

Effect of nickel seed treatment in soybeans

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Abstract. Micronutrients such as molybdenum (Mo), cobalt (Co), and nickel (Ni) have been shown to be necessary for maximizing the benefits of biological nitrogen fixation (BNF) in soybeans by mediating enzymatic processes of transforming gaseous nitrogen into mineral nitrogen. The objective of this literature review was to present a research based on a bibliographic review on the influence of nickel application on soybean seeds and its impact on increasing the productivity of this important legume in Brazilian agribusiness. A better understanding of the biochemical processes involved in biological nitrogen fixation in soybeans allowed the recognition of the relevance of nutrients such as Co, Mo, and Ni, especially in the constitution of enzymes such as urease and hydrogenase involved in this important symbiotic relationship between microorganisms and plants. As for nickel, a safe recommended dose for soybean seed treatment with this micronutrient has not yet been established, and relatively few studies have been conducted under Brazilian conditions. When nickel was applied to soybean seeds under various cultivation conditions, positive results were observed (greater nodulation, nodule weight, and enzymatic activity), higher nitrogen content in the plant and protein in the seeds, and higher productivity. Further research involving variables that directly affect nickel availability for soybeans, either through seed treatment or foliar application, still needs to be conducted before recommended dosages can be established.

Keywords: *Rhizobium*. *Bradyrhizobium*. Nodules. Soybeans. Biological Nitrogen Fixation (BNF)

Contextualization

The factors that have contributed to the expansion and success of soybean cultivation, especially under the soil and climate conditions of the Brazilian Cerrado, has been research about biological nitrogen fixation. Through symbiosis between bacteria of the gender *Rhizobium* and *Bradyrhizobium* that form nodules on soybean roots, plants can meet their nitrogen demand by capturing this nutrient from the air and transforming it into usable nitrogenous forms. The biological nitrogen fixation achieved in this culture means that large amount of nitrogen do not need to be applied, which

could increase production costs and reduce microbial activity in the soil (Franco, 2015).

Some micronutrients such as molybdenum (Mo), cobalt (Co), and nickel (Ni) have been shown to be necessary to maximize the benefits of biological nitrogen fixation in soybeans, as they mediate enzymatic processes that transform atmospheric nitrogen into mineral nitrogen that can be readily assimilated by plants. In the case of nickel, it is known to be a constituent of urease (the enzyme responsible for hydrolysis of urea) and hydrogenase (responsible for reprocessing and reducing losses of H₂ generated during biological nitrogen fixation) in bacteria, and its

deficiency is a limiting factor for nitrogen fixation (Reis et al., 2014). Despite the recognized need for nickel in the symbiotic system, there are relatively few studies on the subject, and a recommended dose for soybean seed treatment with this micronutrient has not yet been established. In the case of molybdenum and cobalt, appropriate doses for seed application have already been defined, and they are common practices with significant increases in productivity in Brazilian agricultural systems (Rodak et al., 2013; Rodak, 2014).

It becomes of great relevance to carry out studies that can provide references for technicians and farmers on the levels of nickel necessary both for the application in soybean seeds and on its availability in soils in Brazil. This can also contribute to avoiding problems of dosage errors and inadequate fertilizer recommendations, reducing the risks of nutrient deficiency or excess and consequently reducing crop productivity.

Biological nitrogen fixation is a natural process by which most of the atmospheric nitrogen is incorporated into living matter in ecosystems on the surface of the earth, mediated by diazotrophic bacteria. Nitrogen recycling is carried out by fixing and nitrifying microorganisms that act on organic matter in the soil, ensuring an inexhaustible reservoir of this element in the atmosphere (Alves et al., 2003; Lucca et al., 2014).

Although there are several biological nitrogen fixation systems, all are restricted to heterotrophic prokaryotic microorganisms that require a source of reduced carbon. Diazotrophic bacteria are particularly noteworthy in this regard, as they can be free-living, associative, or symbiotic. The latter establish a close relationship with plants and can form specialized structures called nodules. This is the case with soybean cultivation, in which most of the nitrogen is supplied by biological fixation, in a symbiotic process with *Bradyrhizobium* bacteria that infect the roots via root hairs and form nodules, where N_2 is converted to NH_3 . Additionally, nitrogen is the most demanded nutrient by soybean plants, with its content in the seed ranging from 45 to 65 g kg^{-1} . Around 240 kg of nitrogen ha^{-1} are required to achieve an average yield of 3000 kg ha^{-1} of soybeans, emphasizing the importance of biological nitrogen fixation in terms of saving nitrogen fertilizers in Brazilian soils (Reis et al., 2006; Oliveira Junior et al., 2010; Lucca et al., 2014).

Biological nitrogen fixation and nitrogen assimilation

The efficiency of biological nitrogen fixation is influenced by micronutrients such as molybdenum (Mo), cobalt (Co), boron (B), and nickel (Ni). All of these nutrients, together with iron (Fe) and sulfur (S), are mainly involved in the process of action of the two subunits of nitrogenase, which is a redox-active enzymatic complex that hydrolyzes ATP to perform the reduction of N_2 to NH_3 . Therefore, legumes such as soybeans, which are highly dependent on biological nitrogen fixation, have a relatively high

requirement for molybdenum, mainly for the formation and functioning of nodules in the roots and multiplication of bacteroids. Cobalt is involved in this process and is part of vitamin B12 (cobalamin), which participates in the biosynthesis of leghemoglobin. Boron (B) is also relevant for nodule formation, and its deficiency can limit its functioning. Another important micronutrient in this system is nickel (Ni), which has a stimulating effect on the nitrification and mineralization of compounds containing organic nitrogen and bacteria of the genera *Rhizobium* and *Bradyrhizobium*, which contain the enzyme hydrogenase. This enzyme plays an important role in the reprocessing of part of the H_2 gas generated during biological nitrogen fixation, contributing to the recovery of some of the energy spent on breaking the triple bonds that ensure the stability of the N_2 molecule (Reis et al., 2006; Ureta et al., 2005).

Nickel is essential for the functioning of the hydrogenase enzyme, and its activity is reduced or not detected when cells are incubated in the absence of this nutrient. Because it is a constituent of the enzyme, nickel has the important function of reprocessing H_2 inside the nodule, since in the process of reducing H^+ to H_2 , there may be competition for electrons from hydrogenase. Thus, the hydrogenase allows the symbiont (*Rhizobium* or *Bradyrhizobium*) to reuse the hydrogen produced in the biological nitrogen fixation process, reducing energy loss and maximizing the benefits of symbiosis. Therefore, the application of nickel at a dosage of 10 mg dm^{-3} , in soil conditions with low availability (0.2 mg kg^{-1}) with deficient plants, promotes the reactivation of hydrogenase and protein synthesis (Evans et al., 1987; Ureta et al., 2005).

Nickel also participates in the metalloenzyme urease, which catalyzes the degradation of urea into carbon dioxide and ammonia, making this element of great importance for nitrogen metabolism in plants. Plants deficient in nickel may present necrotic spots on the leaves due to the accumulation of urea, reduced development, or even death in more severe cases. In the case of soybeans, positive effects of nickel on amino acid metabolism and reduction of the negative effect of excessive foliar urea application were observed. This demonstrates the potential for the application of nickel in maximizing the effects of biological nitrogen fixation and nitrogen fertilizers.

In soybean plants, more than 90% of the fixed nitrogen is exported from the nodules to the roots and shoot parts in the form of ureides (allantoin and allantoic acid). The catabolism of ureides produces urea, making the action of urease necessary for nitrogen recycling into other amino acids and nitrogen assimilation. Therefore, leaves deficient in nickel have high levels of urea, as well as lactic and oxalic acid, due to reduced urease activity. This micronutrient may also be involved in the synthesis of phytoalexins and plant resistance to diseases (Malavolta, 2006; Witte, 2011; Kutman et al., 2013).

The use of nickel in soybean seeds

Nickel was the last element to be considered essential for plant life, therefore, a micronutrient that actively participates in nitrogen metabolism and other biochemical processes of great relevance to plant nutrition. It was long considered a heavy metal and toxic to most cultivated species. However, despite the growing use of micronutrients in modern agriculture, there are relatively few studies to determine the requirements of plants for this nutrient. There are few studies that address the supply of this micronutrient via soil, seed or leaves, especially for legumes.

Although defined doses for the application of cobalt and molybdenum in soybean seeds already exist, these dosages have not been defined for nickel. Considering its relevance for biological nitrogen fixation, further research is needed to safely obtain these definitions for Brazilian soil conditions (Malavolta, 2006; Reis et al., 2006).

In Brazil, nickel application can be made using various sources such as salts, sulfates, nitrate, and chloride, up to formulations with EDTA, such as chelates. According to Instruction Normative 05, of February 23, 2007 and Instruction Normative 53 (amended on January 15, 2020), which controls the registration of fertilizers and mineral correctives, there is a list of products for application via soil or foliar and establishes the minimum concentration for registration. For foliar, soil, or fertigation application products, the minimum amount for registration is 0.005% and 0.01% if the fertilizer is exclusively a source of micronutrients. In the case of products for seed treatment application, they must contain at least one micronutrient, without minimum concentration (Brasil, 2007; Brasil, 2013).

Alovisi et al. (2011) studied the efficiency of the use of nickel sulfate foliar application in soybean cultivation and concluded that although foliar application considerably increased nickel levels in plant leaves, there was no effect on crop productivity.

Levy (2013) observed that the application of nickel in plants grown with ammonium nitrate contributed to increasing the activity of the urease enzyme in leaves seven to ten times. This benefited nitrogen metabolism and reduced the risks of urea toxicity in leaves and its possible necrotic damage. The author emphasized, however, that despite the increasing evidence of the benefits of nickel application, mainly in legume seeds, there are few studies on this micronutrient in agricultural soils in Brazil, and most of these are studies on nutrient adsorption in the soil.

The initial content of available nickel in the soil should be considered regarding the chosen application form, as the dosage used in seed treatment may prove toxic and reduce plant growth and increase the content of this nutrient in the grain above the permitted consumption limit.

Kutman et al. (2013) observed a positive and significant response in soybean plants with the application of nickel and urea, especially when their

levels were low and medium in seeds (0.04 mg kg^{-1} and 0.62 mg kg^{-1}). Nickel reserves in seeds, together with foliar supply of this micronutrient, can improve the use of nitrogen in the form of urea applied to the leaves. They also observed that high levels of nickel in the seed (8.32 mg kg^{-1}) can prevent necrotic spots in plants as a consequence of foliar application of urea, improving its hydrolysis and consequent remobilization of nitrogen via the phloem.

García et al. (2013) evaluated the effect of micronutrient application via seeds on the nodulation of two soybean varieties. Two commercial products (Profol Comol® 225 FIX liquid, containing 15% Mo, 1.5% Co, and NiComo® Dry with 22, 2.2, and 1.0% of Mo, Co, and nickel, respectively) were used. The application of the Co and Mo source product positively influenced the analyzed variables (number and dry weight of nodules) regardless of the variety. However, the application of the Mo, Co, and nickel source product positively influenced only the number of nodules of one of the analyzed varieties.

Franco (2015) reported that treating soybean seeds with nickel (45 to 90 mg kg^{-1}) increased biological nitrogen fixation by 12%, accumulation of nickel and total nitrogen in the grain (99% came from biological nitrogen fixation), and urease activity by up to 77%. It also increased the dry weight of the shoot and nodules, chlorophyll levels, total nitrogen in the plants, and soybean grain yield. However, high doses of this micronutrient reduced shoot dry weight and grain yield. The author established that the ideal dose of nickel for soybean seeds is 45 to 90 mg kg^{-1} , which corresponds to the application of 2.5 to 5 grams of nickel per hectare.

Lavres et al. (2016) conducted a study involving the treatment of soybean seeds, measuring urease and nitrogenase activity, as well as biomass production of roots, shoots, and grains. According to these authors, little research has been done on the influence of nickel on the symbiotic system of biological nitrogen fixation. They observed that low nickel rates caused higher plant and grain dry matter yield and higher nitrogen content in the seeds. The nickel applied to the seeds increased biological nitrogen fixation by 12% compared to control plants. Regarding nodule mass, the fresh weight of the nodules increased with the application of nickel to the seeds. It also positively influenced the accumulation of nitrogen in the seeds, correlated with nodulation, and in the shoot of soybean plants as well. The total nitrogen accumulation in the grains increased by 35% with the seed treatment with nickel. The dry weight of the shoot and seeds increased by 64% and 63%, respectively.

Felippe et al. (2018) evaluated different doses (0, 1, 2, 3, and 4 ml/kg of seeds) of a product based on Co, Mo, Ni, and Zn and their effects on productive performance in soybean plants. They reported that doses of 3 and 4 ml/kg of seeds promoted plant growth delay and reduced grain yield, possibly due to the toxic effects of nutrients at high doses for the crop. They observed that seed

treatment with the micronutrient containing product (Co – 0.5%, Ni – 1%, Mo – 5%, Zn – 20%), at a dose of 2.04 ml/kg, maximized soybean grain productivity.

Oliveira et al. (2018) evaluated the effect of different nutrients on the yield components and protein content of soybeans. The experiment had five treatments (nickel, molybdenum, sulfur, nitrogen, and control). The applications were carried out on leaf 25 days after plant emergence. No effect of the treatments on plant height and insertion of the first pod, stem diameter, thousand grain weight, and yield were observed. The highest number of pods per plant was obtained with nitrogen treatment, and the lowest number of seeds per pod was observed in the control treatment. The treatment with nickel application showed 3% more protein in grains compared to other treatments.

Santos Neto et al. (2018) evaluated the effect of nickel, Co, and Mo application, considering the appropriate time for the supply of important micronutrients to the biological nitrogen fixation process. The first factor consisted of a product based on nickel (Ni), cobalt (Co), and molybdenum (Mo), called NiCoMo, at the percentage of 2.4, 1.4, and 26.0% w/w, respectively, at doses of 160, 200, 240, and 280 g ha⁻¹. The second factor was the either application, seed treatment (ST) or vegetative stage (V3). In the vegetative application stage, NiCoMo resulted in higher soybean grain yields under no-tillage (minimum tillage) for three years. The area under no-tillage (minimum tillage) for 15 years provided higher yields with NiCoMo application in seed treatment.

Carlím et al. (2019) evaluated the effect of Mo and nickel application on yield components, productivity, nitrogen assimilation, and protein production in soybean. The application of nickel associated with molybdenum resulted in a 12% increase in yield compared to the control, as well as a 6% higher protein content. Therefore, the application of nickel associated with molybdenum showed to be a viable alternative in soybean production, considering that the highest protein content was obtained with the combination of the two nutrients.

Manfro (2020) evaluated the use of micronutrients in seed treatment (ST) to enhance nitrogen fixation in soybean plants. For this purpose, three different experiments were implemented. In the first field cultivated, no micronutrients were added to the ST. In the second, 200 ml ha⁻¹ of Co+Mo micronutrients were applied. In the third, the planted seeds received treatment with the application of 200 ml ha⁻¹ of Co, Mo, and nickel. At the V4 phenological stage, the evaluation was carried out by counting and weighing the nodules of five randomly harvested plants in each plot. The results obtained showed a difference in the quantity and weight of nodules and, consequently, in the final production. Therefore, soybean plants treated with the application of the biofertilizer composed of Co+Mo+nickel showed higher productive and nodulation performance

compared to the control treatment with only Co+Mo application.

Discussion

The soybean crop represents a significant part of the Brazilian agribusiness export agenda. It is the main agricultural commodity and has the largest planted area, especially in the Central-West and Southern regions of Brazil. As a result, it has become the focus of numerous studies, particularly those aiming to reduce production costs and achieve significant increases in productivity.

The success of soybean cultivation is directly related to the development of conditions that benefit soil fertility while reducing the use of fertilizers, particularly nitrogen fertilizers. Since the pioneering research conducted in the 1970s, there has been a concern to promote the biological fixation of nitrogen in soybean plants in symbiosis with diazotrophic bacteria (Sediyama et al., 2009). According to Franco (2015), this has ensured the development of varieties that are not only better adapted to Cerrado soils but also more productive and resistant to pests and diseases while maximizing the benefits of reducing dependency on nitrogen fertilizer use.

The better understanding of the biochemical processes involved in biological nitrogen fixation in soybeans has allowed the recognition of the relevance of nutrients such as B, Co, Mo, and Ni, particularly in the constitution of enzymes such as urease and hydrogenase involved in this important symbiotic relationship between microorganisms and plants.

In the case of B, Co, and Mo, there are studies defining the appropriate doses for seed application, which are widespread practices and have been proven to significantly increase productivity in various Brazilian agricultural regions (Rodak et al., 2013; Rodak, 2014). Regarding nickel, it is known that its deficiency in the soil-plant system can be a limiting factor for atmospheric N₂ fixation (Reis et al., 2014). Nevertheless, according to Malavolta (2006), Reis et al. (2006), and Franco (2015), a safe and recommended dose for soybean seed treatment with this micronutrient has not been established under Brazilian conditions, and relatively few studies have been conducted on the subject.

Research on determining appropriate doses of nickel, whether applied in the form of salts (sulfate, nitrate, and chloride) or chelated formulations with EDTA, has encountered some variables that hinder the determination of safe results (Brasil, 2013). Issues related to nickel levels in the soil, different nutritional requirements among soybean varieties, and planting systems adopted in the region require more attention in research planning.

According to Levy (2013), the initial level of available nickel in the soil should be considered when choosing the application form because the dosage used in seed treatment can be toxic, reducing plant growth and increasing the nickel content in the grain above the allowable limit for consumption. These

difficulties, according to Malavolta (2006) and Manfro (2020), have made it challenging to reach a consensus on the effectiveness of seed treatment with nickel-containing products.

There are still doubts about the best way to apply nickel in soybean cultivation, which can be done by leaf, soil or fertigation, and seed treatment. Alovisei et al. (2011) and Oliveira et al. (2018) found that although leaf application considerably increased the nickel content in plant leaves, there was no effect on crop productivity. However, Oliveira et al. (2018) observed that treatment with nickel with leaf application resulted in 3% more protein in the grains compared to other treatments. Additionally, Kutman et al. (2013) observed a positive and significant response in soybean yield with nickel and urea when leaf application was did.

When nickel was applied in soybean seed treatment under different cultivation conditions, positive results were observed in biological nitrogen fixation (higher nodulation, nodule weight, and enzymatic activity), higher nitrogen levels in the plant and protein in the grains, and increased productivity (Garcia et al., 2013; Franco, 2015; Lavres et al., 2016, Felipe et al., 2018; Santos Neto et al., 2018; Carlim et al., 2019; Manfro, 2020).

Appropriate dosages for nickel application in seeds were proposed by Franco (2015) with 45 to 90mg Kg⁻¹ of fertilizer containing nickel (2.5 to 5 g of nickel ha⁻¹) and by Felipe et al. (2018) with 3 to 4 ml kg⁻¹ of organic fertilizer containing nickel, Co, Zn, and Mo. It should be noted that both studies used different methodologies and cultivation conditions, as well as different products containing nickel, which makes it difficult to establish these references for use in other cultivation regions.

Considering that nickel is a micronutrient and the quantity required by soybean is relatively lower than other nutrients, it becomes relevant to consider the aspects presented and discussed by Levy (2013) regarding dosage errors and the possibility of reaching levels above the permitted amount of the nutrient in plants. Another aggravating factor is the difficulty of safely knowing the nickel levels present in the soil cultivated with soybean, both in availability to roots and adsorbed or present in common crop residues in no-till systems. All these viewpoints need to be considered when planning seed treatment not only with nickel but also with B, Co, Mo, and Zn, which are directly linked to the good performance of the biological nitrogen fixation system in soybean.

As studies by Garcia et al. (2013), Felipe et al. (2018), Lavres et al. (2016), Santos Neto et al. (2018), and Manfro (2020) have shown, the use of organic fertilizers containing a complex of nutrients, including nickel, has become increasingly common in soybean cultivation. However, due to the variable formulations and imprecise dosages of these products, standardized application of nickel in large areas is challenging. The variability of biofertilizer products, coupled with the complexity of cultivation systems, environmental conditions, and factors

involved in biological nitrogen fixation, makes the direct or indirect benefits of using nickel in soybean crops unpredictable.

Further research is necessary to better understand the variables that directly affect the availability of nickel to soybean, whether through seed treatment or leaf application, before recommended dosages can be defined. This is a crucial issue for Brazilian agriculture, as one of the world's largest soybean producers must leverage technology to maximize the sustainability benefits of nutrient supply to crops, particularly through biological nitrogen fixation. Nickel plays an active role in the enzymatic process that captures gaseous nitrogen through symbiosis with microorganisms and deserves the same attention as other micronutrients involved in the process, such as boron, molybdenum, cobalt, iron, and zinc.

Conclusions and recommendations

The soybean crop is of great importance to Brazilian agribusiness and one of its main export commodities, ensuring a significant contribution to the country's economy. This importance of the legume both for the domestic and international markets has motivated constant research aimed at ensuring increasing productivity with reduced production costs. Over the last decades, the strategy adopted in Brazil to seek productive soybean varieties with resistance to pests and diseases and less dependence on nitrogen fertilization through the stimulation of biological nitrogen fixation has been successful.

Biological nitrogen fixation integrates a complex process of symbiotic interaction between plant/microorganisms in which the involved enzymes depend on the availability of micronutrients such as B, Co, Mo, Fe, and Ni. This last nutrient has been relatively the least studied under soil and environmental conditions in Brazil, which is why safe dosage recommendations to be applied both by leaf, and seed treatment, are not yet available.

From the available studies presented in this work, it was possible to observe that the appropriate dosages for nickel application in seeds usually range from 45 to 90 mg Kg⁻¹ of fertilizer containing nickel (2.5 to 5 g of Nickel ha⁻¹). However, given the specific characteristics of nickel availability in the soil, variable nutritional requirements among soybean varieties, variations in the environment, and risks of nutrient accumulation in grains above the permitted levels, generalizing this recommended dosage is not safe.

Several studies show that nickel, when supplied through seed treatment, presents significant benefits in increasing and improving nodulation efficiency, nitrogen and protein accumulation in grains, and soybean plant productivity. Although these studies have been conducted in different soil and environmental conditions and cropping systems, the results show the potential of nickel supply in

increasing the efficiency of capturing gaseous nitrogen through biological nitrogen fixation.

An aggravating factor in obtaining safe recommendations for nickel dosages, as well as better sources and application methods, is that there is little knowledge about the levels of this nutrient under soil and crop residues, mainly in regions where soybeans are cultivated under no-till systems.

More research investments are necessary to obtain more knowledge about the potential use of biofertilizers (organic fertilizers), salts (sulfates, nitrates, and chloride), and chelates as sources of nickel, both by leaf and seed treatments. More knowledge on the combined use of Nickel with B, Mo, Co, and Zn in various regions and soybean cultivation systems is also needed, mainly to determine the best dosages and the impacts of this practice on gains in nodulation efficiency, nitrogen assimilation, and soybean plant productivity.

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