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Liming, fertilization, and rhizobia inoculation on cowpea yield in a Brazilian Amazon upland forest environment

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Abstract – The objective of this work was to evaluate the response of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar to liming and to phosphorus and potassium fertilization in the absence of inoculation or nitrogen fertilization, as well as to inoculation with selected *Bradyrhizobium* strains, in an upland forest area in the state of Amapá, eastern Amazon, Brazil. Seven experiments were conducted in field conditions, in order to evaluate six limestone, four phosphorus, and four potassium rates, besides the inoculation with four *Bradyrhizobium* strains. Leaf nitrogen concentrations, pod and grain dry mass per plant, grain yield, and soil pH, Al^{+3}, and Ca^{+2} + Mg^{+2} were evaluated. The obtained data were subjected to the analysis of variance, to the regression analysis, or to test to compare means. The soil presented a high buffer power, but liming reduced its chemical limitations. Leaf nitrogen concentrations were correlated to cowpea grain yield. However, there is no effect of the interaction between potassium and phosphorus rates on grain yield. In addition, grain yield is not influenced by liming and potassium fertilization, but is increased by phosphorus fertilization. Inoculation with the UFLA 3-84 and INPA 03-11B strains promotes a greater grain yield than that with BR 3262 and BR 3267, as well as a similar grain yield to that of the control without inoculation.

Index terms: *Bradyrhizobium*, *Vigna unguiculata*, nitrogen, phosphorus, potassium.

Calagem, adubação e inoculação com rizóbio sobre a produtividade de feijão-caupi em ambiente de floresta de terra firme na Amazônia brasileira

Resumo – O objetivo deste trabalho foi avaliar a resposta da cultivar BRS Tumucumaque de feijão-caupi (*Vigna unguiculata*) à calagem e à adubação com fósforo e potássio na ausência de inoculação ou adubação nitrogenada, bem como à inoculação com estirpes selecionadas de *Bradyrhizobium*, em área de floresta de terra firme no estado do Amapá, na Amazônia Oriental, Brasil. Foram conduzidos sete experimentos em condição de campo, para avaliar seis níveis de calcário, quatro de fósforo e quatro de potássio, além da inoculação com quatro estirpes de *Bradyrhizobium*. Foram avaliados teores foliares de nitrogênio, massa seca de vagens e de grãos por planta, produtividade de grãos, e pH, Al^{+3} e Ca^{+2} + Mg^{+2} no solo. Os dados obtidos foram submetidos à análise de variância, à análise de regressão ou a teste para comparação de médias. O solo apresentou alto poder tampão, mas a calagem reduziu as suas limitações químicas. Os teores foliares de nitrogênio se correlacionaram com a produtividade de grãos de feijão-caupi. No entanto, não há efeito da interação entre os níveis de potássio e fósforo sobre a produtividade de grãos. Além disso, a produtividade de grãos não é influenciada pela calagem e pela adubação com potássio, mas aumenta com a adubação com fósforo. A inoculação com as estirpes UFLA 3-84 e INPA 03-11B promove maior rendimento de grãos do que a com as BR 3262 e BR 3267, bem como produtividade de grãos semelhante à do controle sem inoculação.

Termos para indexação: *Bradyrhizobium*, *Vigna unguiculata*, nitrogênio, fósforo, potássio.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] cultivation is historically associated with smallholders (family farmers) in Brazil, receiving a low investment, but providing an important source of protein and income (Freire Filho et al., 2005). The crop accounts for virtually the entire pulse production in the state of Amapá, since the cultivation of common bean (*Phaseolus vulgaris* L.) is limited by phytosanitary issues (Costa-Coelho et al., 2012). However, in the state, located in the eastern Amazon, there is a predominance of heavy rainfall and high temperatures, as well as acidic and weathered soils with low nutrient levels (Melem Junior et al., 2008).

To increase yield under different edaphoclimatic conditions, improvements have been made, although not yet widely adopted in Brazil, using: efficient rhizobia strains for inoculation (Soares et al., 2006; Leite et al., 2018; Ferreira et al., 2019), cowpea cultivars with superior performance (Bastos et al., 2011; Santana et al., 2019), inputs to correct soil acidity and supply nutrients (Farias et al., 2016; Manzeke et al., 2017), and cropping practices. There is also still a need for scientifically-based technical information for the recommendation of inoculants, liming, and fertilizers for soils from the state of Amapá, which commonly follow the recommendations for soils from other states. These limitations make the differences between the real average yield (obtained by farmers) and potential yield (productivity in experimental conditions) of cowpea genotypes high.

The difference between actual and potential cowpea yield can be reduced by the optimization of an inexpensive nitrogen source: biological nitrogen fixation (BNF). However, although cowpea forms nodules with several rhizobia species, the presence of a high and diverse rhizobia population in the soil hampers the introduction of efficient strains. The rhizobia population is particularly large in regions with a high precipitation (Thies et al., 1995) and in soils with high organic matter contents, such as those in areas that have been forested and with successive cropping with inoculant application (Zilli et al., 2013).

Rhizobia symbiosis may be improved by liming, which reduces soil chemical limitations, causing a systemic effect and resulting in an increase in cowpea root nodulation (Farias et al., 2016). Several authors, for example, have reported an increase in the grain yield of cowpea with phosphorus application (Silva et al., 2010; Melo et al., 2018). In addition, physiological feedback mechanisms in phosphorus, nitrogen, and carbon metabolisms, as well as phenological alterations in the plant, may also affect the contribution of BNF, and practices that raise BNF rates at the start of the crop cycle and/or keep them high for longer periods during the cycle can assure high leaf nitrogen concentrations, increasing BNF contribution to cowpea biomass production and grain yield (Silva Júnior et al., 2018).

The objective of this work was to evaluate the response of the BRS Tumucumaque cowpea cultivar to liming and to phosphorus and potassium fertilization in the absence of inoculation or nitrogen fertilization, as well as to inoculation with selected *Bradyrhizobium* strains, in an upland forest area in the state of Amapá, eastern Amazon, Brazil.

Materials and Methods

Seven experiments were carried out to evaluate the effects of liming, fertilization, and rhizobia inoculation on soil and cowpea yield, in 2012, 2013, and 2014, in an upland forest environment, in the experimental area of Embrapa Amapá, located in the municipality of Mazagão, in the state of Amapá, Brazil (0º07'19"S, 51º17'57"W, at 7 m altitude). According to the Köppen-Geiger classification, the climate is of the Am type, with an average annual temperature of 27.3ºC and an average annual rainfall of 2,410 mm. The soil in the experimental area is a Latossolo Amarelo, according to the Brazilian soil classification system (Santos et al., 2018), i.e., an Oxisol. The physicochemical properties of the soil are shown in Table 1.

To assess the effects of liming on the soil and cowpea yield, three field experiments (sample 1) were carried out in 2012, 2013, and 2014. The soil of the experimental area had been previously left fallow (Table 1). The treatments consisted of the following six limestone rates, applied 60 days before sowing (DBS) in 2012: 0 kg ha^{-1}, corresponding to the original soil base saturation (V) of 13%; 280 kg ha^{-1}, V of 25%; 2,020 kg ha^{-1}, V of 50%; 3,760 kg ha^{-1}, V of 75%; 5,490 kg ha^{-1}, V of 100%; and 7,230 kg ha^{-1}, V of 125%. Filler limestone with 91% total relative neutralizing power was used. The experimental plots received: 120 kg ha^{-1} P_2O_5 as simple superphosphate (18% P_2O_5) and 25 kg ha^{-1} FTE BR12 micronutrient fertilizer at sowing; and 80 kg ha^{-1} K_2O as potassium chloride (KCl, 60% K_2O), applied 50% at sowing and 50% as topdressing 25 days after sowing (DAS). The experimental design was of randomized complete blocks, with four blocks. The experimental plots measured 45 m^2, consisting of nine rows with 0.5 m spacing between rows and five seeds per linear meter of the BRS Tumucumaque cultivar, totaling 100,000 plants per hectare.

To evaluate the effects of phosphorus and potassium fertilization on the soil and cowpea yield, three field experiments were also carried out in 2012, 2013, and 2014, in an upland forest environment soil (sample 2) in an area that had previously left fallow (Table 1). In 2012, at 60 DBS, the soil received 2,700 kg ha^{-1} limestone to increase base saturation. The treatments consisted of: four rates of P_2O_5 (0, 40, 80, and 120 kg ha^{-1} P_2O_5 as triple superphosphate) applied at sowing; and four rates of K_2O (0, 35, 70, and 105 kg ha^{-1} K_2O as potassium chloride), 35 kg ha^{-1} applied at sowing and 70 and 105 kg ha^{-1} as topdressing 25 DAS. Each plot received 25 kg ha^{-1} FTE BR12 micronutrient fertilizer at sowing. The experiment had a 4×4 factorial arrangement in a randomized complete block design, with three blocks. The experimental plots measured 15.75 m^2, consisting of six rows with 0.5 m spacing between rows and five seeds per linear meter, also of the BRS Tumucumaque cultivar, totaling 100,000 plants per hectare.

To assess the effects of inoculation with the strains recommended by Ministério da Agricultura, Pecuária e Abastecimento for cowpea under the edaphoclimatic conditions of the state of Amapá, a field experiment was carried out in 2013, in a soil of an upland forest environment (sample 3) (Table 1). The area had been frequently used for cowpea cultivar selection. At 60 DBS, the soil received 2,700 kg ha^{-1} limestone to increase base saturation. The treatments, carried out in a randomized complete block design with three blocks, consisted of: inoculation with the UFLA 3-84, BR 3267, INPA 03-11B, and BR 3262 *Bradyrhizobium* strains; and a control without inoculation and without nitrogen application. The used strains were obtained from Embrapa Agrobiologia, and a peat-based inoculant was added at a rate of 250 g per 35 kg seed with sucrose solution (10% w/v) to increase adherence. The experimental plots measured 15 m^2, composed of six rows with 0.5 m spacing between rows and five seeds per linear meter of the BRS Tumucumaque cultivar. Each plot received 80 kg ha^{-1} P_2O_5 as triple superphosphate, 25 kg ha^{-1} FTE BR 12 at sowing, and 70 kg ha^{-1} K_2O as potassium chloride – 50% at sowing and 50% as topdressing at 25 DAS.

The following parameters, measured in response to limestone rates, were evaluated: grain yield at 60 DAS; leaf nitrogen concentrations at 30 DAS; and pH, Al^{+3}, and Ca^{+2} + Mg^{+2} from compound samples collected after pod harvesting (ten single samples were used to obtain compound samples from each experimental plot). The parameters assessed in response to phosphorus and potassium rates were grain yield and leaf nitrogen concentrations at 30 DAS, and in response to inoculation, pod and grain dry mass per plant, as well as grain yield. The data were subjected to

Table 1. Physicochemical properties in the 0–20 cm layer of a Latossolo Amarelo (Oxisol) of the experimental area, located in the municipality of Mazagão, in the state of Amapá, Brazil[(1)].

Soil sample	pH in water (1:2.5)	OM (g kg^{-1})	P (mg dm^{-3})	K^+	Ca^{2+} + Mg^{2+} (cmol$_c$ dm^{-3})	Al^{3+}	SB	Texture	Sand (g kg^{-1})	Silt	Clay
1	5.0	13.62	4.0	7.8	0.7	1.2	0.7	Clay loam	438	266	296
2	4.9	19.70	2.0	23.0	1.2	1.1	1.3	Loam	441	318	241
3	4.8	22.93	45.0	27.3	0.6	1.6	0.7	Sandy Clay	468	146	386

[(1)]OM, organic matter; and SB, sum of bases.

the analysis of variance, regression analysis, or Scott-Knott's test to compare means at 5% probability.

The experiments were registered in Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado, Brazil's system for management of genetic heritage and associated traditional knowledge, managed by Ministério do Meio Ambiente, under number A0627F8, as required by Law 13,123/2015 (Brasil, 2015).

Results and Discussion

Limestone application did not significantly affect cowpea grain yield, except in 2014, the third year of cultivation, when there was a slight decline in productivity with increasing rates (Table 2). Therefore, cowpea responds weakly to liming. In two field experiments carried out in the state of Pará in areas with a Latossolo Amarelo (Oxisol), a response to limestone application was only observed during the third and fourth years in the municipality of Terra Alta, with a soil with 120 g kg^{-1} clay, and from the third to sixth years in the municipality of Tracuateua, with a soil with 90 g kg^{-1} clay (Cravo et al., 2012). In these studies, maximum grain yield was obtained with the application of low limestone rates of approximately 1,000 kg ha^{-1} (Cravo et al., 2012). It should be noted that liming has a systemic effect that results in an increase in cowpea root nodulation, as previously mentioned. The BRS Guariba cultivar grown in an Oxisol had a higher yield of 201 kg ha^{-1} when receiving liming and rhizobia inoculation (1,160 kg ha^{-1}), instead of the treatment without liming but with inoculation (959 kg ha^{-1}) (Farias et al., 2016).

Table 2. Grain yield of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar grown in a Latossolo Amarelo (Oxisol), in a upland forest area, in 2012, 2013, and 2014, in function of limestone rates applied to the soil, in the municipality of Mazagão, in the state of Amapá, Brazil[(1)].

Limestone rate (kg ha^{-1})	Grain yield (kg ha^{-1})		
	2012	2013	2014
0	496	1,288	1,141
280	491	1,322	1,571
2,020	503	1,306	1,231
3,760	574	1,556	1,278
5,490	376	1,272	1,238
7,230	444	1,271	1,102
Mean	480	1,336	1,260
Regression	ns	ns	y = 1,347.3 - 0.0279x
R^2			23.73
CV (%)	36.54	14.71	14.33

[(1)]R^2, coefficient of determination; and CV, coefficient of variation. [ns]Nonsignificant.

Limestone application statistically attenuated soil limitations related to parameters pH, Al^{+3}, and Ca^{+2} + Mg^{+2} in the first two years of cultivation (Table 3). However, the observed effects were limited. For example, the application of 5,490 kg ha^{-1} raised soil pH level from 4.48 to 4.88 (only by 0.40 unit), increased Ca^{+2} + Mg^{+2} concentration from 1.45 to 3.13 cmol$_c$ dm^{-3}, and reduced Al^{+3} from 1.5 to 0.68 (only by 0.82 cmol$_c$ dm^{-3}). Furthermore, the values observed between rates after the third harvest were no longer statistically different and were nearly similar to those measured at the start of the experiment. The obtained results are indicative of a high buffering power of the soil, characterized by a resistance to chemical alterations with limestone application, minimizing the residual effect.

No significant effects were related to potassium rates or to the interaction of phosphorus and potassium on grain yield; however, there was a significant effect of phosphorus rates alone during the three experimental years (Table 4). According to Oliveira et al. (2009), cowpea responded to the application of potassium rates in a Regolithic Neosol with loamy texture, resulting in a grain yield of 1,890 kg ha^{-1} with a rate of 170 kg ha^{-1} K_2O. In the present study, grain yield varied from 373 to 984 kg ha^{-1}, and the highest increments were obtained between the rates of 0–40 and 40–80 kg ha^{-1} P_2O_5, when compared with that of 80–120 kg ha^{-1} (Table 4). The grain yield of the BRS Guariba and BRS Aracê cowpea cultivars increased with the rates of phosphorus, up to 120 kg ha^{-1}, and of zinc, up to 3.1 kg ha^{-1}, in an Oxisol in the state of Maranhão (Melo et al., 2018). In another Oxisol in the state of Roraima, cowpea yield and leaf phosphorus concentrations increased in response to the applied phosphorus rate until 90 kg ha^{-1} P_2O_5 (Silva et al., 2010). Phosphorus and nitrogen are the nutrients whose shortage most limits grain yield in tropical environments, and the response to phosphorus fertilization of legume species that are dependent on BNF is high given the energy demand of the fixation process.

Limestone rates did not affect nitrogen leaf concentrations (Table 5). However, phosphorus and potassium rates caused a reduction in these

Table 3. Chemical properties of a Latossolo Amarelo (Oxisol) after the cultivation of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar in an upland forest area, in 2012, 2013, and 2014, in function of limestone rates applied to the soil, in the municipality of Mazagão, in the state of Amapá, Brazil(1).

Limestone rate (kg ha^{-1})	2012	2013	2014
		pH in water (1:2.5)	
0	4.48	4.68	4.60
280	4.48	4.63	4.60
2,020	4.70	4.65	4.60
3,760	4.68	4.70	4.63
5,490	4.88	4.85	4.68
7,230	4.80	4.85	4.75
Regression	y = 4.506 + 0.00005x	y = 4.6267 + 0.00003x	ns
R^2	81.37	82.67	
CV (%)	2.57	2.16	2.47
		Al^{+3} (cmol$_c$ dm^{-3})	
0	1.50	1.05	1.60
280	1.43	1.43	1.68
2,020	0.93	1.15	1.58
3,760	1.30	1.18	1.68
5,490	0.68	0.95	1.55
7,230	0.93	0.84	1.43
Regression	y = 1.3921 - 0.00009x	y = 1.2628 - 0.00005x	ns
R^2	56.17	56.01	
CV (%)	23.76	17.54	15.36
		Ca^{+2} + Mg^{+2} (cmol$_c$ dm^{-3})	
0	1.45	0.80	0.80
280	2.10	0.63	0.70
2,020	2.10	1.03	0.83
3,760	2.05	0.98	0.75
5,490	3.13	1.53	0.88
7,230	3.10	1.60	1.00
Regression	y = 1.6847 + 0.0002x	y = 0.6923 + 0.0001x	ns
R^2	79.35	89.00	
CV (%)	30.34	31.12	20.28

(1)R^2, coefficient of determination; and CV, coefficient of variation. nsNonsignificant.

Table 4. Grain yield of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar grown in a Latossolo Amarelo (Oxisol), in a upland forest area, in 2012, 2013, and 2014, in function of P_2O_5 rates, in the municipality of Mazagão, in the state of Amapá, Brazil(1).

P_2O_5 rate (kg ha^{-1} per year)	Grain yield (kg ha^{-1})		
	2012	2013	2014
0	373	360	695
40	558	739	780
80	943	812	984
120	973	805	978
Mean	712	679	859
Regression	y = 384.06 + 5.4609x	y = 467.92 + 3.5195x	y = 701.07 + 2.6349x
R^2	91.8	71.22	87.98
CV (%)	27.36	25.08	24.42

(1)R^2, coefficient of determination; and CV, coefficient of variation.

Table 5. Nitrogen concentration in leaves of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar grown in a Latossolo Amarelo (Oxisol), in a upland forest area, in 2012 and 2013, in function of liming, phosphorus, and potassium fertilization, in the municipality of Mazagão, in the state of Amapá, Brazil(1).

Limestone rate (kg ha^{-1})	N in leaves (mg g^{-1})	
	2012	2013
0	45.0	48.0
280	46.2	43.3
2,020	48.2	41.2
3,760	47.2	42.5
5,490	44.1	42.8
7,230	44.9	41.9
Regression	ns	ns
CV (%)	8.11	6.6
P_2O_5 rate (kg ha^{-1} per year)		
0	38.3	43.1
40	36.2	39.4
80	34.7	40.6
120	34.4	42.2
Regression	y = 37.90 - 0.0033x	ns
R^2	90	
CV (%)	9.81	9.35
K_2O rate (kg ha^{-1} per year)		
0	36.6	42.6
35	36.3	42.2
70	34.9	42.1
105	35.8	38.4
Regression	ns	y = 43.26 - 0.0037x
R^2		70.41
CV (%)	9.81	9.35

(1)R^2, coefficient of determination; and CV, coefficient of variation. nsNonsignificant

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concentrations, with a significant effect in the first and second years, respectively. These results indicate the occurrence of a nitrogen dilution effect (Jarrell & Beverly, 1981) in cowpea as a function of the increased productivity promoted by phosphorus rates. Moreover, since leaf nitrogen concentration at 30 DAS was closely related to cowpea grain yield, leaf nitrogen could be considered as an indicator for the selection of cowpea genotypes responsive to BNF (Alcantara et al., 2014; Burridge et al., 2016).

Inoculation with the BR 3262, BR 3267, UFLA 3-84, and INPA 03-11B strains did not affect pod and grain dry mass per plant of the BRS Tumucumaque cowpea cultivar, compared with the control without inoculation (Table 6). Inoculation with UFLA 3-84 and INPA 03-11B promoted a greater grain yield than that with BR 3262 and BR 3267, besides a similar grain yield to that of the control without inoculation or nitrogen application. In two experiments carried out in the pre-Amazon region in the state of Maranhão, inoculation with the BR 3262, INPA 03-11B, and BR 3299 strains promoted a greater shoot dry mass accumulation and cowpea grain yield, in comparison with that of the control without inoculation, and BR 3299 showed the best result (Gualter et al., 2011).

Table 6. Pod and grain dry biomass and grain yield of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar grown in a Latossolo Amarelo (Oxisol), in a upland forest area, in 2013, in function of the inoculation with the INPA 03-11B, UFLA 3-84, BR 3262, and BR 3267 *Bradyrhizobium* strains, in the municipality of Mazagão, in the state of Amapá, Brazil[(1)].

Bradyrhizobium strain	Pod biomass	Grain biomass	Grain yield ($kg\ ha^{-1}$)
	----------(g per plant)----------		
INPA 03-11B	35.34	28.21	1,460a
UFLA 3-84	38.45	30.25	1,243a
BR 3262	43.88	34.47	996b
BR 3267	38.54	30.26	991b
Control	34.72	27.76	1,357a
CV (%)	24.96	25.22	13.85
	ns	ns	

[(1)]Means followed by equal letters, in the column, do not differ by Scott-Knott's test, at 5% probability. Control, treatment without inoculation or nitrogen application; and CV, coefficient of variation. [ns]Nonsignificant.

Conclusions

1. Liming reduces soil chemical limitations but does not influence the grain yield of the BRS Tumucumaque cowpea (*Vigna unguiculata*) cultivar grown in an upland forest environment in the Brazilian eastern Amazon region.
2. Fertilization with phosphorus increases the grain yield of the BRS Tumucumaque cowpea cultivar, but fertilization with potassium has no effect.
3. Inoculation with the UFLA 3-84 and INPA 03-11B *Bradyrhizobium* strains promotes a greater grain yield for the BRS Tumucumaque cowpea cultivar than inoculation with the BR 3262 and BR 3267 strains, as well as a similar grain yield to that obtained with the control without inoculation or nitrogen application.

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ERRATA: Liming, fertilization, and rhizobia inoculation on cowpea yield in a Brazilian Amazon upland forest environment

In the paper "Liming, fertilization, and rhizobia inoculation on cowpea yield in a Brazilian Amazon upland forest environment", DOI: 10.1590/S1678-3921.pab2021.v56.02191, published in Pesquisa Agropecuária Brasileira, v.56, e02191, 2021, on page 1, left column, line 1, in the address note of Wardsson Lustrino Borges, where it reads:

"[(1)]",

it should read:

"[(1, 2)]".