



Revista Brasileira de Ciência do Solo

ISSN: 0100-0683

revista@sbcs.org.br

Sociedade Brasileira de Ciência do Solo  
Brasil

Rufini, Márcia; Pádua Oliveira, Dâmianny; Trochmann, André; Lima Soares, Bruno; Bastos de Andrade, Messias José; de Souza Moreira, Fatima Maria  
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Greenhouse and Field Conditions  
Revista Brasileira de Ciência do Solo, vol. 40, 2016, pp. 1-14  
Sociedade Brasileira de Ciência do Solo  
Viçosa, Brasil

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# ***Bradyrhizobium* spp. Strains in Symbiosis with Pigeon Pea cv. Fava-Larga under Greenhouse and Field Conditions**

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**Received:** April 1<sup>st</sup>, 2016

**Approved:** June 4, 2016

**How to cite:** Rufini M, Oliveira DP, Trochmann A, Soares BL, Andrade MJB, Moreira FMS. *Bradyrhizobium* spp. Strains in Symbiosis with Pigeon Pea cv. Fava-Larga under Greenhouse and Field Conditions. Rev Bras Cienc Solo. 2016;40:e0160156.

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**ABSTRACT:** Optimization of symbiosis between nitrogen-fixing bacteria and legumes has been extensively studied, seeking agricultural sustainability. To evaluate the symbiotic efficiency of nitrogen-fixing bacterial strains belonging to the *Bradyrhizobium* genus with pigeon pea (*Cajanus cajan* (L.) Millsp.) cv. *Fava-Larga*, experiments were conducted in Leonard jars (axenic conditions), pots with soil, and in the field. Ten strains were tested in Leonard jars, and three strains, in addition to BR 29, were selected according to their ability to promote the growth of pigeon pea, for further tests in pots with different soil types (Inceptisol and Oxisol) and in the field (Oxisol). Treatments were compared with strains BR 2003 and BR 2801 (approved as inoculants for pigeon pea), with a non-inoculated control with mineral N fertilization, and with another non-inoculated control (absolute control) with low mineral N concentration (Leonard jars) or without mineral N fertilization (soil). The efficiency of *Bradyrhizobium* strains in axenic conditions varies among strains, being higher when pigeon pea cv. *Fava-Larga* establishes symbiosis with the strains UFLA 03-320, UFLA 03-321, UFLA 04-212, BR 2801, and BR 2003. The soil type influences the symbiotic efficiency of *Bradyrhizobium*-pigeon pea in soil in the greenhouse, mainly in Inceptisol, in which strains UFLA 04-212, BR 2801, and BR 2003 increased N accumulation in the plant. The strain UFLA 03-320 increased shoot dry matter and N accumulation in the shoot equivalent to the mineral N treatment under field conditions. UFLA 03-320, BR 29, UFLA 03-321, and UFLA 04-212 promoted yields similar to those of the reference strain (BR 2801), and of the mineral N treatment with 70 kg ha<sup>-1</sup> urea-N. These results confirm that pigeon pea establishes efficient symbiosis, which provides the N required for its growth. All strains, except for BR 2003, show potential for recommendation as inoculants for grain production. The strain UFLA 03-320 also shows potential for use in green manure crops.

**Keywords:** *Cajanus cajan*, inoculant, biological nitrogen fixation, selection.

## INTRODUCTION

Nitrogen (N) is one of the most important nutrients for plants, and also one of the most limiting for agricultural production in the tropics. The main N sources for plants are organic matter of the soil, N fertilizers, and biological nitrogen fixation (BNF). Nitrogen fertilizers, in addition to their high cost and contribution to environmental pollution, have low assimilation efficiency (maximum of 50 %) due to losses caused by inadequate crop practices and to processes such as leaching, denitrification, and  $\text{NH}_3$  volatilization (Cantarella, 2007).

Biological nitrogen fixation is a process of great environmental and economic importance, performed by bacteria that are able to reduce atmospheric nitrogen ( $\text{N}_2$ ) to ammonia ( $\text{NH}_3$ ), and when in symbiosis with plants, they provide  $\text{NH}_3$ , which is readily converted to other forms, such as amides and ureides. This is an alternative for increasing yield in legumes or for use in integrated farming systems since it reduces or eliminates the costs and impacts of N fertilizers and thus contributes to the sustainability of agricultural systems and to conservation of natural resources (Moreira and Siqueira, 2006).

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is a Fabaceae that benefits from BNF, obtaining most of the N required for its development from this process. This was confirmed by experiments in axenic conditions (Fernandes and Fernandes, 2000; Fernandes et al., 2003; Martins et al., 2012), in pots with soil (La Favre and Focht, 1983; Valarini and Godoy, 1994; Sanginga et al., 1996; Paz et al., 2000), or under field conditions (Espanã et al., 2006; Ahmed et al., 2014; Rufini et al., 2014b). This crop can be used for several purposes, such as for green manure (Heinrichs et al., 2005), animal feed, and human consumption (Mizubuti et al., 1995; Canniatti-Brazaca et al., 1996); for phytoremediation (Pires et al., 2006); and for recovery of degraded areas (Beltrame and Rodrigues, 2007). In Brazil, pigeon pea has no economic importance, and it lacks attention from the field of agricultural research. However, it is an important food due to its high protein value (20-25 %) (Paz et al., 2000). Moreover, it can be grown in depleted soils, and can serve as a means of soil fertilization for subsequent crops by the BNF process (Osman et al., 2011).

Symbiosis between pigeon pea and  $\text{N}_2$ -fixing nodulating bacteria (NFNB) can provide approximately 90 % of the N required for plant development (La Favre and Focht, 1983). Sanginga et al. (1996) found that approximately 77 % of the N in the plant was derived from BNF, which was close to the 79 % value found by Espanã et al. (2006). However, several factors related to the environment and to the symbionts can interfere in successful symbiosis, causing lack of response to inoculation. Among them, native rhizobia populations, physical and chemical properties of the soil, the planting season, and the cultivar may interfere with plant response to BNF (Herridge and Holland, 1993; Sanginga et al., 1996; Mapfumo et al., 2000; Bidlack et al., 2001; Freitas et al., 2003; Lombardi et al., 2009). When evaluating the effectiveness of *Bradyrhizobium yuanmingense* strains in different sites in the Dominican Republic, Araujo et al. (2015) observed significant interaction between the sites where the experiments were carried out and the treatments.

Although pigeon pea is a promiscuous legume, results show that *Bradyrhizobium* strains are more efficient for  $\text{N}_2$  fixation in pigeon pea than *Rhizobium* strains (Anand and Dogra, 1997). Both NFNB strains (Brasil, 2011a), approved as inoculants by the Ministry of Agriculture (*Ministério da Agricultura, Pecuária e Abastecimento* - MAPA), belong to the *Bradyrhizobium* genus: strains BR 2003 (SEMIA 6156) and BR 2801 (SEMIA 6157). However, published data that generated this recommendation have not been found, and consequently the criteria used for recommending them are not known.

Given the few studies on this symbiosis in Brazil, especially under field conditions, and for the reasons mentioned above, it is of great interest and relevance to analyze pigeon pea symbiosis with NFNB under different conditions, in order to develop appropriate inoculants. Thus, selection of NFNB strains in regard to efficiency and competitiveness in BNF under specific soil and climatic conditions is still an important step in increasing crop yield with fewer inputs.

The hypothesis of this study is that some strains tested are effective in axenic conditions and in the field, and that symbiotic efficiency may vary according to soil type. Thus, the objective of this study was to evaluate the symbiotic efficiency of NFNB strains of the *Bradyrhizobium* genus, which are efficient in  $N_2$  fixation in symbiosis with legume species, such as pigeon pea cv. IAC *Fava-Larga*, under different conditions, aiming at maximizing the contribution of symbiosis to this species.

## MATERIALS AND METHODS

Three experiments were carried out to evaluate the symbiotic efficiency of *Bradyrhizobium* strains with pigeon pea (*Cajanus cajan* (L.) Millsp.): one in the axenic conditions of Leonard jars (Vincent, 1970), one in pots with soil, and one under field conditions. The pigeon pea cultivar used in the experiments was IAC *Fava-Larga*, which has upright architecture, determined growth habit, and a long cycle, and it is commonly used for green manure, windbreaks, or a temporary shading plant for perennial crops, in addition to its use as animal feed (pasture in the winter to provide forage or hay and grain) and human food (ingested as green or dried grains) (Wutke, 1987; IAC, 2013).

For inoculum preparation, bacteria were cultivated in liquid culture medium 79 (Fred and Waksman, 1928) and shaken at 110 rpm at 28 °C for about 120 h. Then, in each treatment with inoculation in Leonard jars and in pots with soil, 1 mL per seed of the inoculant was added at a concentration of  $10^8$  cells NFNB mL<sup>-1</sup>. The inoculant to be used in the field was prepared with peat (sterilized in an autoclave) mixed at a ratio of 3:2 (w:v) of peat and log phase cultures in liquid medium 79.

At the vegetative stage, in periods pre-determined for each experiment, the following parameters were evaluated: plant height, number of nodules (NN), nodule dry matter (NDM), shoot fresh matter (SFM), root dry matter (RDM), shoot dry matter (SDM), nitrogen (N) content in the shoot (NCS), and N accumulation in the shoot (NAS). Specifically in the field experiment, the determination of N accumulation in the shoot occurred per plant (NASpl) and per hectare (NASha). At the maturity stage, the following determinations were made: grain yield and its main components [pods per plant (PP), grains per pod (GP), and 100-grain weight (W100)], and N content (NCG) and N accumulation (NAG) in the grain.

Plant height was recorded as the distance from the base to the apical meristem. Shoot fresh matter was obtained immediately after collection. The nodules collected and the plant and root materials were allowed to dry in a forced air circulation oven until constant weight. After drying, they were weighed on a precision scale.

Nitrogen content in the shoot was determined by the semi-micro Kjeldhal method (total N), according to Sarruge and Haag (1979). Nitrogen accumulation in the shoot was calculated by multiplying the SDM by NCS and dividing the product by 100. Nitrogen content in the grain and NAG were determined by adopting the same method used for the shoot samples, substituting the values of the SDM by the values of grain yield. To calculate the NASha, the average stand of the trial (53,000 plants ha<sup>-1</sup>) was used. Grain yield was corrected to 130 g kg<sup>-1</sup> moisture.

### Evaluation of symbiotic efficiency in Leonard jars

The experiment was carried out in Leonard jars in a greenhouse in the period from August to October 2012. The experimental design was completely randomized with three replications and 12 treatments with *Bradyrhizobium* spp. strains, plus two uninoculated controls [one with high mineral nitrogen concentration (+N) and another with low mineral nitrogen concentration (-N)].

Strains were selected based on the efficiency of  $N_2$  fixation in symbiosis with other legume species, such as siratro (strain UFLA 04-212) and cowpea (strains UFLA 03-153,

UFLA 03-164, UFLA 03-320, UFLA 03-321, and UFLA 03-325), as described in the studies mentioned in table 1. *Bradyrhizobium* strains approved as inoculants for soybean (strain BR 29/SEMIA 5019), for cowpea (strains UFLA 03-84/SEMIA 6461, INPA 03-11B/SEMIA 6462, and BR 3267/SEMIA 6463), and for pigeon pea (reference strains BR 2003/SEMIA6156 and BR 2801/SEMIA6157) were also tested.

Leonard jars contained a mixture of sand and vermiculite at a ratio of 1:1 at the top and a modified Hoagland and Arnon nutrient solution (Hoagland and Arnon, 1950) at the bottom. The complete solution with 52.5 mg L<sup>-1</sup> N in the forms of NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O

**Table 1.** Origin and characteristics of *Bradyrhizobium* strains tested in the experiments

Strain	Origin			Symbiotic efficiency <sup>(1)</sup>	Species	Source and reference <sup>(3)</sup>
	State/Region	LUS	Host Plant			
UFLA 03-153	Minas Gerais	Bauxite mining	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Soares et al. (2014); Guimarães et al. (2015)
UFLA 03-164	Minas Gerais	Bauxite mining	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Soares et al. (2014); Guimarães et al. (2015)
UFLA 03-320	Minas Gerais	Agriculture	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Rufini et al. (2014a); Guimarães et al. (2015)
UFLA 03-321	Minas Gerais	Agriculture	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Rufini et al. (2014a); Guimarães et al. (2015)
UFLA 03-325	Minas Gerais	Agriculture	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Rufini et al. (2014a); Guimarães et al. (2015)
UFLA 04-212	Amazônia	Agriculture	<i>Macroptilium atropurpureum</i>	<i>Macroptilium atropurpureum</i> <sup>(E)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Florentino et al. (2009); Guimarães et al. (2015)
INPA 03-11B	Amazônia	Forest	<i>Centrosema</i> sp.	<i>Vigna unguiculata</i> <sup>(I)</sup>	<i>B. elkanii</i>	SBMPBS/UFLA; Soares et al. (2006); Brasil (2011a); Guimarães et al. (2015)
UFLA 03-84	Rondônia	Pasture	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(I)</sup>	<i>Bradyrhizobium</i> sp. <sup>(2)</sup>	SBMPBS/UFLA; Soares et al. (2006); Brasil (2011a); Guimarães et al. (2015)
BR 3267	Semi-arid Northeast	-	<i>Vigna unguiculata</i>	<i>Vigna unguiculata</i> <sup>(I)</sup>	<i>Bradyrhizobium</i> sp.	Embrapa Agrobiologia; Martins et al. (2003); Brasil (2011a)
BR 29	Rio de Janeiro	-	<i>Glycine max</i>	<i>Glycine max</i> <sup>(I)</sup>	<i>B. elkanii</i>	Fepagro; Peres e Vidor (1980); Brasil (2011a); Menna et al. (2006)
BR 2801	Rio de Janeiro	-	<i>Crotalaria</i> spp	<i>Cajanus cajan</i> <sup>(I)</sup>	<i>Bradyrhizobium</i> sp.	Embrapa Agrobiologia; Brasil (2011a); Menna et al. (2006)
BR 2003	Brasília, DF	-	<i>Stylosantes</i> spp.	<i>Cajanus cajan</i> <sup>(I)</sup>	<i>B. elkanii</i>	Embrapa Cerrados; Brasil (2011a); Menna et al. (2006)

LUS: Land use system from which the strain was isolated. <sup>(1)</sup> E: effective (shoot dry matter of the treatment inoculated with the strain tested = non-inoculated control fertilized with mineral N); I: approved as inoculant for *Vigna unguiculata* UFLA 03-84, INPA 03-11B, and BR 3267), *Glycine max* (BR 29), and *Cajanus cajan* (BR 2801, BR 2003) by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA). <sup>(2)</sup> Strains at the stage of species identification. <sup>(3)</sup> SBMPBS/UFLA: Bacteria Collection of the Biology, Microbiology and Soil Biological Processes Department of the Federal University of Lavras; Fepagro: Fundação Estadual de Pesquisa Agropecuária.

was used in the +N treatment. In the other treatments, including the -N control, a solution with 5.25 mg L<sup>-1</sup> N in the forms of NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O was used. Leonard jars and nutrient solutions were autoclaved for 60 and 40 min, respectively, at a pressure of 1.5 kg cm<sup>-2</sup> at 127 °C before use.

Seed surfaces were disinfected with 92.8° ethanol (30 s) and 2 % sodium hypochlorite (2 min) and then washed six times with sterilized distilled water to remove residues from previous treatments. After these procedures, seeds were immersed in sterile distilled water for 2 h and germinated in Petri dishes containing moistened and sterilized filter paper and cotton at 28 °C in a growth chamber. Four pre-germinated seeds were sown per jar, and after emergence, they were thinned, leaving two plants per jar. Each seed was inoculated with 1 mL of the inoculant, containing approximately 10<sup>8</sup> cells NFNB mL<sup>-1</sup>. A thin layer of a sterile mixture of sand, chloroform, and paraffin was placed on the surface of the jar in order to avoid possible contaminations.

The level of nutrient solution in the jars was maintained, and was periodically replaced according to plant needs. Jars received nutrient solution with ¼ strength for 45 days, and from that period on, the strength of the nutrient solution was increased to ⅓.

At 60 days after emergence (DAE), at the basic vegetative stage reported by Carberry et al. (2001), determinations of NN, NDM, RDM, SDM, NCS, and NAS were carried out.

### Evaluation of symbiotic efficiency in pots with soil

The experiment in the pots with soil was carried out in a greenhouse in the period from January to May 2013 using the strains that had been most effective in promoting pigeon pea growth in the experiment with Leonard jars.

A randomized block experimental design was used, with four replications and an 8 × 2 factorial arrangement consisting of eight treatments and two soil types. The eight treatments were inoculation with the strains UFLA 03-320, UFLA 03-321, UFLA 04-212, and BR 29; inoculation with the strains approved as inoculant for pigeon pea (strains BR 2003 and BR 2801); and two uninoculated controls [one with mineral N (+N) and another without mineral N (-N)]. The soils used in the experiment were classified as Inceptisol and Oxisol [*Cambissolo* and *Latossolo Vermelho-Amarelo*, respectively, according to the Brazilian System of Soil Classification (Santos et al., 2013)], both clayey, collected in the municipalities of *Luminárias* and *Lavras*, Minas Gerais, respectively, at a depth of 0.00-0.20 m. Both had a history of maize cultivation and no record of inoculation (Table 2). The soil collected in *Lavras* was taken from the experimental area of the Federal University of Lavras, and consequently has a history of use of agricultural inputs. Before being used as substrates in 1.6 dm<sup>3</sup> pots, soils were air dried, homogenized, and sieved in a 4 mm mesh. The native rhizobium population in both experiments was 10<sup>3</sup> colony forming units (CFU) per g of soil. This most probable number (MPN) was determined according to the method described in Rufini et al. (2014a), using the Hoagland and Arnon nutrient solution (Hoagland and Arnon, 1950) and the dwarf pigeon pea cultivar *lapar 43* (Aratã) as a trap plant.

Fertilization of the pots was carried out according to Malavolta et al. (1989) with 300, 300, 40, 0.8, 1.5, 3.6, 5.0, and 0.15 mg dm<sup>-3</sup> of K, P, S, B, Cu, Mn, Zn, and Mo, respectively. The nitrogen control (+N) received 300 mg dm<sup>-3</sup> NH<sub>4</sub>NO<sub>3</sub>-N applied three times, 10 days after every emergence. Irrigation of the pots was carried out by keeping the moisture at approximately 60 % of field capacity.

Pigeon pea cv. IAC *Fava-Larga* seeds were disinfected, inoculated, and sown following the method described in the experiment with Leonard jars. After emergence, plants were thinned, leaving two seedlings per pot.

Plants were harvested at approximately 120 DAE for evaluation of height, NN, NDM, SDM, NCS, and NAS.

## Evaluation of efficiency in the field

The field experiment was carried out from November 2012 to July 2013 in an Oxisol in the municipality of Lavras, MG (Table 2), in an area previously planted to maize, with no previous record of inoculation of leguminous species. The experimental design was a randomized block with four replications and six treatments with *Bradyrhizobium* spp. (strains UFLA 03-320, UFLA 03-321, UFLA 04-212, BR 29, BR 2003, and BR 2801, plus two non-inoculated controls: one with mineral N (+N), and another without mineral N (-N).

The inoculant was applied at a rate of 250 g per 10 kg seed. Sowing was performed immediately after seed inoculation, at a density of 10 seeds per meter.

Each experimental unit (30 m<sup>2</sup>) consisted of six 5-m rows, spaced 1.0 m apart, and the useful area was the four central rows. A conventional cropping system was used. All plots received basic fertilization equivalent to 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (triple superphosphate) and 50 kg ha<sup>-1</sup> K<sub>2</sub>O (potassium chloride), mechanically applied during plowing. In addition to this fertilization, N controls received 70 kg ha<sup>-1</sup> urea-N, applied ½ at sowing and ½ in topdressing at 35 days after emergence. Weed control was carried out by hand hoeing, and there was no need for other phytosanitary treatments. In the experimental period, average temperature was 20.8 °C and accumulated rainfall was 1,021 mm.

At full flowering (165 days after planting), the stage in which at least 50 % of the plants exhibited open flowers (Carberry et al., 2001), five plants were randomly collected in each plot from the third and fourth rows for determination of height, SFM, SDM, NCS, NASpl, and NASHa. It was not possible to evaluate NN and NDM since the root system of this cultivar reached a depth of nearly 0.90 m. This situation did not allow removal of nodules, as in other crops of lower architecture, or even in other varieties of the same species (Rufini et al., 2014b).

**Table 2.** Chemical and physical properties of soil samples, taken at the 0.00-0.20 m depth layer, and geographic coordinates of the collection sites

Property	Inceptisol	Oxisol
pH(H <sub>2</sub> O) (1:2.5)	4.6	5.9
P (mg dm <sup>-3</sup> )	0.56	5.81
K (mg dm <sup>-3</sup> )	68	128
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.4	3.5
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.2	1.1
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.8	0.1
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> )	5.05	4.04
SB (cmol <sub>c</sub> dm <sup>-3</sup> )	0.77	4.93
T (cmol <sub>c</sub> dm <sup>-3</sup> )	5.82	8.97
t (cmol <sub>c</sub> dm <sup>-3</sup> )	1.57	5.03
m (%)	50.96	1.99
V (%)	13.31	54.94
OM (dag kg <sup>-1</sup> )	3.41	2.61
Sand (g kg <sup>-1</sup> )	400	590
Silt (g kg <sup>-1</sup> )	250	70
Clay (g kg <sup>-1</sup> )	350	340
Geographic coordinates	21° 32' S; 44° 57' W	21° 12' S; 44° 58' W

pH in water (v/v 1:2.5); P and K: extractor Mehlich-1; Ca, Mg, Al: extractor 1 mol L<sup>-1</sup> KCl; H+Al: potential acidity, extracted by calcium acetate 0.5 mol L<sup>-1</sup> at pH 7; SB: sum of bases; T: cation exchange capacity at pH 7; t: cation exchange capacity; m: aluminum saturation; V: base saturation; OM: organic matter, Walkey-Black method; sand, silt, and clay: pipette method.

Since the production period of the pigeon pea cultivar used in this study is long, it was carried out a single harvest, at 234 days after planting, when grain yield and its primary components (PP, GP, and W100) were evaluated, as well as NCG and NAG. Grain yield was obtained from the total weight of grains produced in rows 2 and 5 of the useful area of the plot.

### Statistical analysis

All data were subjected to analysis of variance using the Sisvar 4.0 software (Ferreira, 2011), after being subjected to normality (Shapiro-Wilks test) and variance (homoscedasticity) [Bartlett test] testing, using the R software (R Development Core Team, 2011). To meet the assumptions of analysis of variance, the data for the number of nodules in the pot with soil were previously transformed into  $(x+0.5)^{0.5}$ . For evaluations in the field, yield adjustment as a function of stand was carried out using analysis of covariance with correction for average stand, according to the method of Cruz and Carneiro (2003). The Genes software (Cruz, 2013) was used to obtain this adjustment.

In cases of significant effect of treatments and soils, the means were grouped by the Scott-Knott test at the 5 % probability level (in the three tests) and at the 10 % probability level (in the field test), according to the official protocol for assessment of viability and agronomic efficiency of the strains, inoculants, and technologies related to the BFN process in legumes [Instrução Normativa No. 13 (Brasil, 2011b)].

## RESULTS AND DISCUSSION

### Evaluation of symbiotic efficiency in Leonard jars

All the strains tested nodulated pigeon pea (Table 3). The strain UFLA 04-212, followed by the strains UFLA 03-320, UFLA 03-321, and UFLA 03-325 exhibited the highest ( $p < 0.05$ ) number of nodules (NN), surpassing the other strains, including the reference strains

**Table 3.** Plant height, number of nodules (NN), nodule dry matter (NDM), root dry matter (RDM), shoot dry matter (SDM), nitrogen content in the shoot (NCS), and nitrogen accumulation in the shoot (NAS) of pigeon pea cv. IAC Fava-Larga cultivated in axenic conditions (Leonard jars)

Treatment <sup>(1)</sup>	Plant height	NN	NDM	RDM	SDM	NCS	NAS
	cm		g per jar			%	mg per jar
BR 2003	48.33 a	99 c	0.257 a	1.68 a	4.51 a	3.12 a	140.30 a
BR 2801	45.58 a	89 c	0.254 a	1.53 a	4.40 a	3.21 a	141.83 a
BR 29	34.67 b	93 c	0.312 a	0.70 c	2.60 c	2.95 a	76.49 b
UFLA 03-84	40.83 a	106 c	0.245 a	0.98 b	3.27 b	3.47 a	114.54 a
INPA 03-11B	43.33 a	78 c	0.374 a	1.17 b	3.83 b	2.82 a	107.87 a
BR 3267	33.33 b	100 c	0.198 a	0.65 c	2.80 c	2.77 a	77.30 b
UFLA 03-153	38.58 a	106 c	0.279 a	0.92 c	3.40 b	3.51 a	120.66 a
UFLA 03-164	29.92 b	71 c	0.158 a	0.57 c	2.13 c	2.99 a	64.69 b
UFLA 03-320	47.33 a	159 b	0.271 a	1.23 b	4.28 a	3.25 a	140.79 a
UFLA 03-321	44.25 a	138 b	0.213 a	1.04 b	4.18 a	3.73 a	152.10 a
UFLA 03-325	42.42 a	156 b	0.184 a	0.80 c	3.44 b	3.34 a	115.32 a
UFLA 04-212	46.50 a	285 a	0.273 a	1.26 b	4.14 a	3.51 a	145.90 a
+N <sup>(2)</sup>	33.92 b	0 d	0 b	1.05 b	3.02 c	1.47 b	44.05 c
-N	22.50 b	0 d	0 b	0.29 c	0.74 d	1.86 b	13.48 c
p value	0.0002	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000
CV (%)	14.78	40.08	32.77	29.42	16.80	15.06	23.67

Means followed by the same letter in the column belong to the same group by the Scott-Knott test at 5 % probability. <sup>(1)</sup> Except for +N, all treatment, including the uninoculated control without nitrogen fertilization (-N) received 5.25 mg L<sup>-1</sup> N in the form of NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O. <sup>(2)</sup> +N: non-inoculated control fertilized with 52.5 mg L<sup>-1</sup> N in the form of NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O.

approved as inoculant for pigeon pea (BR 2003 and BR 2801). There was no presence of nodules in the controls (+N and -N), indicating no contamination of the experiment. In the analysis of nodule dry matter (NDM), there was no statistical difference among the inoculated treatments (Table 3).

The strains BR 2801 and BR 2003 provided higher ( $p < 0.05$ ) root dry matter (RDM) than the other strains and the control treatment that received high mineral N (+N) concentration.

In relation to shoot dry matter (SDM), the strains UFLA 03-320, UFLA 03-321, and UFLA 04-212 were similar to the reference stains (BR 2003 and BR 2801), surpassing ( $p < 0.05$ ) the other treatments. The second group was composed of the strains UFLA 03-84, INPA 03-11B, UFLA 03-153, and UFLA 03-325, which were also superior to the controls +N and -N and to the strains BR 29, BR 3267, and UFLA 03-164. The control -N had the lowest value for this parameter.

All the inoculated treatments were superior to the controls in relation to N content in the shoot (NCS). For N accumulation in the shoot (NAS) and plant height, the strains UFLA 03-84, INPA 03-11B, UFLA 03-153, UFLA 03-320, UFLA 03-321, UFLA 03-325, and UFLA 04-212, along with the reference strains (BR 2801 and BR 2003) exhibited the highest values, surpassing the other treatments. The strains BR 29, BR 3267, and UFLA 03-164 showed the lowest values for these parameters among all strains; even so, their mean values were equivalent (height) or superior (NAS) to either control treatment.

The strains UFLA 03-320, UFLA 03-321, and UFLA 04-212 were the most effective in growth promotion (plant height and SDM) and N nutrition (NAS) of pigeon pea cv. *Fava-Larga* under the conditions evaluated, providing values similar to the strains approved as inoculants for the crop, and higher than the control +N.

The strain UFLA 04-212 was also effective in establishing symbiosis and in fixing  $N_2$  in siratro (*Macroptilium atropurpureum*) under greenhouse conditions, with flasks containing nutrient solution (Florentino et al., 2009). The strains UFLA 03-320 and UFLA 03-321 also proved to be effective in promoting growth in cowpea (*Vigna unguiculata*) under greenhouse conditions with Leonard jars (Rufini et al., 2014a).

In a study carried out in Leonard jars with NFNB isolates from Mato Grosso do Sul, Martins et al. (2012) found differences between NFNB in relation to the ability of promoting plant growth of pigeon pea. Moreover, some isolates were superior to the strains approved as inoculant and to the treatment that received mineral N.

### Evaluation of symbiotic efficiency in pots with soil

There was significant interaction ( $p < 0.05$ ) between soil types and treatments for most variables (Table 4), except for SDM and plant height (Table 5). In the Inceptisol, the reference strains (BR 2003 and BR 2801) and the strains BR 29 and UFLA 04-212 exhibited higher NN, followed by the strains UFLA 03-320 and UFLA 03-321. For NDM, all the inoculated treatments were superior to the controls +N and -N. For the Oxisol, inoculated treatments and the control -N had greater nodulation and NDM than the control +N.

For N content in the shoot (NCS) and N accumulation in the shoot (NAS), the control with mineral N was superior to the others in the Inceptisol. The strains UFLA 03-320 and UFLA 04-212 were similar to the reference strains (BR 2003 and BR 2801) in relation to NCS, and were superior to the other strains and to the control -N. This also happened for NAS, except for the strain UFLA 03-320. For the Oxisol, there was no significant difference ( $p > 0.05$ ) among treatments for NCS. For NAS, all treatments were similar to each other and higher than the strain BR 29.

In the Oxisol (high pH - 5.9), there was no significant difference for NCS and NAS among the inoculated treatments, except for BR 29 in NAS, and for the treatment that received mineral N. In the Inceptisol (acid soil - pH 4.6), the difference among treatments for these

**Table 4.** Number of nodules (NN), nodule dry matter (NDM), nitrogen content in the shoot (NCS), and nitrogen accumulation in the shoot (NAS) of pigeon pea cv. IAC *Fava-Larga* cultivated in pots with soil, as a function of treatments and soil

Soil	Treatment	NN	NDM	NCS	NAS
			g per pot	%	mg per pot
Inceptisol	BR 2003	19 a	0.329 a	2.63 b	142.36 b
	BR 2801	19 a	0.402 a	2.63 b	145.11 b
	BR 29	26 a	0.324 a	2.21 c	103.11 c
	UFLA 03-320	8 b	0.236 a	2.44 b	116.11 c
	UFLA 03-321	9 b	0.204 a	2.21 c	100.75 c
	UFLA 04-212	21 a	0.222 a	2.70 b	146.30 b
	+N <sup>(1)</sup>	1 c	0.004 b	3.84 a	218.16 a
	-N <sup>(2)</sup>	1 c	0.011 b	2.02 c	88.35 c
Oxisol	BR 2003	29 a	0.365 a	3.02 a	178.99 a
	BR 2801	38 a	0.358 a	2.99 a	166.71 a
	BR 29	47 a	0.485 a	2.57 a	112.52 b
	UFLA 03-320	46 a	0.506 a	3.25 a	204.41 a
	UFLA 03-321	36 a	0.480 a	3.35 a	191.76 a
	UFLA 04-212	71 a	0.439 a	2.96 a	162.73 a
	+N <sup>(1)</sup>	10 b	0.081 b	2.99 a	218.57 a
	-N <sup>(2)</sup>	33 a	0.427 a	2.99 a	164.10 a
p value	-	0.0480	0.0045	0.0000	0.0225
CV (%)	-	27.15	37.10	11.31	20.94

Within each soil, means followed by the same letter in the column belong to the same group by the Scott-Knott test at 5 % probability. <sup>(1)</sup> +N: uninoculated control, fertilized with 300 mg dm<sup>-3</sup> NH<sub>4</sub>NO<sub>3</sub>-N. <sup>(2)</sup> -N: uninoculated control, without nitrogen fertilization.

**Table 5.** Plant height and shoot dry matter (SDM) of pigeon pea cv. IAC *Fava-Larga* cultivated in pots with soil

Treatment	Plant height	SDM
	cm	g per pot
BR 2003	54.43	5.62
BR 2801	53.48	5.49
BR 29	48.70	4.51
UFLA 03-320	50.63	5.50
UFLA 03-321	51.80	5.14
UFLA 04-212	53.61	5.39
+N <sup>(1)</sup>	55.16	6.54
-N <sup>(2)</sup>	48.52	4.90
p value	0.2323	0.0172
Soil		
Inceptisol	48.82 b	5.02 b
Oxisol	55.26 a	5.75 a
p value	0.0001	0.0057
CV (%)	11.75	18.69

Within each soil, means followed by the same letter in the column belong to the same group by the Scott-Knott test at 5 % probability. <sup>(1)</sup> +N: uninoculated control, fertilized with 300 mg dm<sup>-3</sup> NH<sub>4</sub>NO<sub>3</sub>-N. <sup>(2)</sup> -N: uninoculated control, without nitrogen fertilization.

variables was higher. This confirms that symbiosis is more sensitive to environmental stresses, such as acidity, than plants fertilized with mineral nitrogen. Other authors have also found that in the absence of liming, growth and nodulation of pigeon pea were drastically limited, mainly due to aluminum toxicity (Costa et al., 1989).

In relation to the main effects, there was no significant difference ( $p>0.05$ ) between the strains and the controls +N and -N for plant height and SDM. For the types of soil, these variables were higher in Oxisol (Table 5), which may also be related to the acidity and to the lower fertility of the Inceptisol, affecting plant development and symbiosis, as previously mentioned.

Although pigeon pea is a rustic plant, which adapts well to low soil fertility, it responds well to fertilization (Rodrigues et al., 2004) or to higher natural fertility and pH close to neutral (Benedetti, 2005), which may have influenced the differences between the soils.

### Evaluation of efficiency in the field

In evaluations of full flowering under field conditions, the strains BR 29 and UFLA 03-320 provided SDM similar to the control +N, surpassing the other treatments ( $p<0.10$ ). Except for BR 29, these same treatments exhibited the highest NASpl and NASHa (Table 6).

For the variables evaluated at the maturity stage, there was a significant difference only for NAG ( $p<0.05$ ) and grain yield ( $p<0.10$ ). The strains tested provided accumulations and grain yields equivalent not only to that obtained from the reference strain BR 2801, but also from the nitrogen treatment (+N), surpassing the reference strain BR 2003 and the control -N (Table 7).

A grain yield of  $2721 \text{ kg ha}^{-1}$  was obtained, which was higher than that reported by IAC (2013) for this cultivar (1,200 to  $1,800 \text{ kg ha}^{-1}$ ). These yields, however, could be even greater if the harvest period was extended.

By testing these same treatments in dwarf pigeon pea 'Iapar 43' (Aratã), Rufini et al. (2014b) observed that the strain UFLA 03-320 also stood out, as was the case in this study. Thus, since it was effective in forming symbiosis with different cultivars, it can be recommended for this species.

In the experiment in the field, except for BR 2003, the strains tested are effective in fixing  $\text{N}_2$  and in promoting growth of pigeon pea, associated with good fertility conditions and good levels of soil organic matter, which allowed its establishment and survival.

**Table 6.** Plant height, shoot fresh matter (SFM), shoot dry matter (SDM), nitrogen content in the shoot (NCS), and nitrogen accumulation in the shoot per plant (NASpl) and per hectare (NASHa) of pigeon pea cv. IAC Fava-Larga cultivated in the field

Treatment	Plant height	SFM	SDM	NCS	NASpl	NASHa
	m	g per plant		%	mg per plant	kg ha <sup>-1</sup> N
BR 2003	3.31 a	744.6 a	269.3 b	3.38 a	9057.75 b	480.06 b
BR 2801	3.25 a	863.4 a	319.8 b	3.25 a	10237.11 b	542.57 b
BR 29	3.42 a	1068.0 a	385.7 a	2.96 a	11746.54 b	622.57 b
UFLA 03-320	3.37 a	1355.0 a	461.3 a	3.41 a	15810.47 a	837.96 a
UFLA 03-321	3.22 a	825.4 a	289.7 b	3.58 a	10340.46 b	548.04 b
UFLA 04-212	3.29 a	918.1 a	322.5 b	3.51 a	11314.42 b	599.66 b
+N <sup>(1)</sup>	3.18 a	1064.4 a	383.5 a	3.58 a	13448.11 a	712.75 a
-N <sup>(2)</sup>	3.33 a	905.8 a	326.3 b	3.41 a	10853.70 b	575.25 b
p value	0.8092	0.0798	0.1046	0.4684	0.0813	0.0813
CV (%)	6.55	26.83	25.49	12.13	24.97	24.97

Means followed by the same letter in the column belong to the same group by the Scott-Knott test at 10 % probability. <sup>(1)</sup> +N: uninoculated control, fertilized with  $70 \text{ kg ha}^{-1}$  urea-N ( $\frac{1}{2}$  at sowing and  $\frac{1}{2}$  as topdressing, at 35 days after emergence). <sup>(2)</sup> -N: uninoculated control, without nitrogen fertilization.

**Table 7.** Mean values of grain yield and its main components [pods per plant (PP), grains per pod (GP), 100-grain weight (W100)], nitrogen content in the grain (NCG), and nitrogen accumulation in the grain (NAG) of pigeon pea cv. IAC Fava-Larga cultivated in the field

Treatment	Grain yield <sup>(1)</sup> kg ha <sup>-1</sup>	PP <sup>(1)</sup>	GP <sup>(1)</sup>	W100 <sup>(1)</sup> g	NCG <sup>(1)</sup> %	NAG <sup>(2)</sup> kg ha <sup>-1</sup>
BR 2003	2148.97 b	134 a	4.7 a	12.00 a	3.88 a	83.34 b
BR 2801	2622.95 a	199 a	4.8 a	12.54 a	4.07 a	107.58 a
BR 29	3109.29 a	183 a	4.6 a	12.22 a	3.82 a	118.67 a
UFLA 03-320	2679.35 a	148 a	4.7 a	12.25 a	4.02 a	107.19 a
UFLA 03-321	2838.27 a	201 a	4.6 a	12.28 a	3.96 a	112.36 a
UFLA 04-212	3101.70 a	124 a	4.6 a	12.83 a	4.02 a	124.67 a
+N <sup>(1)</sup>	2914.12 a	174 a	4.5 a	11.53 a	3.82 a	110.38 a
-N <sup>(2)</sup>	2357.74 b	150 a	4.6 a	12.47 a	3.82 a	90.12 b
p valor	0.0748	0.2192	0.6326	0.0753	0.3623	0.0470
CV (%)	16.87	29.06	4.64	4.24	4.79	16.25

Means of four replications followed by the same letter in the same column belong to the same group by the Scott-Knott test at 5<sup>(2)</sup> % and 10<sup>(1)</sup> % probability. <sup>(1)</sup> +N: Uninoculated control fertilized with 70 kg ha<sup>-1</sup> urea-N (½ at sowing, and ½ as topdressing, at 35 days after emergence). <sup>(2)</sup> -N: uninoculated control, without nitrogen fertilization.

## CONCLUSIONS

In axenic conditions, symbiotic efficiency of *Bradyrhizobium* varies according to the bacterial strain, and is higher when symbiosis of the pigeon pea cv. *Fava-Larga* is established with the strains UFLA 03-320, UFLA 03-321, UFLA 04-212, BR 2801, and BR 2003.

Soil type influences the symbiotic efficiency of *Bradyrhizobium*-pigeon pea in pots with soil, especially with the Inceptisol, where the strains UFLA 04-212, BR 2801, and BR 2003 provide greater shoot N accumulation; in the Oxisol, the treatments have less influence.

In the field, the strain UFLA 03-320 provides shoot dry matter and N accumulation in the shoot equivalent to the control with mineral N. In relation to grain yield, this strain and BR 29, UFLA 03-321, and UFLA 04-212 exhibit performance equivalent to that of the reference strain approved for pigeon pea (BR 2801), and equivalent to the control with 70 kg ha<sup>-1</sup> urea-N.

Pigeon pea establishes symbiotic association, which provides the nitrogen required for its growth, and that the strains tested, except for BR 2003, have potential for recommendation for this crop in regard to grain yield. The strain UFLA 03-320 has potential for grain production, as well as potential for use in green manure crops.

## ACKNOWLEDGMENTS

We thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq/MAPA- Process No. 578635/2008-9), the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), and the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (Fapemig) for funding, scholarships, and research productivity fellowships.

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