

## Phosphate rock biofertiliser with *Acidithiobacillus* and rhizobia improves nodulation and yield of cowpea (*Vigna unguiculata*) in greenhouse and field conditions

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### Abstract

Biofertilisers produced with phosphate rock (PR) plus sulphur (S) inoculated with *Acidithiobacillus* can promote plant growth and increase available P in tropical soils. Pot and field experiments were carried out in a Brazilian tableland soil with low available P to evaluate the suitability of P biofertiliser at different levels as an alternative to soluble P fertiliser for cowpea, with and without rhizobial inoculation. In the pot experiment, P treatments were: PR — 800 kg/ha; PR plus S (S:PR = 100g/kg) — 800 kg/ha; biofertiliser (B) [phosphate rock treated with sulphur (S:PR = 100g/kg) plus *Acidithiobacillus*] — 200, 400, 600 and 800 kg/ha; and triple superphosphate (TSP) — 400 kg/ha. In the field experiment, P treatments were: PR — 800 kg/ha; biofertiliser — 300, 600 and 900 kg/ha; and triple superphosphate (TSP) — 400 kg/ha. A treatment without added P, S or *Acidithiobacillus* ( $P_0$ ) was added as the control in both experiments. Nodulation, plant dry matter yield, grain yield, N and P uptake, soil pH and available P increased when TSP and biofertiliser were applied. The best values for soil available P were obtained with application of P biofertiliser, especially with higher levels. Uninoculated cowpea nodulated with native rhizobia present in the tableland soil, although rhizobial strains selected for acid soils were more effective on inoculated plants. In pot experiments, rhizobial inoculation showed interaction with biofertiliser, although no significant differences were observed under field conditions. The results suggest that P biofertiliser may be

used as an alternative to triple superphosphate in soils with low available P for fertilising tropical crops and pastures.

### Introduction

Cowpea (*Vigna unguiculata*), a legume widely cultivated in tropical countries, has many agricultural uses due to its high concentrations of protein and amino acids and its effectiveness in fixing atmospheric nitrogen when inoculated with rhizobia. Strains of rhizobia previously screened for cowpea under high temperature and acidic conditions proved to be very effective in nitrogen fixation with inoculated cowpea in Brazilian soils, and inoculation with these selected strains may be sufficient to supply the nitrogen input for good yields (Stamford *et al.* 1995; 2004).

The Brazilian tableland soil used in this study belongs to the Typic Fragiudult soils, which represent a significant proportion of the world's arable soils. Since P is important in the symbiotic process and for plant growth, satisfactory agricultural production on these soils in tropical regions depends on the application of fertiliser to maintain acceptable levels of available P in the soil profile since these soils have low P availability, associated with low organic matter content (Burity *et al.* 2000).

The materials used for the production of commercial fertilisers are mainly sedimentary, igneous and biogenic in origin. Conventional, chemically processed fertilisers are largely water-soluble and contain high and immediately available nutrient concentrations. In the production of soluble phosphate fertilisers, *e.g.* superphosphates and thermo-phosphates, powdered rocks are heated to high temperatures, to above the melting point (fusion), requiring high energy consumption. Furthermore, strong acids (sulphuric and phosphoric) are used (van Straaten 2002). Use of other phosphate fertiliser sources, such as untreated rock phosphate (PR), may provide an economical and

energy-efficient alternative to chemically processed fertilisers (Villanueva *et al.* 2006).

The immediate utilisation of rock phosphates by plants is restricted due to low solubility, and they are used mainly with perennial crops in combination with soluble fertilisers to maintain a slow nutrient uptake over extended periods. Efforts to establish the agronomic efficiency and economic use of rocks as fertilisers are not yet conclusive (van Straaten 2002).

Phosphorus in rocks generally needs to be modified physically, chemically or microbiologically to become an effective nutrient source for crops. Studies to isolate and select microorganisms with the ability to promote higher solubilisation of rocks have been carried out under tropical conditions, especially in interaction with microorganisms involved in biological nitrogen fixation (Nahas 1999).

Many species of *Acidithiobacillus* react with elemental sulphur (S) to produce sulphuric acid (Garcia Jr 1992), which may increase available P levels in soil by promoting higher solubility of phosphate rocks, providing P for the symbiotic process and for plant growth (Stamford *et al.* 2004; 2005).

The effects of S inoculated with *Acidithiobacillus* on natural phosphates and their impact in soils and on plant growth need to be evaluated and compared with the benefits from soluble fertilisers, because the sulphuric acid produced in the biological reaction could reduce soil pH, which could be harmful to plants (Stamford *et al.* 2004). Biofertilisers produced with natural rocks have distinct advantages over chemical fertilisers as they can be used in organic agriculture, as part of a sustainable agricultural system, and may reduce the impact of fertilisers in the environment (Stamford *et al.* 2003b; 2004).

This study was carried out to evaluate the effects and effectiveness of biofertilisers, produced through mixing powdered rocks and elemental S inoculated with *Acidithiobacillus*, when applied to a soil with low available P and sown to cowpea. The biofertilisers were compared with triple superphosphate (TSP) and natural rock phosphate. Parameters measured were cowpea shoot biomass, grain yield and total N and P accumulation in the plants, as well as soil pH and available P. The effects of inoculation with rhizobia on nodulation, plant growth and its interaction with the biofertiliser were also studied.

## Materials and methods

### *Rock biofertiliser production*

The P biofertiliser (B) used in the pot and field experiments was produced in the laboratory, by adding 2 kg of phosphate rock (Gafsa apatite) and elemental S (200 g) inoculated with 200 mL of *Acidithiobacillus* at a rate of  $10^6$  viable cells per mL to plastic trays (50 × 30 × 10 cm). A more detailed explanation is given by Lima (2005). Bacterial cultures were grown in Erlenmeyer flasks using medium 9K (Garcia Jr 1991) for 5 days at 180 rpm in a horizontal shaker at 28–30°C. Analysis of the P biofertiliser, using Embrapa (1997) methodology, showed that: pH = 4.0, and available P = 60 g/kg; while the rock phosphate had pH = 5.1 and available P = 10 g/kg. The triple superphosphate used in the study had pH = 5.0 and available P of 63 g/kg.

### *Pot experiment*

In July 2004, a pot experiment was carried out in a greenhouse controlled at 30–35°C, to compare the effects of P biofertiliser, TSP and PR on cowpea growth, nitrogen and phosphorus uptake, soil pH and available P in soil. The soil used was classified as a Typic Fragiudult with low available P. Samples were collected in the District of Carpina, located in the Tropical Rainforest Zone of Pernambuco state, north-east Brazil. Soil samples (0–30 cm layer) were ground to pass through a 5 mm sieve, mixed and placed in plastic pots. Properties of the soil, measured by the Embrapa (1997) methodology, were: pH (H<sub>2</sub>O 1.0:2.5) 5.8; exchangeable cations (mmol<sub>c</sub>/dm<sup>3</sup>) Al<sup>3+</sup> 0.5, Ca<sup>2+</sup> 20.0, Mg<sup>2+</sup> 8.8 and K<sup>+</sup> 1.2; available P — 4.2 mg/kg; total N — 0.8 g/kg; organic C — 8.7 g/kg; particle density — 2.60 g/cm<sup>3</sup>; global density — 1.54 g/cm<sup>3</sup>; and sand, silt and clay contents of 91.0, 4.4 and 4.6 %, respectively.

The experiment was carried out in pots (8 L) each filled with 10 kg of soil and cowpea was used as the test plant. The pot experiment was arranged in a complete randomised factorial design with 4 replicates. Lime was not applied because soil pH (5.8) and exchangeable cations Ca<sup>2+</sup> and Mg<sup>2+</sup> (28.8 mmol<sub>c</sub>/dm<sup>3</sup>) were adequate for cowpea. To reduce the influence of moisture on available P in soil, as described by Ruiz *et al.* (1990), water was added twice a day (08.00 h and 17.00 h), to maintain soil moisture near field capacity, using lateral plastic drains in the pots.

P treatments were: phosphate rock (PR) — 800 kg/ha; PR plus sulphur (S) (S:PR=100g/kg) — 800 kg/ha; P biofertiliser (B<sub>200</sub>, B<sub>400</sub>, B<sub>600</sub> and B<sub>800</sub>) — 200, 400, 600 and 800 kg/ha; and triple superphosphate (TSP) — 400 kg/ha. A treatment without added P, S or *Acidithiobacillus* (P<sub>0</sub>) served as a control. Fertilisers were applied at the levels recommended for cowpea (Stamford *et al.* 1990). Rhizobial treatments were: inoculation with appropriate rhizobia (strains NFB 516 and NFB 700) for acid soils (Stamford *et al.* 1995); and uninoculated controls with no rhizobia applied.

Seeds of cowpea were surface-sterilised with HgCl<sub>2</sub> (1:1000) (for 1 min) and with ethanol (70%) (for 0.5 min), followed by 10 washes with distilled water. In the rhizobial treatment, the inoculant was prepared in YM medium (50 mL) using 125 ml Erlenmeyer flasks maintained in a horizontal shaker at 100 rpm for 5 days, at 28–30°C (containing rhizobial cells in excess of 10<sup>8</sup>/mL). A mixture (1:1) of both strains was used for inoculation and applied in the soil, near the sown seeds, at the rate of 2 mL per pot.

Shoots were harvested 45 days after planting (DAP). Nodule and shoot dry matter (DM) were determined by drying at 65–70°C until constant weight, and soil pH and soil available P by the Embrapa (1997) methodology. Total N in shoots was determined by the Kjeldahl semi-micro method using an autoanalyser (Kjeltec 1030), according to Malavolta *et al.* (1989); and total P in shoots was determined following the methodology described by Malavolta *et al.* (1989).

### Field experiment

In September 2004, a field experiment with cowpea (cv. IPA 206) was conducted in the same area where the soil samples for the pot experiment were collected. Soil moisture was determined daily using tension meters installed at 20 cm soil depth, and water tension was maintained near field capacity, throughout the experimental period by furrow irrigation. During the experiment (2 months), the photoperiod remained close to 12 h, temperature fluctuated between 22 and 30°C and relative humidity was 60–80%.

Fertiliser treatments were: PR — 800 kg/ha; P biofertiliser (B<sub>300</sub>, B<sub>600</sub> and B<sub>900</sub>) — 300, 600 and 900 kg/ha; and TSP — 400 kg/ha as recommended for cowpea (Stamford *et al.* 1990). A P<sub>0</sub> control was also included. The field experiment was arranged

as a factorial using a split-plot randomised block design with 4 replicates. The plots represented the fertiliser treatments and the subplots represented the rhizobial treatments (with and without rhizobial inoculation). Each subplot was 6 m × 4 m and contained 4 rows with plants 0.5 m apart within rows. To avoid contamination, a row was used to separate treatments with and without rhizobial inoculation.

At sowing, 60 mL/row (6 m) of a micronutrient solution (Norris 1964) was dissolved in water and manually applied to all treatments at a rate of 400 L/ha. For treatments with rhizobial inoculation, a mixture of NFB 516 and NFB 700 strains (1:1) was prepared as described in the pot experiment. Peat packages (400 g; pH 6.5), purchased from Empresa Pernambucana de Pesquisa Agropecuária (IPA), were sterilised at 120°C (1 atm) for 1 hour, and 40 mL of rhizobial culture was added to each package using a sterilised syringe. The packages were then incubated for one month under cool conditions to promote bacterial growth. In treatments with rhizobia, seeds were inoculated in the morning by pelleting the seeds mixed with milk with the peat, at one peat package per kg of seeds. Seeds were sown in the afternoon after air-drying.

At 45 DAP, 2 plants from the central 2 rows in each plot were harvested to determine nodulation (nodule DM), shoot DM yield and total N and P uptake in shoots, using the methodology described for the pot experiment. Between 50 and 60 DAP, pods were harvested to determine grain yield. The total numbers of pods containing grain were counted and weighed. Grains were threshed from the pods and weighed. Soil pH and available P were determined following Embrapa (1997) methodology.

### Statistical analyses

Analysis of variance was performed for both pot and field experiments using the software SANEST (Zonta *et al.* 1982). Significant differences at P ≤ 0.05 for main effects of rhizobia, fertiliser treatment and their interactions were compared by the Tukey test (Silva and Silva 1982).

## Results

### Pot experiment

Data for nodule biomass production are presented in Table 1. Both application of P and inoculation

with rhizobia affected nodulation. All treatments receiving P fertiliser produced more nodule DM than the control ( $P < 0.05$ ), with the highest nodule DM obtained from biofertiliser, *i.e.*, with sulphur + *Acidithiobacillus*, applied at 400 kg/ha. Nodule DM was increased when rhizobial inoculation was applied for the TSP, B<sub>200-600</sub> and PR + S treatments. Application of biofertiliser at levels of 600 and 800 kg/ha resulted in lower nodule DM than lower levels of biofertiliser ( $P < 0.05$ ).

**Table 1.** Effects of P treatments on nodule DM of cowpea inoculated and uninoculated with rhizobia in a Brazilian tableland soil with low available P — Pot experiment.

P treatments <sup>1</sup>	Rhizobial inoculation		
	Inoculated	Uninoculated	Mean
	Nodule DM (g/plant)		
Control (P <sub>0</sub> )	0.20dA <sup>2</sup>	0.18dA	0.20d
PR	0.41cA	0.35cA	0.38c
PR + S	0.56bA	0.44bcB	0.45b
B <sub>200</sub>	0.55bA	0.49bB	0.52b
B <sub>400</sub>	0.71aA	0.59aB	0.65a
B <sub>600</sub>	0.39cA	0.31cB	0.35c
B <sub>800</sub>	0.30cA	0.29cA	0.29c
TSP	0.56bA	0.41cB	0.48b

<sup>1</sup> Control (P<sub>0</sub>)—no P applied; PR—phosphate rock at 800 kg/ha; PR+S—phosphate rock treated with sulphur (S:PR = 100 g/kg) at 800 kg/ha; B<sub>200</sub>, B<sub>400</sub>, B<sub>600</sub> and B<sub>800</sub>—biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 200, 400, 600 and 800 kg/ha; and TSP—triple superphosphate at 400 kg/ha.

<sup>2</sup> Values followed by different letters are significantly different ( $P = 0.05$ ) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. CV (%) = 26.78.

Shoot DM results are presented in Table 2. All treatments receiving P produced more shoot DM than the control and TSP plus all B treatments produced more shoot DM than PR ( $P < 0.05$ ) for inoculated cowpea. Inoculation with rhizobia increased shoot DM production ( $P < 0.05$ ) only when TSP or B treatments were applied.

P fertilisation significantly increased total N in shoot DM (Table 3), with significant differences between phosphorus sources. Overall, plants receiving P biofertilisers (B<sub>600</sub>, B<sub>800</sub>) and TSP contained more N than plants in the remaining P treatments and inoculation with rhizobia increased total N accumulation in shoots.

Total P accumulation in shoots of cowpea (Table 3) was higher ( $P < 0.05$ ) for all treatments other than the PR treatment relative to the control. Inoculation with rhizobia gave variable responses in shoot P with significant ( $P < 0.05$ ) responses in only 3 treatments.

**Table 2.** Effects of P treatments on shoot DM of cowpea inoculated and uninoculated with rhizobia in a Brazilian tableland soil with low available P — Pot experiment.

P treatments <sup>1</sup>	Rhizobial inoculation		
	Inoculated	Uninoculated	Means
	Shoot DM (g/plant)		
Control (P <sub>0</sub> )	4.64cA <sup>2</sup>	4.76bA	4.70c
PR	5.65bA	5.42abA	5.53b
PR + S	5.91bA	5.64aA	5.77ab
B <sub>200</sub>	6.38aA	5.87aB	6.13a
B <sub>400</sub>	6.22aA	5.28abB	5.75ab
B <sub>600</sub>	6.59aA	5.54aB	6.06a
B <sub>800</sub>	6.63aA	5.31abB	5.97ab
TSP	6.72aA	5.88aB	6.30a

<sup>1</sup> Control (P<sub>0</sub>)—no P applied; PR—phosphate rock at 800 kg/ha; PR+S—phosphate rock treated with sulphur (S:PR = 100 g/kg) at 800 kg/ha; B<sub>200</sub>, B<sub>400</sub>, B<sub>600</sub> and B<sub>800</sub>—biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR = 100 g/kg) and *Acidithiobacillus* at 200, 400, 600 and 800 kg/ha; and TSP—triple superphosphate at 400 kg/ha.

<sup>2</sup> Values followed by different letters are significantly different ( $P = 0.05$ ) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. CV (%) = 17.25.

For soil determinations (pH and available P), means represent the average of 8 replicates as the rhizobial inoculation had no significant effect on soil parameters. Soil pH, determined at the end of the pot study (Table 4), was lower for the biofertiliser treatments, especially B<sub>400-800</sub> treatments, than for the control, TSP and PR treatments. Available P levels in soil (Table 4) were significantly ( $P < 0.05$ ) increased in all treatments receiving P fertiliser, with highest levels achieved where biofertiliser treatments were applied.

### Field experiment

Shoot DM and grain yields of cowpea in the field experiment (averaged over the inoculation treatments) are presented in Figure 1. All treatments with P fertilisation showed significant ( $P \leq 0.05$ ) increases over the control. Best results were obtained with the P biofertiliser at higher levels (B<sub>600</sub> and B<sub>900</sub>), followed by B<sub>300</sub> and TSP. The lowest response among the fertilisers was obtained with phosphate rock (PR).

Differences in total N accumulation in shoots (averaged over the inoculation treatments) indicated a positive effect of P application (Figure 2), with B<sub>600</sub> and B<sub>900</sub> achieving significantly higher levels than the remaining 3 fertiliser treatments. N concentration in shoot DM (data not shown) was not significantly affected by rhizobial inoculation

**Table 3.** Effects of P treatments on total N and P in shoot DM of cowpea inoculated and uninoculated with rhizobia in a Brazilian tableland soil with low available P — Pot experiment.

P treatments <sup>1</sup>	Rhizobial inoculation					
	With	Without	Mean	With	Without	Mean
			Total N (mg/plant)		Total P (mg/plant)	
Control (P <sub>0</sub> )	79cA <sup>2</sup>	47dB	63c	2.6eA	2.8dA	2.7d
PR	155bA	118cB	137b	2.9deA	3.1cdA	3.0cd
PR + S	140bA	128cB	132b	3.5cA	3.7bcA	3.6bc
B <sub>200</sub>	166bA	144bcB	155b	4.7abcA	3.7bcB	4.2a
B <sub>400</sub>	156bA	156bA	156b	4.9abcA	4.3abA	4.6a
B <sub>600</sub>	209aA	165abB	187a	5.7aA	4.3abB	5.0a
B <sub>800</sub>	203aA	195aA	195a	4.4bcA	4.7aA	4.6a
TSP	191abA	175aB	183a	5.0abA	3.9bB	4.5a
Mean	162A	141B		4.2A	3.8B	

<sup>1</sup> Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; PR + S — phosphate rock treated with sulphur (S:PR=100 g/kg) at 800 kg/ha; B<sub>200</sub>, B<sub>400</sub>, B<sub>600</sub> and B<sub>800</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 200, 400, 600 and 800 kg/ha; and TSP — triple superphosphate at 400 kg/ha.

<sup>2</sup> Values followed by different letters are significantly different (P=0.05) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. CV (%) = total N — 12.09; total P — 11.40.

(P>0.05). N concentration in the control treatment (P<sub>0</sub>) exceeded that obtained for the fertilised treatments (data not shown) showing a dilution effect.

**Table 4.** Soil pH and available P after the harvest of cowpea in the pot experiment with a Brazilian tableland soil.

P treatments <sup>1</sup>	Soil pH	Soil available P
	(water 1:2.5)	(mg/kg)
Control (P <sub>0</sub> )	6.0b <sup>2</sup>	0.3e
PR	6.4a	5.7d
PR + S	6.3a	8.0cd
B <sub>200</sub>	5.7bc	16.9b
B <sub>400</sub>	5.5c	19.1ab
B <sub>600</sub>	5.4cd	21.6a
B <sub>800</sub>	5.3d	24.1a
TSP	5.9b	12.4bc

<sup>1</sup> Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; PR + S — phosphate rock treated with sulphur (S:PR = 100 g/kg) at 800 kg/ha; B<sub>200</sub>, B<sub>400</sub>, B<sub>600</sub> and B<sub>800</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR = 100 g/kg) and *Acidithiobacillus* at 200, 400, 600 and 800 kg/ha; and TSP — triple superphosphate at 400 kg/ha.

<sup>2</sup> Values within columns followed by different letters are significantly different (P=0.05) using the Tukey test. CV (%) = pH — 3.98; soil available P — 11.55. For soil determinations, means represent the average of 8 replicates as the rhizobial inoculation had no significant effect on soil parameters.

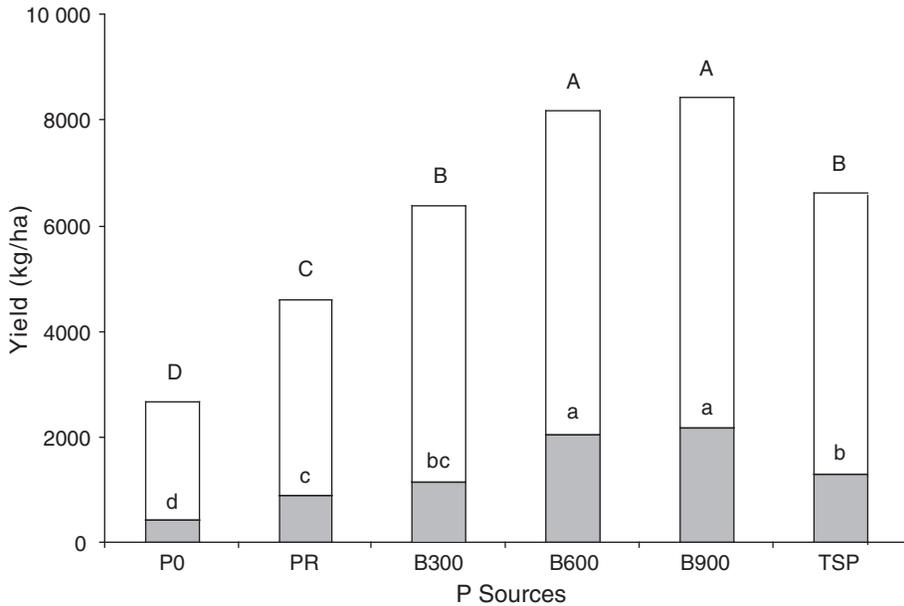
For soil determinations, means represent the average of 8 replicates as the rhizobial inoculation had no significant effect on soil parameters. The soil pH associated with P fertiliser treatments was significantly lower than that for the control at the end of the field experiment, especially when applied at levels of B<sub>600</sub> and B<sub>900</sub> (Figure 3). Amount of available P in the soil 2 months after plant harvest (Figure 4) was higher in all

P fertiliser treatments than in the control, with highest values for B<sub>900</sub>.

The effects of rhizobial inoculation with specific strains selected for use with cowpea under high temperature and acidic conditions were inconsistent in pot and field experiments. In contrast with the pot experiment, there were no significant responses to rhizobial inoculation in the field and no interaction was observed between the inoculation and P fertilisation (data not shown).

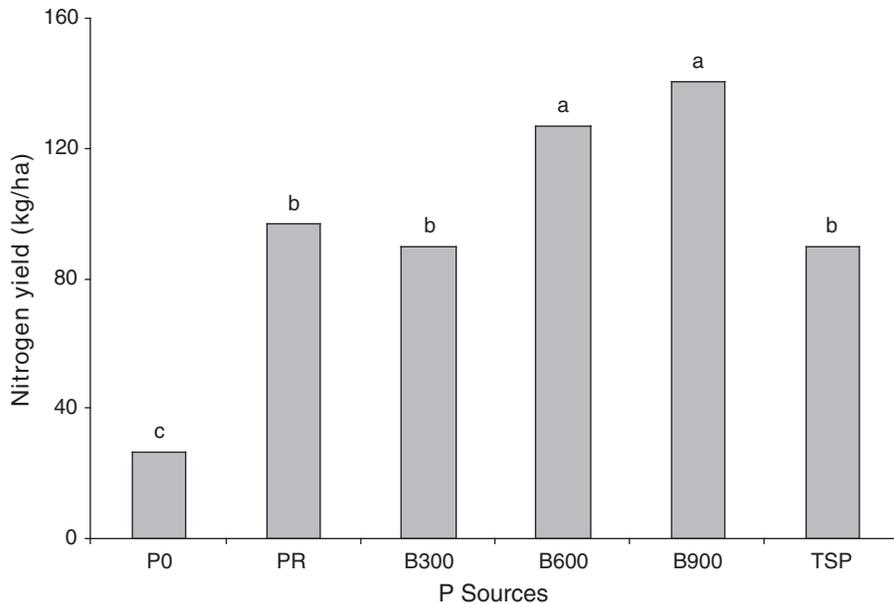
## Discussion

This study has provided useful information on the response of cowpea to application of biofertilisers produced using phosphate rock plus elemental sulphur inoculated with *Acidithiobacillus*. According to Garcia Jr (1992), the production of sulphuric acid by *Acidithiobacillus* should be responsive to addition of elemental sulphur. Other studies by the authors (unpublished data) have shown that adding *Acidithiobacillus* alone produces no effect on P availability, while inclusion of sulphur with *Acidithiobacillus* in increasing amounts leads to responses in forage production and some soil parameters (higher available P and reduced pH). Our results from the addition of sulphur to phosphate rock without *Acidithiobacillus* inoculation differ from those of Lombardi (1981), who suggested that sulphur addition to phosphate rock with or without *Acidithiobacillus* inoculation was equally effective in improving P availability. The significant differences in soil pH and

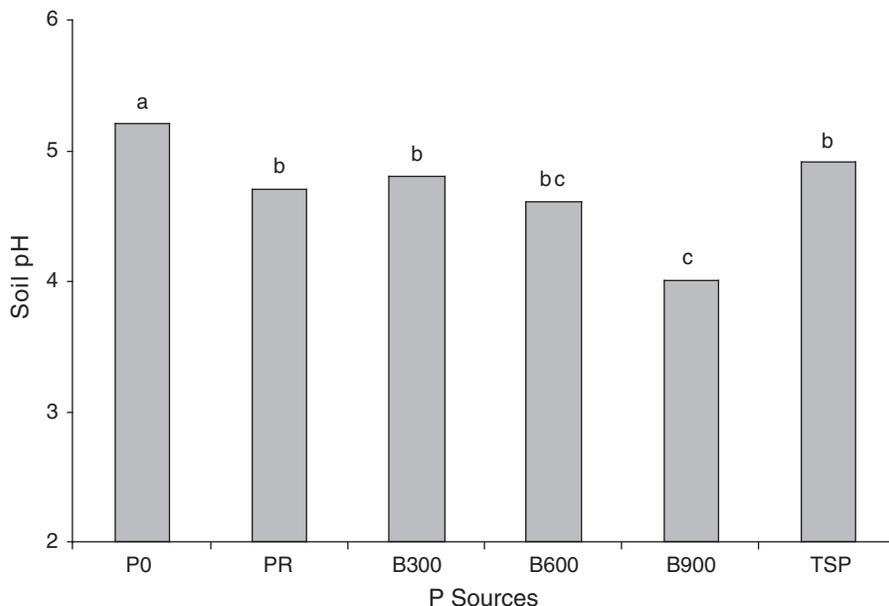


**Figure 1.** Yield of shoot DM (□) and grain (■) of cowpea in a Brazilian soil with low available P with the following P sources: Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; B<sub>300</sub>, B<sub>600</sub> and B<sub>900</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 300, 600 and 900 kg/ha; and TSP — triple superphosphate at 400 kg/ha.

Different letters on columns indicate significant differences at  $P \leq 0.05$  (Tukey test). Upper case letters compare data from shoot DM yield and lower case letters from grain yield. CV (%): shoot DM =10.49; and grain yield = 12.42.

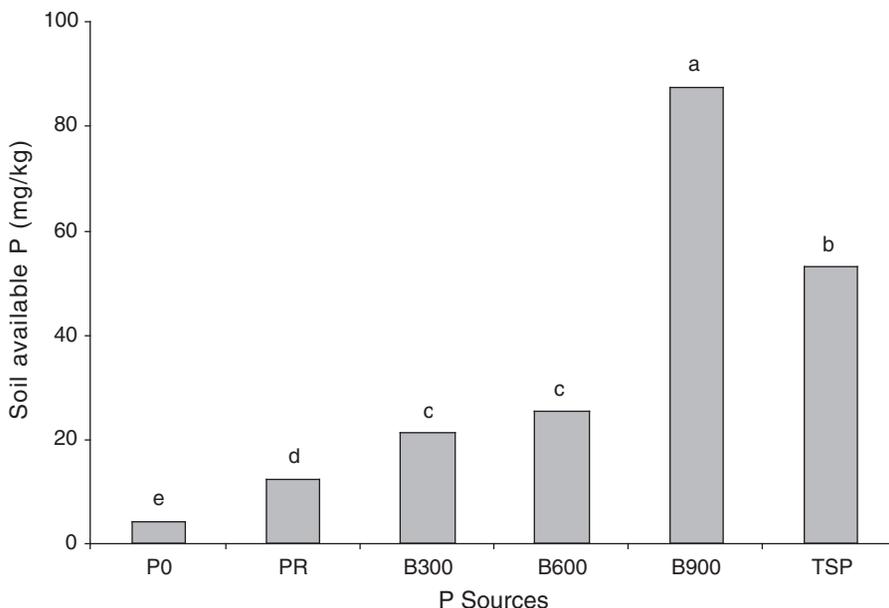


**Figure 2.** Total N in shoot DM of cowpea in a Brazilian soil with low available P with the following P sources: Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; B<sub>300</sub>, B<sub>600</sub> and B<sub>900</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 300, 600 and 900 kg/ha; and TSP — triple superphosphate at 400 kg/ha. Different letters on columns indicate significant differences at  $P \leq 0.05$  (Tukey test). CV (%) = 10.47.



**Figure 3.** Soil pH in a Brazilian tableland soil with low available P after two months of cowpea growth with the following P sources: Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; B<sub>300</sub>, B<sub>600</sub> and B<sub>900</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 300, 600 and 900 kg/ha; and TSP — triple superphosphate at 400 kg/ha.

Different letters on columns indicate significant differences at  $P \leq 0.05$  (Tukey test). (CV %) = 2.2. Means represent the average of 8 replicates as the rhizobial inoculation had no significant effect on soil parameters.



**Figure 4.** Available P in a low-P Brazilian soil after two months of cowpea growth with the following P sources: Control (P<sub>0</sub>) — no P applied; PR — phosphate rock at 800 kg/ha; B<sub>300</sub>, B<sub>600</sub> and B<sub>900</sub> — biofertiliser, *i.e.*, phosphate rock treated with sulphur (S:PR=100 g/kg) and *Acidithiobacillus* at 300, 600 and 900 kg/ha; and TSP — triple superphosphate at 400 kg/ha. Different letters on columns indicate significant differences at  $P \leq 0.05$  (Tukey test). CV (%) = 15.9. Means represent the average of 8 replicates as the rhizobial inoculation had no significant effect on soil parameters.

available P following the application of P biofertiliser and the responses in plant yields in this study strongly support the need for inclusion of both components in producing P biofertilisers.

The effects of P fertiliser treatments on shoot DM and uptake of N and P in plant tops compared with the control are conclusive and strongly indicate the need for additional P for legumes on these low-P soils. The additional responses obtained with biofertilisers relative to TSP provide strong evidence for their use, especially by low-income farmers, who frequently are unable to afford higher solubility P fertilisers, such as recommended by Klepker and Anghinoni (1995), Ernani *et al.* (2001) and Stamford *et al.* (2005).

Stamford *et al.* (2005), in a pot experiment to evaluate the effect of applying phosphate rock pelleted with sulphur inoculated with *Acidithiobacillus*, achieved greater increases in total N and P in plant shoots of mimosa (*Mimosa caesalpinifolia*) than application of triple superphosphate and phosphate rock plus sulphur without *Acidithiobacillus*. While this supports our current results, their responses were of a smaller magnitude, possibly because the pelleting may have limited sulphuric acid production by limiting the input of oxygen and water to oxidise the sulphur.

While application of P fertilisers lowered soil pH, especially when higher levels of the biofertilisers were applied, plant growth and cowpea grain yield were not affected by the reduction in soil pH. This lack of a negative response to low soil pH is most probably due to a combination of increased P availability, at least partly a consequence of low soil pH as related by He *et al.* (1996), and plant adaptation to acid soil conditions, since this species characteristically produces good growth on moderately acid soils. Stamford *et al.* (2002; 2003a) showed that cowpea is tolerant of low pH and of moderate rates of exchangeable aluminium.

The responses to rhizobial inoculation in the pot experiment were in contrast with data obtained in the field experiment. This may be due to the presence of a highly competitive native rhizobial population on the experimental site, as pointed out by Campos *et al.* (2001). Alternatively, the efficiency of the inoculated strains of rhizobia could be influenced by the higher soil acidity found in the field experiment (Figure 3) than in the pot experiment (Table 6), especially when biofertilisers with greater amounts of sulphur with *Acidithiobacillus* were used.

The positive impact of the P biofertiliser, produced with apatite plus sulphur inoculated with *Acidithiobacillus*, on growth and yield of cowpea holds great promise for improving usage of these products as partial or total substitutes for soluble P fertilisers due to the low cost of production. While use of P biofertilisers on low-P soils seems justified, caution must be exercised, since P biofertiliser, produced with phosphate rock plus elemental sulphur inoculated with *Acidithiobacillus*, applied at higher levels, may decrease soil pH to levels unfavourable for efficient action of rhizobia and could reduce nitrogen fixation and plant growth.

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