 10 days, is better than only one application (Campo et al., 2008).

The dose of 400 g.ha^{-1} of Mo applied between the reproductive stages R3 and R5.1 (T1) resulted in a higher Mo enrichment in the seeds among all the studied doses and times, with a mean of 13.5 mg Mo kg^{-1} of seeds. This content fits these seeds into the class of Mo-rich seeds (Campo et al., 2008). It means a 1350% increase in Mo content in relation to the control. In the soybean crop, Mo values above 7.6 mg.kg^{-1} in the seeds do not require the use of Mo at cultivation time, either via seed treatment or leaf application, because the internal reserve of the seed is sufficient for the plant to grow and develop without its external dependence (Moraes et al., 2008).

Co content in the seeds presented a significant difference for the applied doses. The means of the doses 0, 40, and 60 g.ha^{-1} were 1.0, 1.3, and 1.3 mg Co kg^{-1} of seeds, respectively. It is observed a quadratic increase in Co content in the seeds, in which the doses 40 and 60 g.ha^{-1} of Co promoted a 30% increase in Co content of the seeds when compared to the control (Figure 2).

Cobalt can be absorbed through the leaves although it is practically immobile via the phloem (Marcondes and Caires, 2005). We believe that Co enrichment in the seeds, regardless of application times, was due to the deposition of this nutrient on the pods so that only it was transported to the seeds given its low mobility in the plant.

Conclusions

Leaf applications of Co and Mo did not affect productivity, one thousand-grain weight, and germination of soybean seeds, regardless of the dose and application time. However, Mo applications resulted in an enrichment of up to 1350%, being the best dose 400 g.ha^{-1} split in the reproductive stages R3 and R5.1. The short time interval with increasing dose between applications was the least efficient for enrichment. Co applications provided seeds with a content of this element 30% higher than that observed in the controls, with a linear increase regardless of the application time.

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