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Performance of beans after inoculation with *azospirillum brasilense* and *rhizobium tropici*, and nitrogen and molybic fertilizations under amazonian conditions

Desempenho do feijoeiro à inoculação com *azospirillum brasilense* e *rhizobium tropici*, adubação nitrogenada e molíbdica em condições amazônicas

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Highlights:
Inoculation with *A. brasilense* contributes to bean development and yield; if associated with 30 kg ha^{-1}. nitrogen topdressing, such inoculation improves crop productivity components.
Nitrogen topdressing is still critical to high yields in winter beans.

Abstract

Beans are nutrient-demanding plants, with a high demand for nitrogen (N). Nitrogen biological fixation (NBF) is probably the best solution to meet this demand, especially considering losses in the soil-plant-atmosphere system. Molybdenum (Mo), present in the enzymes nitrogenase and nitrate reductase, is fundamental in the metabolism of N, including NBF. This study aims to evaluate if bean seed inoculation with *Azospirillum brasilense*, *Rhizobium tropici*, and possible interactions with nitrogen topdressing and molybdenum leaf application, may affect winter bean development and yield. A randomized complete block design was used in a 4x2x2 factorial scheme. Plant population, production components, and grain yield were evaluated in two crop seasons (2017 and 2018). Seed inoculation with *A. brasilense*, when associated with 30 kg ha^{-1} N topdressing, increased plant population, pod number per plant and grain yield. Nitrogen topdressing is key to increasing winter bean productivity, regardless of inoculation or Mo foliar application.

Key words: *Phaseolus vulgaris* L. Diazotrophic bacteria. Co-inoculation.

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Resumo

O feijoeiro é uma planta exigente em nutrientes, com alta demanda por nitrogênio (N). A fixação biológica de N (FBN) é, provavelmente, a melhor solução para atender essa demanda, especialmente considerando as perdas no sistema solo-planta-atmosfera. O molibdênio (Mo), presente nas enzimas nitrogenase e redutase do nitrato, é fundamental no metabolismo do N, inclusive da FBN. Com objetivo de avaliar se a inoculação das sementes de feijão com *Azospirillum brasilense, Rhizobium tropici*, e possíveis interações com a adubação nitrogenada e molíbdica em cobertura tem efeito no desenvolvimento e na produtividade do feijoeiro, foi conduzida esta pesquisa. Utilizou-se delineamento experimental de blocos casualizados, em um esquema fatorial 4x2x2. Avaliou-se população de plantas, componentes de produção e produtividade de grãos, em dois cultivos (2017 e 2018). A inoculação das sementes, com *A. brasilense*, quando associada com 30 kg ha^{-1} de N em cobertura, incrementa a população de plantas, o número de vagens por planta e o rendimento de grãos. A adubação nitrogenada em cobertura é determinante no aumento de produtividade do feijão de inverno, independente da inoculação ou aplicação de Mo foliar.

Palavras-chave: *Phaseolus vulgaris* L. Bactérias diazotróficas. Coinoculação.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the main components of the Brazilian diet, constituting one of the most important sources of vegetable protein, especially for the low-income population (Arf, Lemos, Soratto, & Ferrari, 2015). Besides being part of the typical Brazilian cuisine, as the main protein source (Melo, Camargo, Leite, & Lima, 2011), this legume stands out economically because it has been grown by small- and large-scale farmers and under diversified production systems (Valadão et al., 2009; Arf et al., 2015), mainly family-farming ones. The Brazilian average yield in the 2016/17 harvest was about 1,069 kg ha^{-1}; however, in the state of Rondônia, production reached on average 970 kg ha^{-1} in the same harvest season (Companhia Nacional de Abastecimento [CONAB], 2017), standing out as one of the smallest in the country when compared to other states.

Among the yield-limiting factors for common beans, the state of Rondônia faces challenges in terms of cultivar disease-susceptibility, low-fertility soils, and low-tech farmers (Valadão et al., 2009). In this sense, better yields and profitability can be achieved mainly by improving soil properties and plant nutrition, especially nitrogen fertilization (Malavolta, 2006). Nitrogen (N) is considered by many authors (Straliotto et al., 2002; Oliveira, Ferreira, Oliveira, & Esteves, 2012) as one of the nutrients required in larger amounts by different crops, mainly annual grain legumes, which have a short crop cycle and high N export rates through grains during harvest time.

The main sources of N for beans are the soil (through organic matter decomposition), nitrogen fertilizers, and atmospheric N_2 biological fixation, which occurs by an association of bean plants and rhizobia bacteria (Oliveira et al., 2012).

Besides the high production cost, beans also have an additional ecological cost using nitrogen fertilizers, given significant losses of fertilizers in tropical soils mainly by leaching or volatilization (Figueiredo et al., 2016). These soils still present acidification problems since ammonium oxidation reaction can generate acidity when fertilizers such as $(NH_4)_2SO_4$ and NH_4NO_3 are applied, which is proportional to the applied dose.

Biological fixation by diazotrophic bacteria is one of the most important ways for ammonium-based N acquisition by legumes, contributing to increased productivity and cost reductions, as bacteria can be inoculated together with seeds. Such biological fixation of atmospheric N is carried out by prokaryotic microorganisms, known as diazotrophic bacteria, which can be free-living, associated with plant species or even in symbiosis with legumes (Moreira, Silva, Nóbrega, & Carvalho, 2010). Among the genera belonging to this group, *Azospirillum* and *Rhizobium* stand out. These microorganisms, known as growth promoting bacteria (GPB), present as main characteristics: ability of N biological fixation, increased nitrate reductase activity when growing endophytically in plants, production of phytohormones (such as auxins, cytokines, and gibberellins), and phosphate solubilization (Rodriguez, Gonzalez, Goire, & Bashan, 2004; Cassán et al., 2009).

Alternatively, *Azospirillum* co-inoculation with *Rhizobium spp.* can improve *Rhizobium* performance in common beans and increase biological fixation capacity. Several studies have found that *Azospirillum* produces phytohormones stimulators of root growth in several plant species (Hungria, Nogueira, & Araujo, 2015; Peres, Rodrigues, Arf, Portugal, & Corsini, 2016) and can also fixate N and make it available to the bean plants. Positive responses of *Azospirillum* and *Rhizobium* co-inoculation on plant development and growth can be attributed to early nodulation, increasing nodule numbers, and improving root system development (Volpin & Kapulnik, 1994). For Hungria (2011), such an improved root development by *Azospirillum* inoculation may lead to several other effects such as increases in water and mineral absorption, as well as increased tolerance to salinity and drought, resulting in more vigorous and productive plants.

Molybdenum (Mo) plays a key role in nitrogen metabolism as well as in its symbiotic fixation (Figueiredo et al., 2016). This micronutrient is a constituent of three enzymes associated with plant nitrogen nutrition such as nitrogenase, nitrate reductase, and xanthine dehydrogenase. These enzymes act in acquiring N through NBF, using nitrogen absorbed in nitrate form and degrading purine, respectively. Therefore, Mo-deficient beans may have low N levels owning to a decrease in the activity of these enzymes. (Vieira et al., 2015)._

Due to the nitrogen fertilization high cost and low nitrogen-fixing bacteria inoculation in bean crops, which are considered inefficient when compared to other legumes, optimized technologies *Rhizobium* inoculation are fundamental for sustainable bean production. A few studies, mostly in foreign countries, have shown that combining the genus *Azospirillum* with *Rhizobium* can improve bean inoculation results. Nevertheless, such combination must still be studied to solve doubts regarding co-inoculation, suitable N topdressing doses, and Mo foliar application needs in common beans.

Given the above, this study aimed to evaluate if *Azospirillum brasilense* and *Rhizobium tropici* seed inoculation (alone or co-inoculated) and their potential interactions with N topdressing and Mo leaf application may affect development and yield of winter beans.

Materials and Methods

Experimental area and soil characteristics

The study was carried out at an experimental area of the Federal Institute of Education, Science, and Technology of Rondônia, Campus Colorado do Oeste. It is located on BR 435, Km 63, rural area, in the municipality of Colorado do Oeste, southern Rondônia State (13º 07' 01" S latitude, 60º 20' 20" W longitude, and 399-m altitude). Two experiments were performed: one in 2017 and another in 2018.

The local soil is classified as eutrophic Red Argisol and climate as hot and humid tropical (*Awa* type), with two well-defined seasons (Köppen's classification). Annual averages are 25 ºC for temperature and about 2,259 mm for rainfall (Silva, Querino, Santos, Costa Silva, & Saraiva, 2017). Climate conditions during the two cultivation years (2017 and 2018) showed monthly averages of 197 mm 27 ºC, and 85% for rainfall, temperature, and air relative humidity.

Soil chemical and physical properties were determined before the experiments (Table 1), following the methods proposed by Raij and Quaggio (1983).

Table 1
Soil chemical analysis of samples collected from the 0.00 to 0.20 m depth layer in 2017. Colorado do Oeste (RO), Brazil, 2018

MACRONUTRIENTS AND COMPLEMENTARY RESULTS												
Depth Layer	P (1)	OM (2)	pH (3)	K	Ca	Mg	H+ Al	Al	SB (4)	CEC (5)	m (6)	V (7)
(cm)	mg dm^{-3}	g dm^{-3}		----------------------mmol$_c$ dm^{-3}---------------------							----(%)----	
0-20	5	31	4.4	3.5	20	3	64	3	26.5	90.5	10	29

(1) Resin method; (2) Organic matter; (3) Hydrogen potential ($CaCl^2$); (4) Sum of bases; (5) Cation exchange capacity; (6) Aluminum saturation; (7) Base saturation. Particle size analysis showed the following results: 566 g kg^{-1} sand, 224 g kg^{-1} silt, and 210 g kg^{-1} clay.

Treatments and experimental design

The experimental design was randomized blocks arranged in a 4x2x2 factorial scheme, with four replications. Treatments consisted of a combination between seed inoculation (control without inoculation, *Azospirillum brasilense* inoculation, *Rhizobium tropici* inoculation, and *Azospirillum brasilense* + *Rhizobium tropici* co-inoculation), molybdenum foliar application (without application and with 126 g ha^{-1}), and nitrogen topdressing (without application and with 30 kg ha^{-1} N).

Experiment implementation and conduction

Based on the soil properties, the soil was corrected to increase pH and base saturation, following the recommendations for common beans. Filler limestone (100% effective neutralizing power) was cast 30 days before sowing at a dose of 3.7 t ha^{-1} and incorporated by heavy harrow, along with existing crop remnants (Urochloa).

Each experimental unit (12.5 m^2) consisted of five 5-m rows spaced 0.5 m apart, and a useful area corresponding to the 3 central rows, disregarding 0.5 m at either end (borders).

Sowing was carried out on April 21, in the two years of cultivation (2017 and 2018), using BRS Estilo cultivar of the Carioca group. Sowing was conducted manually in furrows, distributing 12 seeds per minute, at a depth range of 3 to 4 cm. In the second year of cultivation, beans were grown in the same area, and sowing was performed directly on *Urochloa ruziziensis* straw, which was cultivated prior to planting and desiccated using glyphosate herbicide.

In the two years of cultivation, before sowing, the plots were base-fertilized with 10 kg ha^{-1} N (ammonium sulfate), 90 kg ha^{-1} P_2O_5 (triple superphosphate), and 20 kg ha^{-1} K_2O (potassium chloride), taking into account the chemical soil analysis and the expected yield range.

Prior to inoculation, seeds were chemically treated with a product containing a mixture of insecticide Fipronil (insecticide of the pyrazole group), and fungicides Pyraclostrobin (strobilurins group) and Methyl-thiophanate (benzimidazole group), also known as “Standak Top”, using the recommended dose of 200 mL c.p. per 100 kg seeds.

Seed inoculation was performed shortly before sowing, using the recommended doses of *Azospirillum brasilense* (100 g or mL inoculant per 25 kg seeds) and *Rhizobium tropici* (100 g inoculant per 25 kg seeds), for both isolated and co-inoculated treatments. The strains of *Azospirillum brasilense* used in the experiment were AbV5 and AbV6, with a count of 2x10^8 CFU g^{-1}. For *Rhizobium tropici*, SEMIA 4080, with 2x10^8 viable cells g^{-1}, was used, being obtained from commercial products registered by the Brazilian Ministry of Agriculture and Livestock (MAPA).

Molybdenum was supplied at the estimated dose for the region (126 g ha^{-1}), according to Melo et al. (2011) and leaf applied, only once, 19 days after seedling emergence (DAE). A manual costal sprayer equipped with a tapered nozzle was used for the foliar spraying.

Nitrogen topdressing (30 kg ha^{-1}) was supplied during the R5 reproductive stage of common beans (flower buds formed), using urea as a nitrogen source. The application was performed manually, distributing the fertilizer under plant canopy and along the plant row.

Weeds, pests, and diseases were controlled according to the infestation level. Pesticides and Mo were applied by a constant pressure sprayer with 20 L capacity. Pest control was done by spraying thiamethoxam, a contact and ingestion systemic insecticide of the neonicotinoid group (125 mL ha^{-1}).

All experimental units received complimentary drip irrigation from the end of the rainy season. Although not recommended for annual grain crops, drip irrigation was used to reduce phytosanitary problems that might affect the crop during the development.

Bean crop assessments

Agronomic traits and yield components were measured. At harvest, 10 plants were collected from the useful area of each plot, determining the following: number of pods per plant (NPP) as the ratio between total number of pods and number of plants; mean number of beans per pod (NBP) as the ratio between total number of beans and total number of pods; 100-grain mass (M100) obtained by random collection and weighing of 2 samples containing 100 grains per plot, corrected to 16% humidity.

During harvest, the number of plants was counted in two rows within the useful area of each plot, so that the final plant population (FPP) per hectare could be estimated.

Plants within the useful area (6 m^2) of each plot were uprooted and left to dry under full sun. After drying, they were selected by a manual trail system, then grains were weighed and data transformed to kg ha^{-1} (at 16% wet basis).

Statistical analysis

Data underwent variance analysis and treatment means were compared by Tukey's test at 5% probability, using the statistical software SISVAR (Ferreira, 2000).

Results and Discussion

Treatments had no isolated effect on plant population, but interaction between inoculation and N topdressing was observed (Table 2). Such results emphasize that without N fertilization neither diazotrophic bacteria nor Mo application influenced bean population density. The breakdown of this interaction showed that topdressing at 30 kg N ha^{-1} increased final plant population (FPP) in treatments inoculated with *Azospirillum brasiliense.* These findings differ from those of M. V. Arf et al. (2011), who analyzed different N sources and doses and observed a linear reduction in winter bean population under no-till system.

Table 2

Averages of final plant population (FPP) and number of pods per plant (NPP) of winter beans as a function of seed inoculation, molybdenum foliar application, and nitrogen topdressing. Colorado do Oeste (RO), Brazil, 2017 and 2018

TREATMENT	Final Population (plants ha^{-1})		Pods per plant^{-1}	
	2017	2018	2017	2018
Inoculation (I)				
Control	240,104	205,937	8.6	16.6
A. brasilense	258,242	203,229	8.1	15.6
R. tropici	239,479	205,208	9.0	15.9
A. brasilense + R. tropici	247,604	205,729	8.7	15.2
Molybdenum (Mo)				
0 g ha^{-1}	246,458	206,510	8.3	15.70
126 g ha^{-1}	246,406	203,541	8.9	16.00
Nitrogen (N)				
0 kg ha^{-1}	246,927	203,697	8.0 b	15.69
30 kg ha^{-1}	245,938	206,354	9.2 a	16.01
CV (%)	9.8	5.42	25.3	14.73
F-test				
I	2.18^{ns}	0.19^{ns}	0.47^{ns}	1.08^{ns}
Mo	0.001^{ns}	1.14^{ns}	1.32^{ns}	0.26^{ns}
N	0.03^{ns}	0.91^{ns}	4.38*	0.30^{ns}
I x Mo	0.61^{ns}	0.38^{ns}	0.56^{ns}	0.70^{ns}
I x N	1.17*	0.99^{ns}	1.48*	0.62^{ns}
Mo x N	1.29^{ns}	0.003^{ns}	3.91*	0.07^{ns}
I x Mo x N	0.05^{ns}	3.11^{ns}	1.33^{ns}	1.06^{ns}

Means followed by the same lowercase letter in the column do not differ from each other at 5% probability by Tukey's test. *, ** = significant at 5% and 1% probability by Tukey's test, respectively. Ns = non-significant. CV = coefficient of variation.

Moreover, sole inoculation with *Azospirillum brasilense* stood out over that with *Rhizobium tropici* for plant population. It can be attributed to a hormonal effect of *Azospirillum brasilense* on the initial plant growth, which may increase seedling establishment in the field. According to Hungria (2011), a great root development by *Azospirillum* inoculation may lead to several other effects such as increased water and mineral absorptions, increased tolerance to salinity and drought, resulting in a more vigorous and productive plant.

Regarding the number of pods per plant (NPP), significant effects were observed for N topdressing alone (Table 2), for interactions between N topdressing with seed inoculation, and N topdressing with Mo leaf application. However, inoculations had no effect on treatments without N topdressing.

Topdressing (30 kg N ha^{-1}) in control had a significant effect on NPP. The same was observed when beans were inoculated with *Azospirillum brasilense*. In other words, N enhances the effects of this microorganism on pod ripening. According to Silva et al. (2009), increasing N topdressing doses may increase NPP, as a result of higher plant heights and branching. Likewise, Silva et al. (2009) observed an isolated effect of Rhizobium seed inoculation on NPP. Otherwise, unlike this study, the same authors noted that N application without inoculation increased NPP up to a maximum dose of 105 kg ha^{-1}.

Molybdenum foliar application only influenced 100-grain mass in the second cultivation year (Table 3). This result corroborates Melo et al. (2011), who studied different Mo doses and found that beans fertilized with 20 g ha^{-1} Mo had an average 1,000-seed mass (240.3 g) about 10.7% higher than seeds produced without Mo (217.0 g). Valadão et al. (2009) also found the 100-grain mass of beans was affected by the interaction of Mo and N and, in the absence of Mo, N increased by 14% in 100-grain mass of beans.

Table 3
Number of grains per pod (NGV), 100-grain mass (M100), and grain yield (GY) of winter beans as a function of seed inoculation, molybdenum foliar application, and nitrogen topdressing. Colorado do Oeste (RO), Brazil, 2017 and 2018

TREATMENT	Grains per pod^{-1}		100-grain mass (g)		Yield (kg ha^{-1})	
	2017	2018	2017	2018	2017	2018
Inoculation						
Control	4.4	4.4	34.0	33.07	2,712	2,960
A. brasilense	4.2	4.5	33.3	33.68	2,741	3,102
R. tropici	4.4	4.5	33.6	32.78	2,699	3,183
A. brasilense + R. tropici	4.3	4.4	33.4	33.35	2,780	3,165
Molybdenum						
0 g ha^{-1}	4.3	4.5	33.3	32.94b	2,669	3,093
126 g ha^{-1}	4.3	4.4	33.9	33.50a	2,799	3,112
Nitrogen						
0 kg ha^{-1}	4.4	4.4	33.7	33.27	2,579 b	3,082
30 kg ha^{-1}	4.3	4.5	33.5	33.17	2,889 a	3,123
CV%	10.1	9.03	5.2	3.68	9.9	17.38
F-test						
I	0.94^{ns}	0.75^{ns}	0.43^{ns}	1.59^{ns}	0.29^{ns}	0.56^{ns}
Mo	0.03^{ns}	1.96^{ns}	2.36^{ns}	3.44^{*}	3.7^{ns}	0.01^{ns}
N	1.25^{ns}	0.01^{ns}	0.06^{ns}	0.11^{ns}	21.26^{**}	0.09^{ns}
I x Mo	1.34^{ns}	0.22^{ns}	0.89^{ns}	1.01^{ns}	0.14^{ns}	1.38^{ns}
I x N	0.35^{ns}	3.43^{*}	1.27^{ns}	0.42^{ns}	3.86^{*}	0.36^{ns}
Mo x N	0.02^{ns}	0.03^{ns}	0.006^{ns}	0.21^{ns}	0.95^{*}	0.002^{ns}
I x Mo x N	0.29^{ns}	1.28^{ns}	0.89^{ns}	0.75^{ns}	1.88^{ns}	1.05^{ns}

Means followed by the same lowercase letter in the column do not differ from each other at 5% probability by Tukey's test. *, ** = significant at 5% and 1% probability by Tukey's test, respectively. Ns = non-significant. CV = coefficient of variation.

In the two cultivation years, mean yields for inoculated treatments were 2,733 Kg ha^{-1} (2017) and 3,102.5 kg ha^{-1} (2018), which are much higher than the national average of 1,158 kg ha^{-1} (CONAB, 2017), with average grain yields 57.62% and 62.67% higher, respectively. Not only were significant effects of N topdressing observed on grain yield (Table 3) in the first cultivation year, but also interaction effects of N topdressing with seed inoculation (Table 3) and with Mo foliar application (Table 4).

A breakdown of the interaction showed that seed inoculation had no variation in crop response between

N topdressed and non-topdressed treatments (Table 4). However, N fertilization increased grain yield in the control and, when inoculated with *Azospirillum brasilense* or *Rhizobium tropici* alone, such effect occurred only in the first year of study (Table 4). This result evidences that, without inoculation, N topdressing is fundamental. On the other hand, when seeds were inoculated, N topdressing enhanced bacterial effect on grain yield. Moreover, co-inoculation was inefficient, given a potential competition between bacterial species within the bean rhizosphere (Table 3). Kaneko et al. (2010) found significant differences in grain yield of winter beans only between treatments with N topdressing in the two years of cultivation.

Table 4
Breakdown of the interaction between seed inoculation and nitrogen topdressing for final plant population (FPP), number of pods per plant (NPP), and grain yield (GY) of winter beans grown under Amazonian conditions in 2017. Colorado do Oeste (RO), Brazil

Seed inoculation	**Nitrogen topdressing**	
	0 kg ha^{-1}	**30 kg ha^{-1}**
	FPP (plants ha^{-1})	
Control	244,583 aA	235,625 abA
Azospirillum brasilense	250,833 aA	266,250 aA
Rhizobium tropici	246,250 aA	232,708 bA
A. brasilense + R. tropici	246,042 aA	249,167 abA
	0 kg ha^{-1}	30 kg ha^{-1}
	NPP	
Control	7.5 aB	9.7 aA
Azospirillum brasilense	6.9 aB	9.3 aA
Rhizobium tropici	9.0 aA	9.0 aA
A. brasilense + R. tropici	8.7 aA	8.7 aA
	0 kg ha^{-1}	30 kg ha^{-1}
	GY (kg ha^{-1})	
Control	2,534 aB	2,890 aA
Azospirillum brasilense	2,444 aB	3,045 aA
Rhizobium tropici	2,536 aB	2,862 aA
A. brasilense + R. tropici	2,801 aA	2,760 aA

Means followed by the same letter, lowercase in the columns and uppercase in the rows, do not differ statistically from each other by Tukey's test at 5% probability.

As Mo foliar application increased grain yield, Mo may play a fundamental role in N metabolism, even without N topdressing. Melo et al. (2011) tested the same Mo dose (126 g ha^{-1}) via leaf application and observed a significant increase in grain yield for Carioca and Rosinha cultivars. This result diverges from Valadão et al. (2009), who noted that Mo influenced only NGP but without affecting grain yield. Moreover, regardless of the Mo leaf application, N topdressing was decisive to increase bean grain yield. However, when N fertilizer was used, Mo had no effect on grain yield, which can be attributed to the suitable organic matter contents in the soil (Table 1).

Regarding the Mo foliar application (Table 5), the lack of N fertilization had no effect on NPP, whereas topdressed plants showed a depressive effect. It can be attributed to the suitable levels of organic matter in the soil (3.1%) (Table 1) since it is the main source of Mo to plants. Such a result is more evident in treatments without Mo foliar application. In this treatment, the application of 30 kg ha^{-1} N as topdressing promoted a significant increase in NPP. Therefore, organic matter content in the soil was enough to supply Mo to the winter beans. This result differs from the findings of Melo et al. (2011), who evaluated several doses of Mo and found that NPP varies with Mo dose. These authors also observed that higher NPP (13.5) was obtained at a dose of 60 g ha^{-1} Mo. This result was about 12.9% higher than that obtained without Mo fertilization.

Table 5

Breakdown of the interaction between molybdenum foliar fertilization and nitrogen topdressing for number of pods per plant (NPP) and grain yield (GY) of winter beans grown under Amazonian conditions. Colorado do Oeste (RO), Brazil, 2017

Foliar molybdenum	**Nitrogen topdressing**	
	0 kg ha^{-1}	**30 kg ha^{-1}**
	NPP	
0 g ha^{-1}	7.8 aB	10.0 aA
126 g ha^{-1}	8.3 aA	8.3 bA
	0 kg ha^{-1}	30 kg ha^{-1}
	GY (kg ha^{-1})	
0 g ha^{-1}	2,471 bB	2,857 aA
126 g ha^{-1}	2,676 aB	2,921 aA

Means followed by the same letter, lowercase in the columns and uppercase in the rows, do not differ statistically from each other by Tukey's test at 5% probability.

Bean seed inoculation had no isolated effect on NPP and 100-grain mass (Table 3), corroborating a study by Valadão et al. (2009). These authors did not find any effect of seed inoculation for the same variables. However, in the present study, inoculation and N-topdressing interactions were significant for number of grains per pod NGP (Table 6).

Table 6

Breakdown of the interaction between seed inoculation and nitrogen topdressing for number of grains per pod (NGP) of winter beans grown under Amazonian conditions in 2018. Colorado do Oeste (RO), Brazil

Seed inoculation	**Nitrogen topdressing**	
	0 kg ha^{-1}	**30 kg ha^{-1}**
	NGP	
Control	4.65aA	4.16bB
Azospirillum brasilense	4.50aA	4.65abA
Rhizobium tropici	4.36aA	4.77aA
A. brasilense* + *R. tropici	4.44aA	4.42abA

Means followed by the same letter, lowercase in the columns and uppercase in the rows, do not differ statistically from each other by Tukey's test at 5% probability.

Conclusion

For winter beans, seed inoculation with *Azospirillum brasilense* increases plant population, number of pods per plant, and grain yield when associated with 30 kg N ha^{-1} as topdressing;

Nitrogen topdressing is a determining factor to increase winter bean yields, regardless of seed inoculation with diazotrophic bacteria or molybdenum leaf application;

Molybdenum leaf application is needed in the lack of nitrogen topdressing to increase yields of winter beans.

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