




## Foliar application of molybdenum on cover crops and response of common beans in successive cultivation<sup>1</sup>

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### ABSTRACT

The effects of foliar application of Mo and use of N from cover crops (*Crotalaria juncea* L. and *Canavalia ensiformis* (L.) DC) on the performance of common beans were investigated in this study. Field experiments were carried out in a succession planting system (cover crops – common beans), and nitrate reductase activities were evaluated in the laboratory. For the production of cover crops, a 2 x 4 factorial scheme was installed, with the two cover crops sunn hemp and jack bean, along with four doses of Mo (0, 40, 80, and 120 g ha<sup>-1</sup>) in the form of sodium molybdate (Na<sub>2</sub>MoO<sub>4</sub>) through foliar application in random blocks with four replications. For bean cultivation, an unfertilized control treatment was evaluated. Sunn hemp showed a linear positive response to Mo application in terms of shoot dry matter and N accumulation, while jack bean showed a quadratic negative response for shoot dry matter; for N accumulation, there was no adjustment of the regression models. Nitrate reductase activity in the common beans was up to three times higher at the Mo dose of 0 g ha<sup>-1</sup> when compared to the treatments with Mo application to both cover crops. Cover crops or Mo doses had no significant effect on bean productivity.

**Keywords:** legumes; nitrogen; enzyme activity; nitrate reductase.

### INTRODUCTION

Despite the potential benefits, the use of legumes in cover crops production is still not a common practice in production systems. Generally, commercial crops occupy most of the agriculturally used area, with potential negative consequences for soil quality. Studies demonstrating the efficiency of the use of legumes, especially in succession planting, serve as stimulus to the expansion of the adoption of this practice by farmers.

Studies on the increase in nitrogen metabolism, generated through the application of molybdenum (Mo) in leguminous crops used for fertilization, can contribute to a better scientific understanding of the processes involved in cover crop application. A recent study has shown that the application of Mo in common beans via seeds raises the mass of nodules formed by native rhizobia

(Matoso & Kusdra, 2014), whereas foliar application increases the formation of nodules, the foliar nitrogen content, the number of grains per pod, the mass of 100 grains, and overall productivity (Albuquerque *et al.*, 2012).

These benefits may be associated with the processes of absorption and assimilation of N in the soil, fixed via BNF (biological nitrogen fixation), since molybdenum (Mo) plays an important role in the regulation of reductase and nitrogenase synthesis, durability, and continuity (Almeida *et al.*, 2013). Thus, plants receiving Mo applications show increased N levels a productivity, based on the higher activities of these enzymes (Almeida *et al.*, 2013; Lopes *et al.*, 2014; Matoso & Kusdra, 2014).

However, there are few studies concerning the application of Mo in leguminous crops used as cover crops. Knowledge about the effects of the application of

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this micronutrient in cover crops and the transfer of N in succession planting significantly contributes to our understanding of the processes associated with a new management strategy for cover crops plants, aiming at a greater availability of nutrients for crops of commercial interest.

In this context, we evaluated effect of Mo application and N from cover crops *Crotalaria juncea* and *Canavalia ensiformis* in terms of common beans performance in succession planting.

## MATERIAL AND METHODS

The experiment took place from October 22, 2014 to June 25, 2015, in the experimental area of the Universidade Tecnológica Federal do Paraná, Pato Branco campus (26° 06' 59.3 S and 52° 40' 59"W, 760 m above sea level). The soil is classified as Typic Hapludox (Soil Survey Staff, 2014), with the following chemical characteristics of the top soil (0-20 cm) layer: pH in CaCl<sub>2</sub> = 5.1; P (Mehlich-1) = 3.28 mg dm<sup>-3</sup>; K, Ca, Mg, H + Al, SB, CTC = 0.18, 5.40, 2.10, 4.61, 7.68, 12.29 cmol<sub>c</sub> dm<sup>-3</sup>, respectively; V = 62.49%, and MO = 37.53 g dm<sup>-3</sup>. The region has a mesothermic humid subtropical climate; precipitation and temperature data for the experimental period are shown in Figure 1.

Cover crops cultivation was conducted in a 2 x 4 factorial scheme, using sunn hemp (*Crotalaria juncea*) and jack bean (*Canavalia ensiformis*), along with four Mo doses (0, 40, 80, and 120 g ha<sup>-1</sup>) in the form of sodium molybdate (Na<sub>2</sub>MoO<sub>4</sub>) through foliar application in random blocks with four replications, totaling 32 experimental units (EU). Each plot corresponded to an area of 2.25 m in width x 5.0 m in length, totaling 11.25 m<sup>2</sup>.

The cover crops were sown on 22 October 2014. The sowing line groove was opened with a no-tillage seeder, with 0.45 m spacing between lines, and the seeds were

distributed manually, without base fertilization. Six jack bean crops and 22 sunn hemp plants were planted per linear meter.

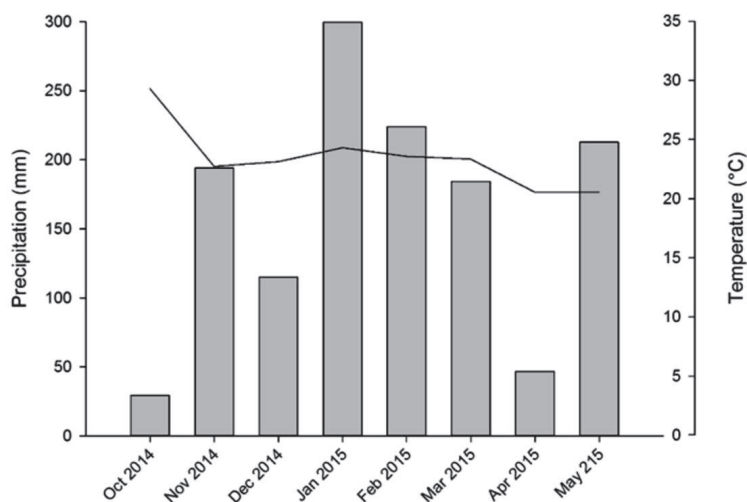
When jack bean reached the stage V4, Mo was applied to both cover crops. This developmental phase was chosen because of the positive results of previous works with Mo application in common beans (Pires *et al.*, 2005; Albuquerque *et al.*, 2012; Lopes *et al.*, 2014). For Mo application, we used a manual sprayer with a flat fan nozzle, and an adjuvant was used to spray 2 L per plot.

At 105 DAS (days after sowing), cover crop samples were collected for quantification of shoot dry matter (DM) and N accumulation. The remaining plants in the plot were harvested using a manual brush cutter and deposited in one piece inside the experimental boundaries established.

The sampling of plant material for DM and N quantification was performed with the help of a metal frame (0.25 m<sup>2</sup>), which was randomly placed in two separate locations within each plot. The species were cut close to the ground, weighed, and oven-dried at 60 °C until reaching a constant mass. After drying, the samples were ground in a Willey-type mill for determination of the total N concentration, using the Kjeldahl method (Tedesco *et al.*, 1995).

Four days after cover crop harvesting, on February 9, 2015, the common beans cultivar IPR 'Tuiuiú' was sown. The cultivar belongs to the group of black beans, has indeterminate type II growth response, and is erect, with an 88-day average cycle and a yield potential of around 3,950 kg ha<sup>-1</sup>; average mass of 1,000 seeds is 227 g.

A (2 x 4) + 1 factorial scheme was used in succession planting; common beans were sown in the plots under the two species of cover crop and four doses of Mo, plus one additional treatment (planned earlier, the



**Figure 1:** Precipitation (mm) and average temperature (°C) for the city of Pato Branco, Paraná, during the experimental period.

corresponding plot received no Mo fertilization), in random blocks with four replications, totaling 36 experimental units (EUs). Sowing was carried out manually without soil preparation and at a spacing of 0.45 m between rows, with 12 seeds per linear meter. No fertilization was performed both before and after sowing, without inoculation with N-fixing strains.

Samples for the quantification of shoot dry matter (DM) were also collected, following the same methodology as described for the cover crops. Bean grain harvesting was performed manually at the stage of physiological maturity, on 14 May 2015, at 94 DAS; threshing was performed mechanically within each parcel. Productivity was estimated for the grains harvested in the three central lines of the plot, calculated with adjustment of humidity to 13%. In the central line, assessment of the yield components was performed in 10 randomly selected plants, determining the following parameters: number of pods per plant, number of grains developed per pod, number of grains developed per plant, and mass of 100 grains.

*In vivo* nitrate reductase activity was evaluated in cover crops and common beans at 90 and 66 DAS, respectively. For this, foliar samples were collected in the early morning from the mid-third parts of the plants and packed with ice in a Styrofoam box (Toledo *et al.*, 2010). The tests followed the methodology proposed by Jaworski (1971), with adaptations.

The data were submitted to analysis of variance. When an interaction between Mo doses and species were observed, polynomial regression analysis was carried out; the models were selected according to the criterion of the greater  $R^2$  and the significance ( $p < 0.05$ ) of the equation's parameters. For the control evaluations, when significant differences between the treatment and control averages were observed, Dunnett's test was used to compare these differences ( $p < 0.05$ ). For all analyses, the software package ASSISTAT was used (Silva & Azevedo, 2016).

## RESULTS AND DISCUSSION

There was a significant interaction between species x doses of Mo for the accumulation of DM and N in the cover crop. For the accumulation of DM in sunn hemp (DM\_sunn hemp), the 120 g ha<sup>-1</sup> dose resulted in the highest response (34.19 t ha<sup>-1</sup>), showing linear adjustment of data, with a nearly 13,7% increase in the accumulation of DM between the doses 0 and 120 g ha<sup>-1</sup>. Jack beans (DM\_jack bean) showed no significance response, with an average of 12.05 t ha<sup>-1</sup> of DM. For all doses studied, the accumulation of sunn hemp shoot dry matter was greater than that of jack beans (Fig. 2a).

The responses to the accumulation of N (N) were similar to those of DM. Sunn hemp showed a positive linear

response to Mo doses, reaching 545,49 kg N ha<sup>-1</sup> at the highest dose of Mo applied. According to the regression equation, for each 100 g of Mo ha<sup>-1</sup> applied, we observed an accumulation of 113 kg of N ha<sup>-1</sup> (Fig. 2b).

Although there is no information in the literature about the application of Mo in cover crops, its purpose is believed to be similar to those observed for other legumes associated with N-fixing bacteria, since various studies base their positive results on the performance of Mo as a cofactor of enzyme nitrogenase and nitrate reductase, which are responsible for biological N fixation and assimilation, respectively (Pessoa *et al.*, 2000; Matoso & Kusdra, 2014; Bertoldo *et al.*, 2015).

However, in this study, we observed differences in the accumulation of DM and N between sunn hemp and jack bean. This finding can be attributed to the sampling time, as the material was collected when the plants were in the phenological phase. Similar results were found by Carvalho *et al.* (2015), who, when assessing the DM of cover plants during the flowering and physiological maturation periods, found 2.32 t ha<sup>-1</sup> of DM in sunn hemp during the flowering period and 2.42 t ha<sup>-1</sup> during maturation. Similarly, for Barbicou-beans (*Canavalia brasiliensis*), 3.30 t ha<sup>-1</sup> of DM were found during flowering and 2.75 t ha<sup>-1</sup> during maturation. Other factors, such as genetic differences in the absorption and use of Mo (Nie *et al.*, 2014; Liu *et al.*, 2017), as well as the time and form of Mo application, may also have contributed to these results.

As the two species showed distinct phenological responses, it was not possible to carry out a compatibilization of the developmental stages at harvest (105 DAS). While jack beans were in the reproductive stage, with flowering and pod formation, sunn hemp was still in the vegetative stage. Sunn hemp is responsive to the photoperiod and has been classified as a short-day plant (Santos & Junior, 2003), and in Pato Branco, it flowers after the first two weeks of February, which was after harvesting. In this way, without flower induction, sunn hemp remained in the vegetative phase throughout the entire experimental period, with high biomass accumulation; Leal *et al.* (2012) have also observed similar results.

Sunn hemp responded linearly to the Mo doses and did not obtain its maximum DM and N accumulation levels. In contrast, jack bean, with its relatively large seeds, showed a different response, possibly due to the considerable Mo amounts in the seeds. In a study by Kubota *et al.* (2008) on bean seeds, the authors found that seeds with high levels of Mo can generate plants that do not respond to the additional application of this element; Almeida *et al.* (2013) when assessing the response of common bean seeds to Mo supplementation have found similar results.

Regardless of the variation caused by Mo application, the results obtained in this study indicate the adaptability of these cover crop to the environmental conditions of southwestern Paraná, as the DM values were similar to those observed by Vargas *et al.* (2011) in Viçosa, MG, where cover crop are adapted to the local conditions, with 30.60 and 19.94 t ha<sup>-1</sup> for sunn hemp and jack bean, respectively, at 118 DAS. Cavalcante *et al.* (2012) in São Vicente da Serra, MT, found lower DM values with 3.0 t ha<sup>-1</sup> for sunn hemp and jack bean.

For all Mo doses examined, the accumulation of N in sunn hemp was greater than in jack bean because of the high DM accumulation of sunn hemp, although its N content (1.5%) is lower than that of jack bean (2.4%), resulting in an average N accumulation of 477.69 and 294.27 kg ha<sup>-1</sup> for sunn hemp and jack bean, respectively. A similar linear response for sunn hemp was obtained by Pessoa *et al.* (2000) for common beans fertilized with Mo, which, according to the authors, is associated with increased amounts of nitrogenase (BNF) and nitrate reductase (reduction of N absorbed from the soil).

The performance of the common beans, in succession to the cover crop grown with different doses of molybdenum, was not affected by the treatments. However, significant differences in relation to the control were found for the following variables: number of pods per plant (sunn hemp 40 g ha<sup>-1</sup> of Mo, jack bean 40 and 80 g ha<sup>-1</sup> of Mo) and number of developed grains per plant (sunn hemp at 40-g ha<sup>-1</sup> of Mo) (Table 1). It should be noted that the addition of 40 g Mo ha<sup>-1</sup> to sunn hemp resulted in the highest rates for both variables, with about 50% more grains and pods per plant when compared to the control.

It is important to highlight that the experimental plots were not fertilized, with the exception of the control. The overall average productivity obtained was 1,917 kg ha<sup>-1</sup>,

less than the maximum potential productive of the cultivar, which is 3,950 kg ha<sup>-1</sup>. However, the value observed is higher than the average for Paraná in the 2014/2015 agricultural year, which was 1,756 kg ha<sup>-1</sup>.

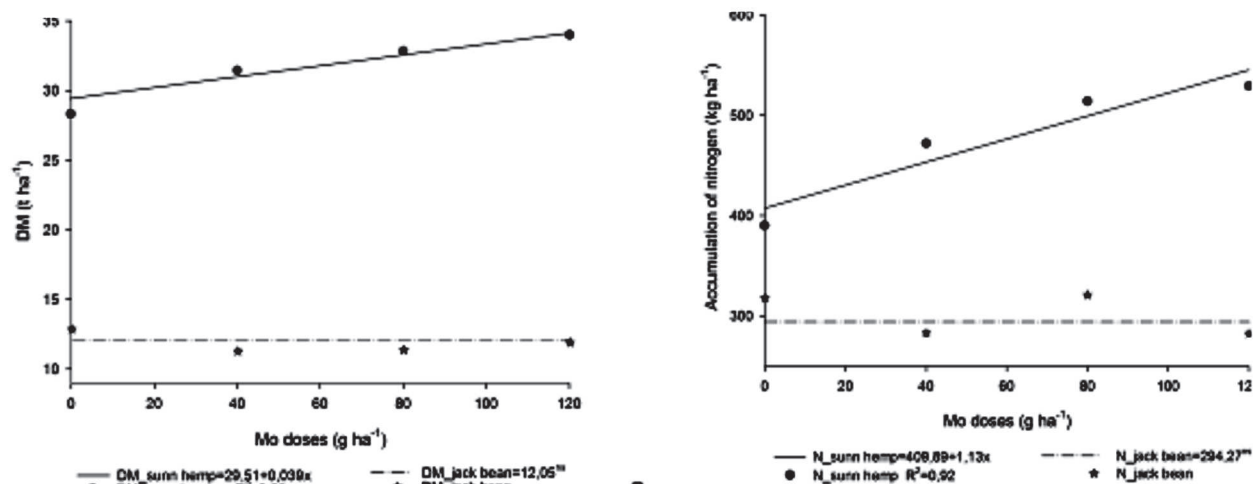
One of the factors that may have contributed to the lack of effect of the treatments on productivity is related to the patchy distribution of rainfall throughout the growing period (Fig. 1), despite the accumulation of 442 mm throughout the crop cycle. In April (phase of flowering and grain filling), the average monthly rainfall was only 48 mm, which was less than the quantity recommended by Pereira *et al.* (2014). In Lima common beans (*Phaseolus lunatus* L.), Oliveira *et al.* (2014) noted that water deficiency during flowering and pod formation causes the fall of flowers and pods. In common beans, stress at this stage may result in a production reduction of up to 40% (Calvache *et al.*, 1997).

In the evaluation of nitrate reductase (NR) in cover crops, we observed a significant interaction between

**Table 1:** Number of pods (NP) and number of grains (NG) developed over two cover crops with different doses of molybdenum in comparison with the control

Treatments	NP	NG
Control	7.62	44.62
Bean_Sunn hemp 0	9.87	65.37
Bean_Sunn hemp 40	15.62*	86.37*
Bean_Sunn hemp 80	11.50	63.62
Bean_Sunn hemp 120	10.00	58.50
Bean_Jack bean 0	9.25	49.25
Bean_Jack bean 40	13.00*	63.75
Bean_Jack bean 80	13.00*	70.50
Bean_Jack bean 120	11.25	58.00
CV(%)	17.79	20.99

Means followed by \* within one column differ from the control at 5% probability of error by the Dunnett test.



**Figure 2:** a) Accumulation of shoot dry matter (t ha<sup>-1</sup>) and b) nitrogen (kg ha<sup>-1</sup>) in cover crop under different molybdenum doses.

species and Mo doses. Both species showed a quadratic response in relation to the Mo doses applied. There was a negative effect for jack beans, with a higher activity at 120 g Mo ha<sup>-1</sup>, whereas sunn hemp exhibited a positive effect, with a maximum of 1.34  $\mu\text{mol NO}_2^- \text{ h}^{-1} \text{ g}^{-1} \text{ MC}$  (moisture content) at a Mo dose of 71.18 g ha<sup>-1</sup> (Fig. 3a).

The activity of an enzyme is governed by several biological factors, which are associated with external stimuli. The development stage is one of these factors, influencing the decrease in NR activity during the plant's reproductive phase (Pessoa *et al.*, 2000). The synthesis of NR is also rapidly induced in the presence of substrate, in this case, nitrate (Almeida *et al.*, 2013), except when the availability of Mo is limited (Bredemeier & Mudstock, 2000), which possibly occurred at the dose of 0 g ha<sup>-1</sup>.

When Mo and substrate are available, enzyme synthesis is induced. The increase in Mo availability through foliar applications of linear doses of Mo may lead to increased absorption and synthesis of nitrate (Pessoa *et al.*, 2000; Matoso & Kusdra, 2014), favoring enzymatic activity as well as DM and N accumulation. However, the accumulation of N was higher at the highest dose of Mo applied (120 g ha<sup>-1</sup>), although enzymatic activity showed a greater response to the dose of 40 g ha<sup>-1</sup>. We therefore assume that the biological N fixation also increased with increasing Mo doses, with a greater contribution of atmospheric N, as noted by Pessoa *et al.* (2000).

When we analyzed the effect of the different Mo doses, we observed a significant interaction between species and doses, with a quadratic negative response for common beans grown under both cover crop (Fig. 3b). The induction of nitrate reductase synthesis can significantly be affected by water deficit (Marur *et al.*, 2000), triggered by the decline of nitrate flow due to a lack of soil moisture. However, the observed values were within the ranges

obtained by Almeida *et al.* (2013) for beans, with activities between 0.3 and 1.7  $\mu\text{mol NO}_2^- \text{ h}^{-1} \text{ g}^{-1} \text{ MC}$ .

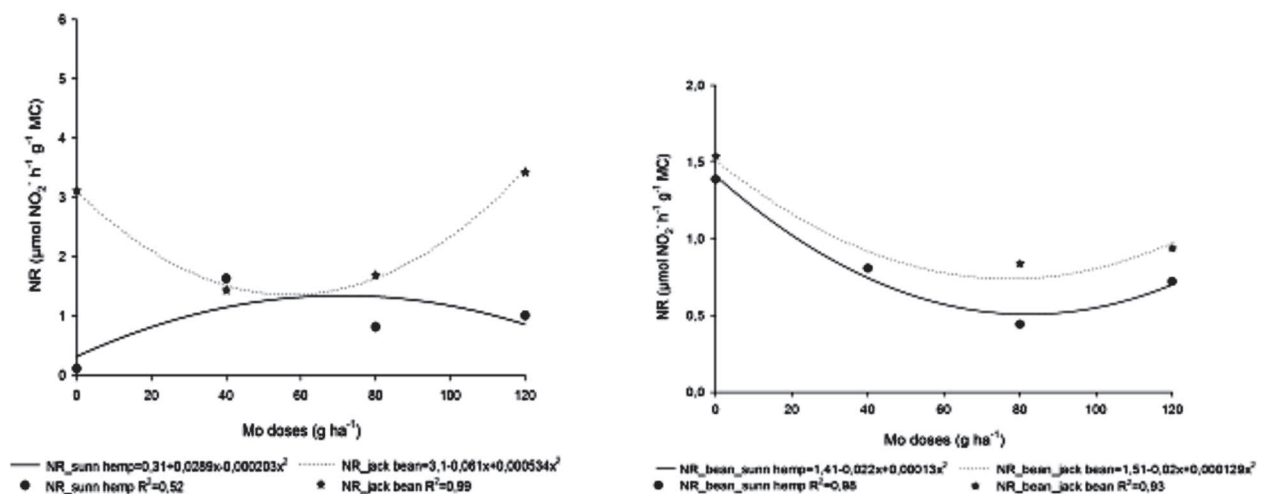
At the time of plant material collection, the common beans were between the developmental stages R7 and R8 (between the early formation of pods and the filling of the first pods). This period, according to a survey carried out by Bredemeier & Musdtock (2000), represents the longest period in which the crops require N, and in the grain-filling phase, the decreased nitrate absorption and assimilation and nodule senescence begin. The plants then start to remobilize the N present in its tissues to fill the grains. However, the presence of Mo prolongs the activity of both enzymes (Almeida *et al.*, 2013).

For a same dose of Mo, NR activity in the common beans was higher when the plants were grown after jack bean cultivation (Fig. 3b), probably as a result of the more accelerated N mineralization in jack beans.

In the evaluation of the activity of NR in the common beans, only three treatments differed significantly from the control without cover crop or Mo application: residues of sunn hemp and jack beans without Mo, and the latter with 120 g ha<sup>-1</sup> of Mo (Table 2).

Nitrate deficiency leads to low enzyme synthesis and, consequently, low activity. This was also found for the control, which did not receive any fertilization and, consequently, had low nitrate levels. However, in the presence of cover crop, decomposition processes occur, along with N mineralization. This results in significant amounts of available nitrate, facilitating NR synthesis and activity (Pessoa *et al.*, 2001; Almeida *et al.*, 2013), which was also observed in our study.

In other treatments, even in the presence of substrate, NR levels were similar to those of the control. Almeida *et al.* (2013) and Pessoa *et al.* (2001) have observed the same response in later developmental stages of plants that



**Figure 3:** a) Nitrate reductase (NR) activity in cover crop species under different doses of molybdenum 90 days after sowing and b) nitrate reductase (NR) activity in common beans 66 days after sowing, grown after the cultivation of two cover crop grown with different molybdenum doses.

**Table 2:** Nitrate reductase (NR) activity in the common beans 66 days after sowing after the cultivation of two cover crop grown with different molybdenum doses.

Treatments	NR (mmol NO <sup>2</sup> h <sup>-1</sup> g <sup>-1</sup> de MC)
Control	0.52
Bean_Sunn hemp 0	1.39 *
Bean_Sunn hemp 40	0.81
Bean_Sunn hemp 80	0.44
Bean_Sunn hemp 120	0.72
Bean_Jack bean 0	1.54 *
Bean_Jack bean 40	0.81
Bean_Jack bean 80	0.84
Bean_Jack bean 120	0.94 *
CV(%)	16.50

Means followed by \* within one column differ from the control at 5% probability of error by the Dunnett test.

received Mo fertilization, due to the maintenance of nitrogenase activity rates.

The activity of nitrate reductase, by itself, does not provide all the explanation necessary for the discussion of the effect of Mo applied to cover crop. However, it can be used to infer about the use of the nitrate made available from the decomposition of plant residue by common beans.

It is not possible to say whether the residual Mo would be sufficient to increase the NR activity; however, the results obtained for common beans indicate that of the addition of cover crop resulted in a higher enzymatic activity compared to the control. Common beans, as well as the cover crop, can form a symbiotic association with N-fixing bacteria; thus, it is possible that part of the nutrients also contributed to nitrogenase activity.

In this way, nitrogenase, responsible for BNF, can explain, in conjunction with nitrate reductase, the processes involved in the assimilation and metabolism of N.

## CONCLUSIONS

The foliar application of Mo resulted in an increased accumulation of shoot dry matter and N in the cultivation of sunn hemp, while this effect was not observed for jack bean.

Common beans productivity was not affected by Mo doses and previous cultivation of cover crops.

Nitrate reductase activity was influenced by the developmental stage of the legume species and by the Mo doses applied.

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## CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interests.

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