



Revista Brasileira de Ciência do Solo

ISSN: 0100-0683

ISSN: 1806-9657

Sociedade Brasileira de Ciência do Solo

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Revista Brasileira de Ciência do Solo, vol. 43, 2019

Sociedade Brasileira de Ciência do Solo

DOI: 10.1590/18069657rbcS20170399

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Rhizobial Inoculation and Molybdenum Fertilization in Peanut Crops Grown in a No Tillage System After 20 Years of Pasture

Carlos Alexandre Costa Crusciol⁽¹⁾, Jayme Ferrari Neto⁽²⁾, Tsai Siu Mui⁽³⁾, Alan Joseph Franzluebbbers⁽⁴⁾, Cláudio Hideo Martins da Costa⁽⁵⁾, Gustavo Spadotti Amaral Castro⁽⁶⁾, Livia Cristina Ribeiro⁽⁷⁾ and Nidia Raquel Costa^{(1)*} 

⁽¹⁾ Universidade Estadual Paulista "Júlio Mesquita Filho", Faculdade de Ciências Agrônômicas, Departamento de Produção e Melhoramento Vegetal, Botucatu, São Paulo, Brasil.

⁽²⁾ Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brasil.

⁽³⁾ Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Centro de Energia Nuclear na Agricultura, Laboratório de Biologia Celular e Molecular, Piracicaba, São Paulo, Brasil.

⁽⁴⁾ United State Department of Agriculture - Agricultural Research Service, North Carolina State University, Raleigh, North Carolina, United States.

⁽⁵⁾ Universidade Federal de Goiás, Unidade Acadêmica Especial de Ciências Agrárias, Jataí, Goiás, Brasil.

⁽⁶⁾ Empresa Brasileira de Pesquisa Agropecuária, Embrapa Monitoramento por Satélite, Campinas, São Paulo, Brasil.

⁽⁷⁾ Universidade Estadual Paulista "Júlio Mesquita Filho", Faculdade de Ciências Agrônômicas, Departamento de Ciência do Solo, Botucatu, São Paulo, Brasil.

* **Corresponding author:**
E-mail: nidiarcosta@gmail.com

Received: December 21, 2017

Approved: August 3, 2018

How to cite: Crusciol CAC, Ferrari Neto J, Mui TS, Franzluebbbers AJ, Costa CHM, Castro GSA, Ribeiro LC, Costa NR. Rhizobial inoculation and molybdenum fertilization in peanut crops grown in a no tillage system after 20 years of pasture. Rev Bras Cienc Solo. 2019;43:e0170399.

<https://doi.org/10.1590/18069657rbcs20170399>

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ABSTRACT: Peanut (*Arachis hypogaea*) is an important legume grain consumed by humans and utilized for effective nutrient cycling in a diverse cropping system. Areas that have been cultivated with perennial pasture for decades may have nutritional deficiencies and lack a sufficient population of atmospheric nitrogen-fixing bacteria. Molybdenum is an essential micronutrient that is part of the enzyme nitrogenase contained within symbiotic *Bradyrhizobium* bacteria, which are responsible for fixing nitrogen in legumes. Our objective was to evaluate the effects of application of Mo at different rates and a rhizobial inoculant on peanut growth characteristics. The experiment was conducted in the 2009/2010 growing season in a no-tillage cropping system following 20-year use as pasture [*Urochloa brizantha* (Syn. *Brachiaria brizantha*)]. The experimental design was a randomized complete block with four replicates. The main plots were characterized by peanut inoculation with *Bradyrhizobium* inoculant or without, and the split plots were characterized by different rates of molybdenum (0, 50, 100, and 200 g ha⁻¹) applied to leaves in the form of ammonium molybdate. The nutritional status of plants, nodulation (number of nodules and nodule dry matter per plant), nitrogenase activity, and nitrogenase specific activity were evaluated at 45 and 64 days after emergence (DAE). The yield components and kernel yield were evaluated at the end of the growing season. Nitrogenase enzyme activity at 64 DAE approximately doubled, and the number of pods per plant was greater with inoculation than without, both of which led to greater yields of pods and kernels. In long-term pasture areas, inoculation and molybdenum fertilization greater than the currently recommended rate appear to be necessary to increase pod and kernel yield per hectare of peanut when managed under no-tillage.

Keywords: *Arachis hypogaea*, *Urochloa brizantha*, symbiotic fixation, nodulation, nitrogenase activity.

INTRODUCTION

The Brazilian Cerrado (tropical savanna) has become one of the main agricultural production areas of the world as a result of relatively recent land development based on its favorable soil and climate characteristics. This biome covers approximately 205 million hectares and approximately 24 % of Brazilian territory (Sano et al., 2008). Most of this area is suitable for farming and is favorable for growing cash crops because 70 % of the biome is flat with good drainage, which is favorable for mechanization (Sano et al., 2008). However, in the Brazilian Cerrado, approximately 80 Mha of pasture are degraded and others 50 Mha are highly degraded (Sano et al., 2008).

Crop systems in tropical regions have been characterized by intensification of the use of soils and pastures, and inadequate management practices have been widely adopted for agriculture and cattle raising. This can lead to a reduction in soil quality and the use of poor pastures that are insufficient for ruminant feed demand. In addition to soil degradation, the use of species not suitable for local conditions, insufficient establishment of plants before grazing operations, and loss of soil fertility due to nutrient extraction by the animals are among the factors that contribute to pasture degradation (Carvalho et al., 2010; FAO, 2017).

Sustainability of crop and livestock systems in tropical regions is at risk (Nascente et al., 2013) because crop grain yields, soil fertility, and carrying capacity of pastures may decline over time if soil erosion and pasture degradation are not controlled (Crusciol et al., 2015). Different regions around the world need to restore degraded areas, reduce production costs, and intensify agricultural activity throughout the year (Crusciol et al., 2014; Franzluebbers and Stuedemann, 2014; Mateus et al., 2016). Legume crops such as peanut (*Arachis hypogea*) in rotation with other crops can help maintain N fertility through biological N fixation, especially if managed in a no-tillage system (NTS) (Jordan et al., 2002; Borges et al., 2007; Crusciol and Soratto, 2007; Godsey et al., 2007; Jordan et al., 2008; Crusciol and Soratto, 2009).

In Brazil, peanut has been promoted as a potential rotation crop with sugarcane (*Saccharum* spp.) or degraded pastures within an integrated crop-livestock system (ICLS) aimed at recovering the productive potential of forage (Crusciol and Soratto, 2009). Long-term pastures with minimal inputs may have nutritional deficiencies, as well as a low natural population of N-fixing rhizobacteria in the soil. The basic principle of NTS is the use of technologies to protect the environment, such as no-till planting, erosion control, efficient herbicide management, maintenance of crop residue on the soil surface, and crop rotation. In this system, the accumulation of crop residues on the soil surface favors recycling of nutrients, soil aggregation, water storage, and maintenance of soil organic matter (SOM), and it has positive effects on soil fertility (Kumar et al., 2012; Soratto et al., 2014). Currently, agricultural research centers in Brazil are placing emphasis on crops adapted to the NTS to enhance production and support environmental initiatives, as NTS is a pillar of sustainability in tropical regions. Many crop systems include use of a legume in rotation to maintain soil fertility from inputs from biological N fixation. Thus, a crop rotation system is a valuable alternative for improving the diversification of pasture and agricultural activities in tropical regions (Oliveira et al., 2011). Moreover, legumes in this crop system represent an alternative for the producer to implement biological nitrogen fixation in the soil, because peanut can fix atmospheric N and can reduce the need for mineral N fertilizer following cereal crops (Myaka et al., 2006) or pasture (Oliveira et al., 2011).

Molybdenum (Mo) fertilization and rhizobial inoculation could potentially be combined in efforts to recover these degraded areas, reduce production costs, and enhance utilization throughout the year, all of which could generate positive socio-economic and environmental outcomes. Molybdenum is a micronutrient required by plants in very small amounts, which suggests that plants use Mo for few functions. However, these

functions are crucial, and most are related to the acquisition or use of N. A Mo complex is part of both nitrate reductase, in which nitrate (NO_3^-) is reduced to nitrite (NO_2^-), and nitrogenase, by which bacteria that fix nitrogen convert dinitrogen gas (N_2) to ammonia (NH_3) (Malavolta et al., 1997; Epstein and Bloom, 2004). Thus, Mo is part of the nitrogenase enzyme that is present in nodular bacteroids and is responsible for symbiotic fixation of atmospheric N_2 by rhizobia. Nitrate reductase is responsible for assimilation of the nitrate absorbed from the soil by the reduction of nitrate to nitrite (Sung and Sun, 1990; Chamber-Perez et al., 1997; Caires and Rosolem, 2000; Quaggio et al., 2004). Biological N_2 fixation requires that Mo and cobalt (Co) are present in the soil in sufficient levels, since these micronutrients are part of nitrogenase and leghemoglobin, respectively (Chamber-Perez et al., 1997; Caires and Rosolem, 2000; Quaggio et al., 2004). Thus, Mo is an especially important part of biological N_2 fixation because it participates as a cofactor in the enzymes nitrogenase, nitrate reductase, and sulfide oxidase, and participates in electron transport during biochemical reactions of plants (Sung and Sun, 1990; Caires and Rosolem, 2000; Vieira et al., 2011).

Rhizobial inoculation (Castro et al., 1999; Anandham et al., 2007; Borges et al., 2007; Crusciol and Soratto, 2007, 2009; Vieira et al., 2011; Kraimat and Bissati, 2017) and Mo fertilization investigations (Caires and Rosolem, 2000; Quaggio et al., 2004; Vieira et al., 2005; Kovács et al., 2015) have found positive effects on seed yield of various leguminous crops in Brazil, such as soybean, common bean, and peanut. Inoculation of seeds leads to greater development of the plant root system and, consequently, also greater uptake of water and nutrients (Hungria et al., 2013; Galindo et al., 2017; Moretti et al., 2018). There may be improvements in the performance of crops in current demand for purposes of agricultural, economic, social, and environmental sustainability (Hungria et al., 2013). Applying a peanut-specific inoculant at planting is recommended to meet the N needs of the plant to maximize yield and revenue (Castro et al., 1999; Lanier et al., 2005; Mokgehele et al., 2014). However, there are few studies combining Mo fertilization and inoculation with *Bradyrhizobium* spp. strains in peanut, especially in a rotation system with pasture in the Brazilian Cerrado. Several questions remain from those studies conducted on rhizobial inoculation and Mo fertilization for peanut crops, such as: What is the best method and time for application of the nutrient? What is the ideal application rate? Would molybdenum levels higher than the recommend rate of 55 g ha^{-1} (Quaggio and Godoy, 1997) be needed in a degraded pasture areas? Is inoculation necessary to express the effect of Mo fertilization on the agronomic, physiological, and nutritional characteristics of the crop? With these questions in mind, we conducted a study to investigate the effects of the inoculation and Mo fertilization rate on yield characteristics of peanut. Soils previously under Cerrado vegetation and degraded pastures may have particular limitations on the concentration of Mo and the natural population of N-fixing rhizobia. We assessed the efficiency of inoculation and Mo fertilization on nodulation, nitrogenase enzyme activity, plant nutrition, yield components, and pod and kernel yield in peanut managed with NTS following long-term pasture use that resulted in degraded pasture in the Brazilian Cerrado.

MATERIALS AND METHODS

Site description

The experiment was conducted in Botucatu in the state of São Paulo, Brazil ($48^\circ 25' 28'' \text{ W}$, $22^\circ 51' 01'' \text{ S}$, 777 m a.s.l.), during the 2009/2010 growing season. The soil was a clayey, kaolinitic, thermic Dystroferic Red Oxisol (IUSS Working Group WRB, 2006), which corresponds to a *Latossolo Vermelho distrófico* (Santos et al., 2013). Throughout the previous 20 years, the experimental area had been cultivated with palisade grass for grazing [*Urochloa brizantha* (Stapf) R. D. Webster cv. Marandu (syn. *Brachiaria brizantha* Stapf cv. Marandu)].

The climate according to the Köppen climate classification system is Cwa, i.e., tropical with dry winters and hot and rainy summers. The long-term (1956-2013) average annual maximum and minimum temperatures were 26.1 and 15.3 °C, respectively, and the average annual mean temperature was 20.7 °C. Average annual rainfall was 1,359 mm (Cepagri, 2013). The rainfall and maximum and minimum temperatures during the period of study were also measured (Table 1). Climatic conditions during the period of study were in agreement with the historical average of the region.

Before initiating the experiment, soil chemical properties were evaluated at 20 points per hectare (0.00-0.20 m) according to the methods of van Raij et al. (2001). Soil pH was 4.9 [CaCl₂ 0.01 mol L⁻¹ suspension (1:2.5 soil/solution)]; total soil organic matter was 31 g dm⁻³ (colorimetric method using a sodium dichromate solution); P was 5 mg kg⁻¹ (ion exchange resin); exchangeable K, Ca, Mg, and total acidity were 2.7, 25.7, 13.3, and 44.4 mmol_c kg⁻¹, respectively, the ions were extracted using an ion exchange resin; and base saturation was 48.4 %. Base saturation was calculated from exchangeable base content, and total acidity was measured at pH 7.0 (H+Al). Soil micronutrients of Fe, Cu, Mn, Zn (extraction with DTPA TEA at pH 7.0), B (hot water), and Mo (resin) were obtained using methods adapted from Dallpai (1996) and were 19.3, 5.67, 18.2, 0.61, 0.19, and 0.003 mg dm⁻³, respectively. It should be noted that limestone was not applied prior to carrying out the experiment.

Experimental design and treatments

A randomized split plot experimental design was used, with four replications. The main plots were the inoculation treatment (with and without the *Bradyrhizobium* inoculant specific for peanut), and the split plots were the amounts of molybdenum (0, 50, 100, and 200 g ha⁻¹) applied to leaves as ammonium molybdate. Each plot consisted of six 8-m-long peanut rows spaced 0.90 m apart, for a total area of 43.2 m². The outer two rows on each side and 0.5 m at the end of each plot constituted a border area, which was not used for sample collection.

Tillage and crop management

On 18 November 2009, before peanut sowing, palisade grass and annual broadleaf weeds were desiccated by applying glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] (1.08 kg acid-equivalent ha⁻¹) and 2,4-D amine (2,4-Dichlorophenoxyacetic acid) (0.67 kg acid-equivalent ha⁻¹), using a spray volume of 200 L ha⁻¹.

Table 1. Rainfall, maximum and minimum temperatures, and photoperiod at Botucatu, state of São Paulo, Brazil, during the study period (November to April) and long-term averages

Climate characteristics	Month											
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
2009-2010												
Monthly rain (mm)	289	331	350	180	135	71	-	-	-	-	-	-
Mean max. temp. (°C)	29.5	26.9	27.5	29.4	28.4	26.4	-	-	-	-	-	-
Mean min. temp. (°C)	19.6	19.1	19.5	20.1	18.7	17.1	-	-	-	-	-	-
Photoperiod (h day ⁻¹)	13.1	13.3	13.2	12.7	12.1	11.5	-	-	-	-	-	-
Long-term (50 yr) avg.												
Monthly rain (mm)	133	185	224	203	141	67	76	56	38	39	71	127
Mean max. temp. (°C)	27.2	27.2	28.1	28.0	28.0	27.0	24.0	23.0	23.0	25.0	26.2	26.7
Mean min. temp. (°C)	15.1	16.4	17.1	17.4	19.0	17.0	15.0	13.0	13.0	14.0	12.4	14.2
Photoperiod (h day ⁻¹)	13.1	13.3	13.2	12.7	12.1	11.5	10.9	10.7	10.8	11.2	11.9	12.5

Surface mulch was assessed after pasture desiccation by sampling every meter in two diagonals per plot. This procedure was performed by three evaluators using a square metal grid (1.0 × 1.0 m). Grass straw was cut at ground level using a hand-held brush cutter at three representative 1.0 m² areas per plot. The collected material was dried by forced-air circulation at 65 °C for 72 h and ground. Grass straw dry matter was equivalent to 14.2 Mg ha⁻¹.

The Peanut Runner IAC 886 cultivar was sown on 26 November 2009. Seed was sown at a 0.90 m row spacing, with 12 seeds m⁻¹ of row length using no-till sowing (Semeato, model Personale Drill 13, Passo Fundo, RS, Brazil). Every 100 kg of seeds was treated with 0.7 g of thiamethoxam [3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine] as active ingredient to control *Enneothrips flavens*. Fertilization in the furrow at the time of peanut sowing was 130 kg ha⁻¹ of P₂O₅ as single superphosphate and 35 kg ha⁻¹ of K₂O as KCl (Ambrosano et al., 1997).

Peanut seeds were inoculated in the shade with *Bradyrhizobium* spp. (SEMIA 6144) just before sowing. A minimum concentration of 10⁹ viable cells kg⁻¹ of seed was applied at the rate recommended by the manufacturer (200 g per 50 kg of seed), and there was no molybdenum in the composition of the inoculant.

Peanut seedlings emerged six days after sowing. Mineral fertilization between peanut rows was topdressed on the soil surface without incorporation (41 kg ha⁻¹ of K₂O as potassium chloride) at 20 days after emergence (DAE), following the recommendation of Ambrosano et al. (1997).

Molybdenum was applied on peanut leaves at the pre-flowering stage of 25 DAE. Different Mo rates were applied using a manual CO₂ pressurized sprayer equipped with a spray boom with four nozzles (Teejet XR 11002 VS) at a pressure of 200 kPa and spray volume of 200 L ha⁻¹.

Sampling and Analyses

Peanut nodulation and nitrogenase activity

Number of nodules per plant and nitrogenase activity were evaluated by collecting five plants per plot. Soil was excavated with a shovel 0.10 m to the side of each plant row to a depth of 0.30 m.

First of all, the root systems of the five plants of each plot, together with the soil adhering to the rhizosphere and the nodules that were detached from the roots, were placed in hermetically sealed vials to measure nitrogenase enzyme activity by the acetylene reduction method (Hardy et al., 1968). Then the plant roots were taken to the laboratory and carefully washed, and the number of nodules were counted. Subsequently, the nodules were oven dried at 65 °C until constant weight and then weighed to determine nodule dry matter per plant.

The acetylene reduction method is a fast and efficient method for quantitative determination of the N₂ fixed, based on the relationship between the reduction of acetylene to ethylene and N₂ to ammonia by the nitrogenase enzyme. Peanut roots without excess soil were placed in 100 mL vials and hermetically sealed. Acetylene at 10 % of vial volume was delivered, taking care to pre-extract this same volume of air so as not to alter the pressure. Vials were incubated in the dark at an average temperature of 25 °C for 30 min. After this incubation period, 0.5 mL of the atmosphere of the vials was withdrawn with a syringe and injected into a gas chromatograph using a hydrogen flame detector at 125 °C and glass column of 0.5 m length and 5 mm diameter containing Poropak N resin of 80 to 100 mesh at 50 °C, with N₂ as entrainment gas at a flow of 40 mL min⁻¹. The ethylene standard was determined by injecting 0.5 mL of pure ethylene into the chromatograph at 500 ppm concentration. The results were

divided by the number of plants used so that the result was converted in $\mu\text{moles C}_2\text{H}_4 \text{ plant}^{-1} \text{ h}^{-1}$. Nitrogenase specific activity was nitrogenase activity divided by nodule dry matter, based on the research of Kubota et al. (2008).

Peanut leaf nutrient contents

When peanut plants were at the full-bloom stage, approximately 30-40 days after emergency (DAE), 40 plants were sampled per plot (apical cluster of the main branch), according to Ambrosano et al. (1997). The material was dried in an oven at 65 °C to constant weight and then ground for macronutrient analyses. Contents of N, P, K, Ca, Mg, and S were determined from ground plant materials using methods described by Malavolta et al. (1997). Nitrogen was determined by the Kjeldahl method, while other nutrients were determined from a nitric-perchloric solution. Concentrations of K, Ca, and Mg were determined using an atomic absorption spectrophotometer. Concentrations of P and S were determined using a spectrophotometer (Malavolta et al., 1997). Concentrations of Mo were determined based on a method adapted from Dallpai (1996).

Peanut agronomic characteristics and yield

Peanut seeds were harvested manually on 10 April 2010. Pod yield was determined by manually harvesting all plants within the middle three rows of 6-m length (usable area). Determination of yield components included plant population (number of plants in three central rows of 6-m length in each plot), number of filled pods per plant (number of pods from 10 randomly selected plants in each plot), number of kernels per pod (total number of kernels from 10 plants divided by total number of pods from 10 plants), 100-kernel weight (random collection and weighing of four samples of 100 kernels from each plot), and hulled-kernel yield (kernel weight divided by pod weight).

Statistical analyses

Data for all variables were analyzed using ANOVA and the statistical software package SISVAR (Ferreira, 2011), following the scheme of split plot analysis. Responses to inoculation were analyzed using the method of unprotected comparison of means (Vieira, 1999), i.e., regardless of the result of the F test using LSD at $p < 0.10$. Responses to Mo application rates were fitted to mathematical functions by adopting the significance of regression coefficients at $p < 0.10$ as a criterion for choosing the model.

RESULTS AND DISCUSSION

During the experimental period, 1,356 mm of rainfall were recorded, more than the historical average of 750 mm. Mean annual temperature was similar to the historical average (Table 1). Thus, weather conditions were favorable for peanut development (Caires and Rosolem, 2000; Crusciol and Soratto, 2009), and the crop developed without water stress.

In general, rhizobial inoculation and the Mo application rate were significant factors for most of the parameters evaluated (Table 2). Number of nodules per plant (64 DAE) and nodule dry matter per plant (45 and 64 DAE) were greater with inoculation than without. The same was observed for nitrogenase activity and nitrogenase specific activity at 45 and 64 DAE (Table 2). Interactions between rhizobial inoculation and Mo rate were not significant. Plant nutrient contents were generally not influenced by inoculation and Mo rate, except for N and Mo (Table 2). Peanut yield components of the number of filled pods per plant, number of kernels perpod, pod yield, hulled-kernel yield, and kernel yield were positively affected by inoculation and Mo rate. A significant interaction of these factors occurred only for number of filled pods per plant, pod yield, and kernel yield (Table 2).

Table 2. Statistical significance ($Pr > F$) of inoculation, Mo rate, and their interaction on number of nodules plant⁻¹, nodule dry matter (NDM) plant⁻¹, nitrogenase activity, nutrient content in leaves, and yield components and kernel yield as a function of days after emergence (DAE) of peanut

	Nodules plant ⁻¹		NDM plant ⁻¹		Nitrogenase activity		Nitrogenase specific activity	
DAE	45	64	45	64	45	64	45	64
ANOVA (<i>F</i> probability)								
Inoculation (I)	0.15	0.05	0.16	0.06	0.04	0.02	0.07	0.003
Mo rates (Mo)	0.31	0.02	0.005	0.02	<0.001	0.88	0.57	<0.001
I × Mo	0.61	0.90	0.95	0.42	0.78	0.39	0.69	0.44
Nutrient content in peanut leaves								
	N	P	K	Ca	Mg	S	Mo	
ANOVA (<i>F</i> probability)								
Inoculation (I)	0.16	0.20	0.59	0.29	0.54	0.87	0.01	
Mo rates (Mo)	<0.001	0.17	0.23	0.74	0.69	0.70	0.005	
I × Mo	0.17	0.89	0.91	0.82	0.98	0.99	0.18	
Yield components and kernel yield								
	No. of plants	No. of filled pods plant ⁻¹	Kernels pod ⁻¹	100-kernel weight	Pod yield	Hulled-kernel yield	Kernel yield	
ANOVA (<i>F</i> probability)								
Inoculation (I)	0.43	0.008	0.08	0.99	0.03	0.004	0.02	
Mo rates (Mo)	0.15	<0.001	0.86	0.29	0.10	0.34	0.05	
I × Mo	0.15	0.03	0.64	0.46	0.10	0.18	0.05	

Number of nodules per plant at 45 DAE was not affected by the application of Mo in treatments without inoculation, which had an average of 357 nodules per plant (Figure 1). In a study conducted by Fernandes (2008), the author reported that Mo fertilization ratio up to 160 g ha⁻¹ did not influence the number of nodules in the roots of peanut evaluated at the beginning of flowering, but it is important to mention that this study was carried out in an area with a history of growing annual crops. In contrast, in our study, the area under cultivation had a 20-year history under pasture that exhibited a certain degree of degradation. These authors also reported that fertilization with molybdenum can be performed in several ways - through seed coating, foliar application, direct application to the soil, and the use of seeds rich in this micronutrient. However, one advantage of molybdenum foliar application is that it can be associated with a spraying operation during the crop cycle. Not only the use of a selected and efficient rhizobial inoculant decisively contributes to biological N₂ fixation, but also Mo application, which affects the synthesis of leghemoglobin and determines nodule activity. Furthermore, since it is a structural component of nitrogenase, it catalyzes the reduction of atmospheric N₂ to NH₃ (Epstein and Bloom, 2004; Galindo et al., 2017).

The negative effect of inoculation on nodulation in the peanut crop in the treatments without Mo application may be due to the native population present in the soil, which is able to colonize and promote nodulation, but they might not be as effective as the strains just added via inoculants. Moreover, the nodulation process is usually delayed when relying only on the rhizobial population established in the soil, which may affect plant performance and kernel yield (Cerezini et al., 2016), as observed at 64 DAE. In addition, low soil fertility affects the effectiveness of inoculation (Hungria et al., 2015), especially regarding low MO content, as in the present experiment. So, the absence of Mo treatments may have impaired nodulation development, since this element is extremely important in the efficiency of biological N fixation.

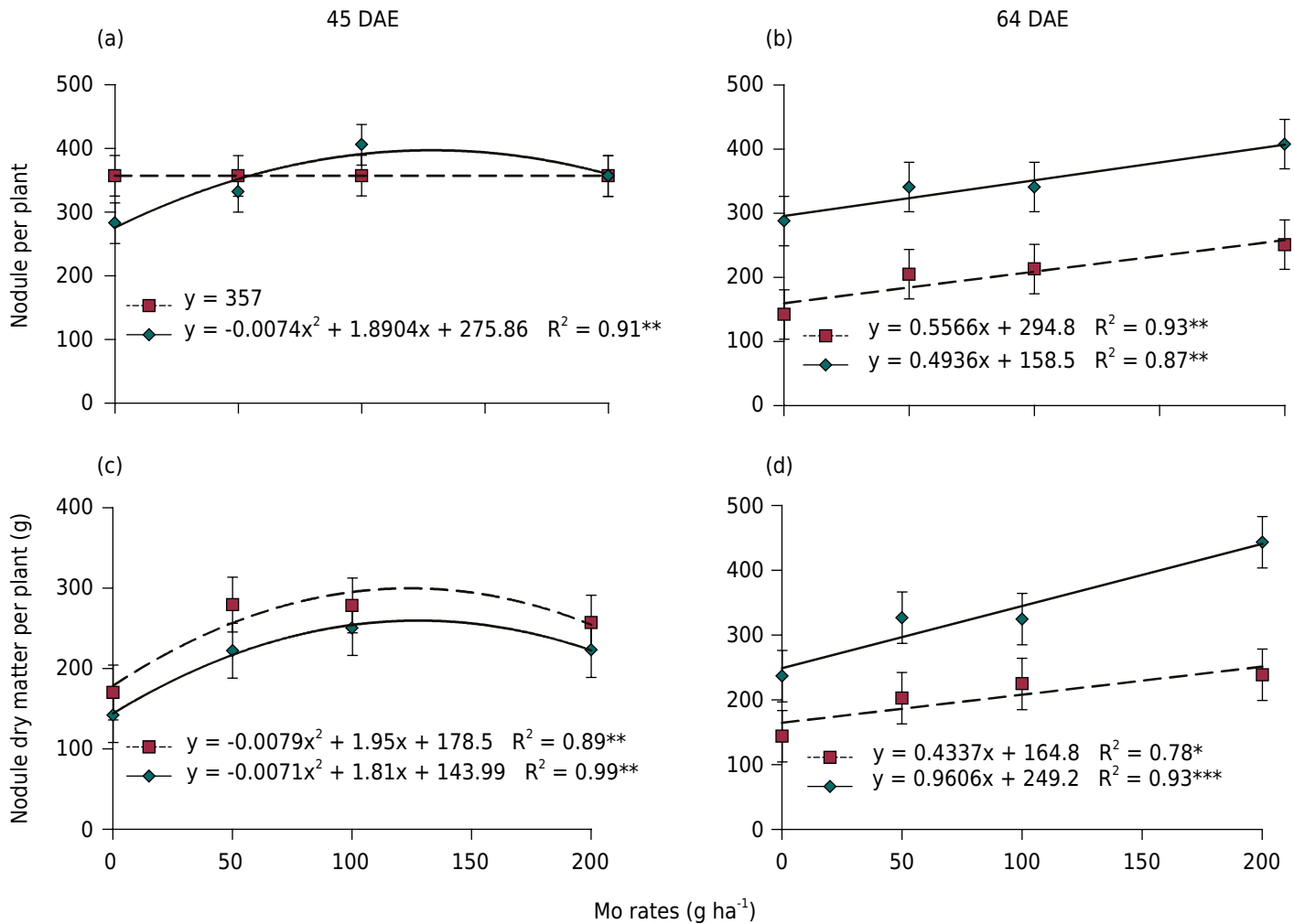


Figure 1. Number of nodules per plant (a and b) and nodule dry matter (c and d) as a function of Mo rate and rhizobial inoculation [without (\square) and with (\blacklozenge) inoculation] at two different stages of peanut growth (45 and 64 days after emergence, DAE). ***, **, and * are significant at $p < 0.01$, $p < 0.05$, and $p < 0.10$, respectively.

With inoculation, Mo fertilization increased the number of nodules per plant up to at least the 100 g ha⁻¹ rate. However, without Mo application, the number of nodules per plant was greater without than with inoculation (357 vs 283 nodules per plant). At 64 DAE, the number of nodules and nodule dry matter per plant were always greater with inoculation than without inoculation (Figures 1b and 1d). These results suggest that the strain used for inoculation was efficient in fixing N₂. Application of Mo increased the number of nodules and nodule dry matter per plant, regardless of inoculation. These results demonstrate the importance of the Mo supply in the peanut crop, because this nutrient acts directly on nitrate reductase and the nitrogen fixation process. It is an element of fundamental importance when aiming to increase efficiency and nitrogen uptake in the peanut crop. According to Vieira et al. (2005), Mo fertilization is another key management practice to ensure that resident soil rhizobia are efficient when peanut is grown.

Peanut seed inoculation is also an important management practice to ensure adequate nodulation, which is a prerequisite for biological N₂ fixation. However, it should be emphasized that peanut response to inoculation depends on how frequently peanut is placed in the crop rotation scheme, as well as the soil and climatic conditions in the agricultural region (Crusciol and Soratto, 2009). These effects can come about through various mechanisms, which include anticipation in biological N fixation of the nodules, an increase in nodule dry matter (NDM), promotion of the occurrence of heterologous nodulation through an increase in the formation of root hairs and secondary roots (with an increase in infection sites, inhibition of phytopathogens, and production of

phytohormones), as well as effects on the partition of dry matter between roots and shoots (Hungria et al., 2013; Galindo et al., 2017; Moretti et al., 2018).

Molybdenum fertilization increased NDM, which reached maximum values of 299 and 259 mg NDM per plant (without and with inoculation, respectively) at the optimized Mo application rate of $\sim 125 \text{ g ha}^{-1}$ (Figure 1c). At 45 DAE, the number of nodules per plant was around 350, but if rhizobial inoculation had not had a negative effect on this parameter, there would be no differences between treatments. At 64 DAE, although the number of nodules in the inoculated treatment remained high it decreased significantly to levels between 250 and 158 nodules per plant. Molybdenum application always had a positive effect. Nodule dry matter followed the behavior of the number of nodules. Caires and Rosolem (2000) found that application of Mo to peanut increased the NDM but did not affect the number of nodules per plant at 74 DAE. Peanut seed rhizobial inoculation, regardless of Mo rate, resulted in lower nitrogenase enzyme activity at 45 DAE than without inoculation (Figure 2a). The results were fitted to second-degree equations, where the highest values were obtained with the maximum calculated rates of 111 and 125 g ha^{-1} of Mo in the treatments with and without inoculation, respectively. Nitrogenase specific activity at 45 DAE was also not altered by Mo rate, but values with and without inoculant were 36.3 and $52.5 \text{ } \mu\text{moles of C}_2\text{H}_4 \text{ g dry matter}^{-1} \text{ of nodules h}^{-1}$, respectively (Figure 2c). Calculating the results per g of NDM resulted in a high coefficient of variation (CV, %), making it difficult to identify a statistically significant difference even at $p < 0.10$.

The specific activity values of nodules at 45 DAE exhibited a mean of 45, about 20 times greater than at 64 DAE, where values less than $2 \text{ } \mu\text{moles of C}_2\text{H}_4 \text{ g dry matter}^{-1} \text{ of nodules h}^{-1}$ were observed (Figures 2a and 2b). At 45 DAE, inoculation alone had a positive effect on the biological nitrogen fixation process (BNF). At 64 DAE, inoculation had a favorable effect, but in all cases, Mo decreased nitrogenase activity. Thus, as nitrogenase activity is responsible for BNF, it can be inferred that this process would be more important at 45 DAE and that inoculation was efficient.

In contrast to the results at 45 DAE, nitrogenase enzyme activity at 64 DAE approximately doubled with inoculation compared to without inoculation (Figure 2b). Similarly, strong differences due to inoculation occurred for nitrogenase specific activity, and this result also contrasted with that obtained at 45 DAE. Studies on nitrogenase enzyme activity in peanut are scarce in the literature. However, activity of the nitrogenase enzyme was greater with inoculation than without in the period of high peanut nutritional demand, which is at the beginning of seed formation (Figures 2b and 2d). Reduction in nitrogenase specific activity as a function of Mo fertilization rate likely occurred due to an increase in nodule dry matter (Figures 1d and 2d). The reciprocal relationship of nitrate reduction and N fixation suggests that nitrogen fixation plays a crucial role in N assimilation during the post flowering stages of peanut plant development (Sung and Sun, 1990).

According to Souza et al. (2011), inoculation of common bean seeds did not affect the number of nodules and NDM at the time of full flowering of this crop. Common bean nodule formation without inoculation is an indication of the presence of native strains of N_2 -fixing microorganisms, and these native strains can limit the establishment of inoculated strains, which are often more efficient (Souza et al., 2011; Mokgehele et al., 2014; Moretti et al., 2018). Thus, similarity in nodulation at the beginning of peanut fruiting (45 DAE), regardless of inoculant application, indicates that native rhizobia were present in the soil of the experimental area. The presence of N_2 -fixing nodules throughout the growth cycle might represent a key strategy for supplying the nutrient for high yielding plant genotypes (Moretti et al., 2018).

In general, contents of P, K, Ca, Mg, and S in peanut leaves (Figure 3) were considered adequate for this crop (Ambrosano et al., 1997; Quaggio et al. 2004). However, N content

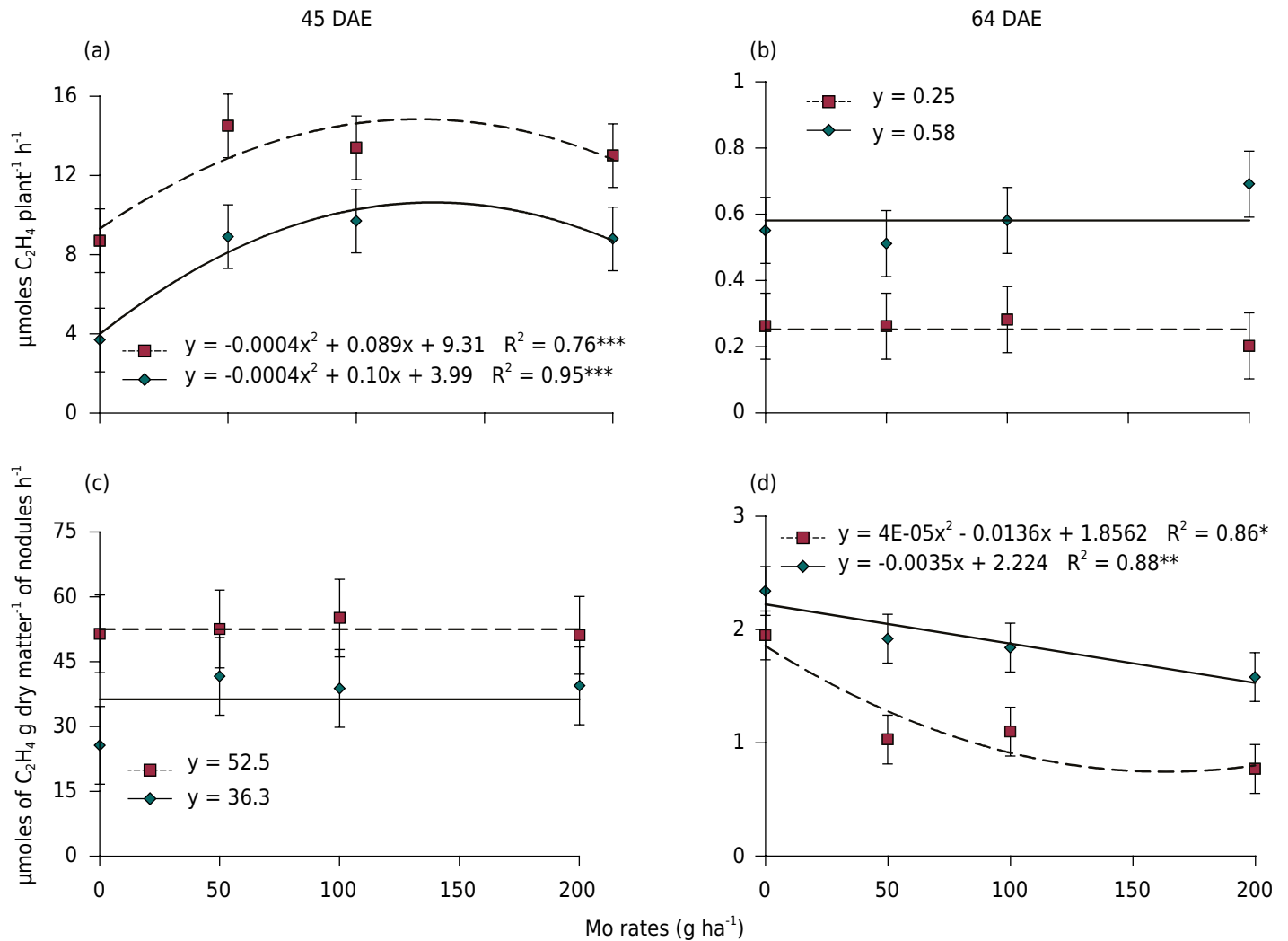


Figure 2. Nitrogenase enzyme activity (a and b) and nitrogenase specific activity (c and d) as a function of Mo rate and rhizobial inoculation [without (□) and with (♦) inoculation] at two different stages of peanut growth (45 and 64 days after emergence, DAE). ***, **, and * are significant at $p < 0.01$, $p < 0.05$, and $p < 0.10$, respectively.

in the absence of Mo fertilization, irrespective of inoculation, was considered deficient for peanut. Only with application of $>50 \text{ g ha}^{-1}$ of Mo was N concentration within the range considered adequate (Figure 3). It is noteworthy that the recommended content of Mo is around 55 g ha^{-1} for the peanut crop in Brazil (Quaggio and Godoy, 1997), demonstrating the importance of this micronutrient application, especially for legume crops in succession to pastures.

Content of Mo in peanut leaves increased linearly with Mo fertilization when peanut was not inoculated (Figure 3). The ideal leaf Mo content in peanut is between 0.1 and 5.0 mg kg^{-1} (Gascho and Davis, 1995). When peanut was inoculated with *Bradyrhizobium* spp., Mo fertilization $>100 \text{ g ha}^{-1}$ saturated Mo content in the leaves. However, when inoculated, peanut appeared to synthesize N in leaf tissue more efficiently at lower rates of Mo fertilization. Nodules of inoculated plants may have had greater Mo content than uninoculated plants, since nodules often have greater content of Mo relative to leaves. Under conditions of Mo deficiency, the tendency is to accumulate Mo in nodules instead of plant leaves. When plants depend on N_2 fixation, molybdenum deficiency renders them deficient in nitrogen, with symptoms characteristic of deficiency of this element, such as little nodulation, less biological N_2 fixation, chlorosis, bending or choking of the limb, may appear on older leaves or physiological middle age, inhibition of nitrate reductase activity, and subsequent nitrate accumulation (Epstein and Bloom, 2004). Thus, in

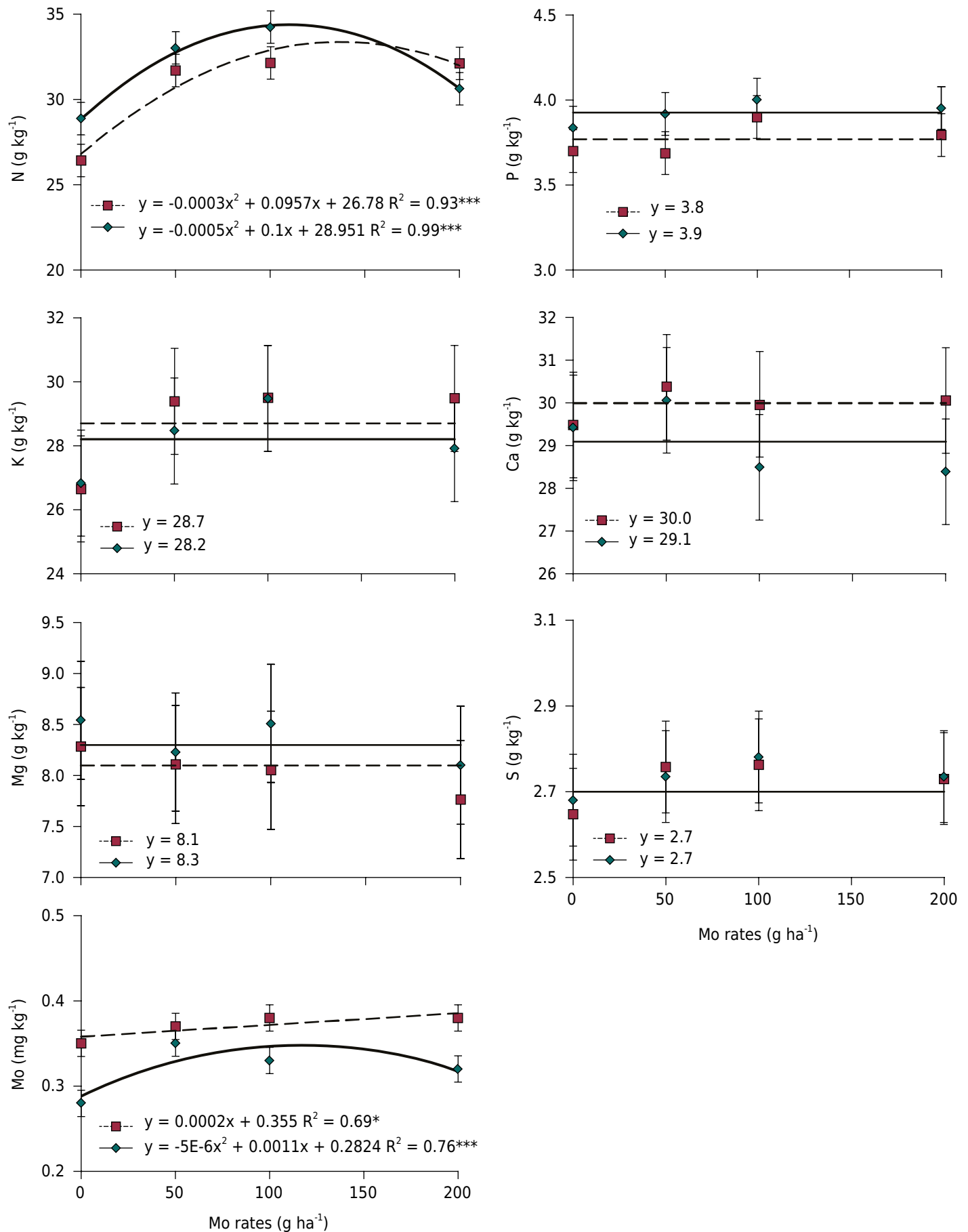


Figure 3. Nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and molybdenum contents in peanut leaves at the full flowering stage of peanut as a function of Mo rate and rhizobial inoculation [without (□) and with (♦) inoculation]. ***, **, and * are significant at $p < 0.01$, $p < 0.05$, and $p < 0.10$, respectively.

general, the growth and development of the peanut crop is affected. Souza et al. (2011) also observed greater leaf N content in common bean plants as a function of inoculant application when preceded by corn intercropped with palisade grass (*U. brizantha*). A positive correlation between content of N and Mo in peanut leaves was observed ($r = 0.84$ and 0.96 with and without inoculation, respectively). Content of Mo is directly related to metabolism of N, so this relationship was to be expected. Vieira et al. (1998) described the importance of Mo for the activity of nitrogenase and nitrate reductase, resulting in a better supply of N to plants, with a direct effect on yield components of common bean. Quaggio et al. (2004) obtained positive results with Mo application to peanut, resulting in greater N content in leaves and greater seed yield.

In general, values for most yield components of peanut were greater for inoculated than uninoculated plants at all Mo rates. Number of filled pods per plant was greater with inoculation than without and also increased at greater Mo rates (Figure 4). Although the interaction between the inoculant and Mo rate was significant, it was due to low variation and slightly greater response with increasing Mo rate when inoculated than without. Pod yield also increased along with the Mo application rate. However, when the crop was inoculated, kernel yield was adequate with Mo rate of $\sim 125 \text{ g ha}^{-1}$, whereas without inoculation, Mo fertilization led to a linear increase in pod yield (Figure 4). Thus, correct Mo fertilization management and inoculation in peanut seeds can favor the development and production of the crop.

The recommendation for Mo fertilization of peanut as proposed by Quaggio and Godoy (1997) is 55 g ha^{-1} . However, it was verified in our study that the effect of fertilization occurred only at Mo rates above that recommended by these authors. The soil in our study had low content of Mo, averaging 0.003 mg kg^{-1} (Table 1). The Brazilian Cerrado regions are characterized by extensive areas with soils of low natural fertility (Sano et al., 2008). Intensive and indiscriminate use of these areas can promote degradation of soils and pastures. Thus, peanut cultivation in rotation with pasture was an efficient alternative, since satisfactory crop yields were obtained, even in adverse fertility conditions. This can perhaps be attributed to the fact that Mo availability and uptake are directly influenced by soil pH and constituent clay minerals (Epstein and Bloom, 2004). It should be noted that this was an area in pasture for 20 consecutive years and exhibited some degree of degradation. These low soil Mo conditions created an opportunity for large peanut yield responses to Mo fertilization.

With 50 g ha^{-1} of Mo fertilization, pod yield was maximized at $\sim 5000 \text{ kg ha}^{-1}$ (Figure 4). This yield level is considered good for the region (Conab, 2017). Without inoculation, application of 200 g ha^{-1} of Mo fertilizer was needed to maximize pod yield at $\sim 3950 \text{ kg ha}^{-1}$ (Figure 4). Our results for yield components, hulled-kernel yield, and pod and kernel yield were better than those obtained by Crusciol and Soratto (2009), who also used the peanut cultivar IAC Runner 886 in a NTS with soil and climate conditions similar to those of our study.

Hulled-kernel yield was not influenced by Mo fertilization rate and inoculation, and it averaged 80 % for all treatments (Figure 4). Kernel yield response to inoculant and Mo fertilization were therefore essentially the same as for pod yield (Table 2 and Figure 4). Kernel yield increased with enhanced Mo rates, especially in treatments without inoculation. Number of filled pods per plant was the peanut yield component that most directly influenced pod yield and, consequently, kernel yield (Figure 4). A greater number of filled pods per plant occurred due to better N nutrition of peanut plants in response to inoculation and adequate Mo fertilization. With the high kernel yield obtained in this study, we confirm and highlight the ability of a Cerrado soil in a NTS with high residual straw production from *Urochloa brizantha* (14.2 Mg ha^{-1}) to successfully produce peanut. As in previous research, a large quantity of surface straw was not a detriment to establishing peanut plants and achieving pod yield in a NTS (Crusciol and Soratto, 2009). The organic

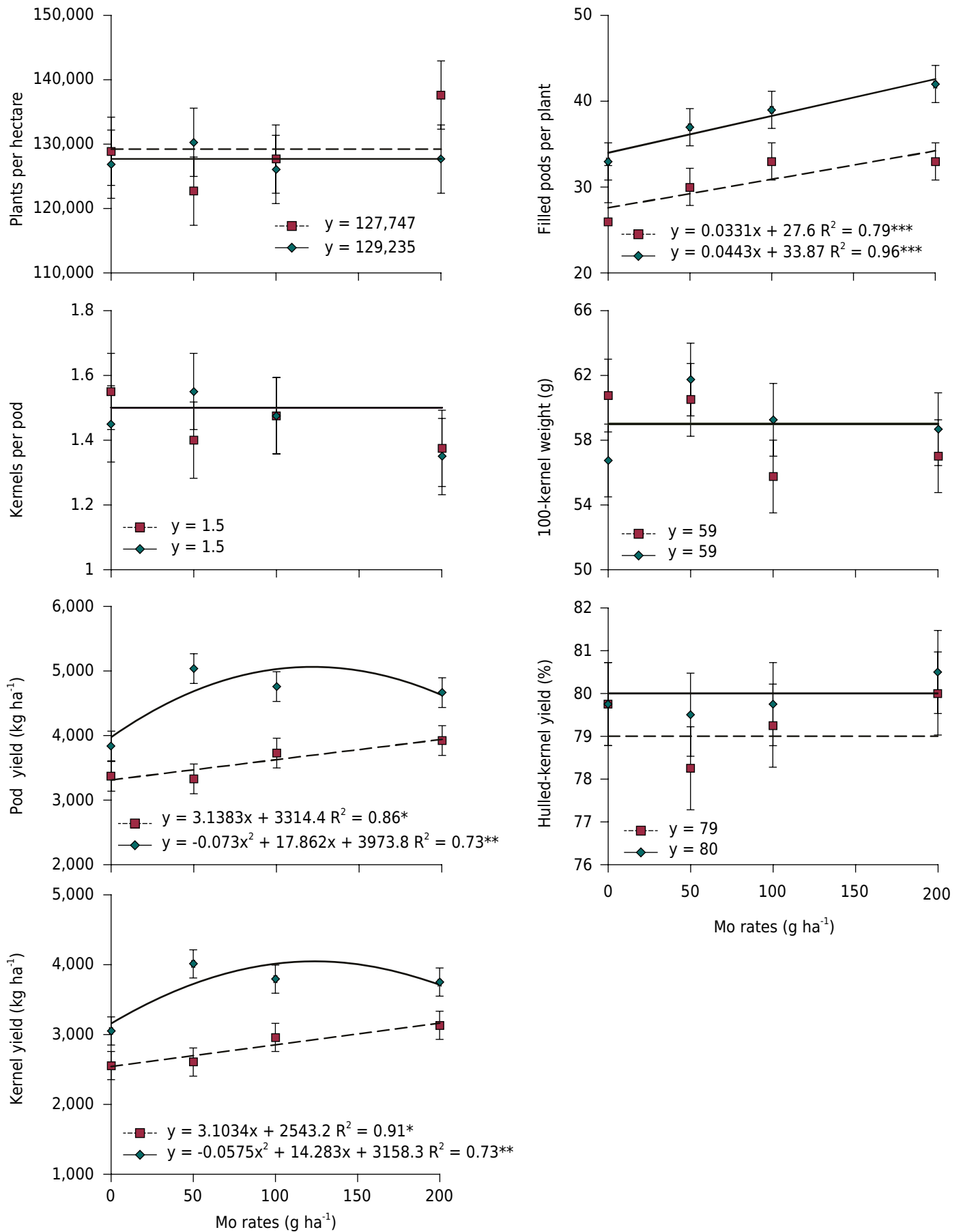


Figure 4. Number of plants per hectare, number of filled pods per plant, kernels per pod, 100-kernel weight, pod yield, hulled-kernel yield, and kernel yield as a function of Mo rate, and rhizobial inoculation [without (□) and with (◆) inoculation]. ***, **, and *, are significant at $p < 0.01$, $p < 0.05$, and $p < 0.10$, respectively.

matter from the high residual straw production likely contributed to the high kernel yield obtained in this study.

The results for peanut yield components, hulled-kernel yield, and pod and kernel yield were better than those obtained by Crusciol and Soratto (2009) and Fernandes (2008), who also used the peanut cultivar IAC Runner 886 in a NTS under soil and climate conditions similar to those of our study. These positive results are due to adequate peanut crop management practices and the benefits of straw residue in the area from the cultivated grass, which may have favored nutrient cycling and improved soil structure due to the abundant root system. Thus, some years after adopting a NTS, an equilibrium between the amounts of immobilized and mineralized N in the soil is reached. The direction of the mineralization-immobilization turnover will determine nutrient availability in the soil (Anghinoni, 2007). Over time, the N contribution through crop residue decomposition becomes greater than the quantity of N immobilized by soil microorganisms, which may permit application of less N fertilizer (Soratto et al., 2014). Several studies emphasize the importance of straw remaining on the soil surface because this promotes soil maintenance, protects the soil-plant system, and favors soil biota and nutrient cycling (Franzluebbers and Stuedemann, 2014). Thus, these crop systems proved to be excellent alternatives for the present agricultural conditions, especially in tropical regions (Crusciol et al., 2015).

Studies on soybean and common bean have demonstrated that the application of molybdenum on the leaves from flowering to the filling of the bean grains is efficient in increase the final yield of these crops (Jacob-Neto and Franco, 1986, 1988, 1989). Leaf fertilization is widely used in agricultural production as an alternative or complementary strategy to soil fertilization and is increasing in importance in agricultural production worldwide. This practice can be used as a supplement for soil applications or under conditions of limited soil nutrient availability. The most practical and effective form of fertilization with the Mo amounts required by plants is application through seed pelleting (Sfredo and Oliveira, 2010). There are no indications that toxicity to *Bradyrhizobium* in the soybean crop occurs immediately prior to sowing when pelleting with low Mo quantities. In this case, there was excellent nodulation and a considerable increase in the yield of bean grains. However, Hungria et al. (2007) detected some problems in the application of Mo and Co on the seeds. According to these authors, application of saline formulations or formulations with low pH may drastically affect survival of the bacteria, nodulation, and fixation of N₂. These problems can be avoided with application of these micronutrients on the leaves, just as practiced in the present study, because the application of this element via the soil is difficult. Only a small amount is required by the crop, making it difficult to distribute in an agricultural area. Under acidic soil conditions, seed pelleting or treatment of soil with Mo can increase N₂ fixation rates in legumes (Cooper and Scherer, 2012). According to these authors, pelleting with 100 g ha⁻¹ Mo increased nitrogenase activity, leaf N content, and, particularly, nodule dry weight, whereas addition of mineral N decreased nodule dry weight and suppressed nitrogenase activity. An adequate Mo supply may be favorable to the development of crops under adverse environmental conditions. According to Cooper and Scherer (2012), supplying Mo may not only enhance N₂ fixation, total N uptake, and drought tolerance, but also increase pod yield more than application of 60 kg ha⁻¹ N of mineral fertilizer.

There are many positive results of increases in crop grain yields as a function of Mo fertilization (Jacob-Neto et al., 1988, 1997; Caires and Rosolem, 2000; Quaggio et al., 2004; Borges et al., 2007; Campo et al., 2009; Vieira et al., 2011; Pacheco et al., 2012; Kovács et al., 2015) and rhizobial inoculation (Anandham et al., 2007; Tubbs et al., 2012; Ibañez et al., 2014; Kraimat and Bissati, 2017; Moretti et al., 2018). Galindo et al. (2017) evaluated the technical and economic viability of soybean in the Cerrado according to the mode of application of Co and Mo, as well as seed or leaf co-inoculation with *Azospirillum brasilense* in contributing to biological nitrogen fixation. They found that seed application of Co + Mo associated with seed inoculation with *A. brasilense* promoted the highest leaf

N content, hundred-grain weight, yield, and profitability in the soybean crop, and proved to be important management techniques for crop production. However, there have been few studies assessing Mo fertilization and inoculation with *Bradyrhizobium* spp. strains on peanut yield components when peanut is grown in succession to pasture cultivation in tropical regions. These crop systems can promote soil nitrogen accumulation (Oliveira et al., 2011), which favors the pasture ecosystem (Boddey et al., 1997) via biological fixation of atmospheric nitrogen (N_2) (Oliveira et al., 2011).

It is important to note that several environmental factors can positively or negatively influence the uptake and availability of nutrients. These factors are related to soil properties such as moisture, aeration, organic matter, pH, temperature, presence of other elements, competitive inhibition, synergism, presence of roots, exudation, enzymes, and microorganisms, as well as factors related to plant genetic potential, internal ionic state, carbohydrate level, intensity transpiration, and root morphology (Malavolta et al., 1997; Epstein and Bloom, 2004), making nutritional evaluation in crop systems very complex. In this study, we have demonstrated positive effects of *Bradyrhizobium* inoculation, Mo fertilization, and their interaction on peanut kernel yield. Under these conditions, *Bradyrhizobium* inoculant and at least 50 g ha⁻¹ of Mo must be applied to allow maximum biological N_2 fixation, and consequently, to obtain the most economical return on investment in a cropping endeavor following pasture. Thus, evaluation of these properties in this configuration and arrangement of crop systems is very important to clarify the benefits provided to the soil for continuous improvement of crop systems in tropical regions.

CONCLUSIONS

The use of inoculum combined with molybdenum (Mo) fertilizer on the peanut crop showed great potential for enhancing nodulation and production of this legume. Nitrogenase enzyme activity was greatest at 64 DAE when inoculated and fertilized with a high Mo content. The number of pods per plant was the yield component that most directly influenced pod and kernel yield in the treatments with inoculation. In agricultural areas under *Urochloa brizantha* pastures for several years, *Bradyrhizobium* inoculation and Mo fertilization with at least 50 g ha⁻¹ can increase the yield of peanut pods and kernels in a no-tillage system with a large quantity of surface straw.

ACKNOWLEDGMENTS

The authors would like to thank the Office of Improvement of Higher Education Personnel (CAPES) for the grant to the first author and the National Council for Scientific and Technological Development (CNPq) for a fellowship for excellence in research to the second author.

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