



Nitrogen-fixing and vesicular–arbuscular mycorrhizal symbioses in some tropical legume trees of tribe Mimoseae

Camila Maistro Patreze*, Lázara Cordeiro

Departamento de Botânica, Universidade Estadual Paulista, IB, Caixa Postal 199, Av. 24A,
1515 Bela Vista, 13506-900 Rio Claro, SP, Brazil

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Abstract

Response to mineral fertilization and inoculation with rhizobia and/or arbuscular mycorrhiza fungi (AMF) of the *Anadenanthera colubrina*, *Mimosa bimucronata* and *Parapiptadenia rigida* (Leguminosae–Mimosoideae) native trees from Brazilian riparian forests, were studied in nursery conditions. Each species was submitted to seven treatments, varying nitrogen and phosphorous fertilization and inoculation with rhizobia (r), mycorrhiza (m) or both (rm): NP, P, P + r, P + rm, N, N + m and N + rm. Results showed that AMF inoculations did not enhance the mycorrhizal colonization, and P uptake was not sufficient to sustain good growth of plants. The level of P mineral added affected negatively the AMF colonization in *A. colubrina* and *M. bimucronata*, but not in *P. rigida*. Native fungi infected the three legume hosts. The absence of mineral N limited growth of *A. colubrina* and *P. rigida*, but in *M. bimucronata* the lack of N was corrected by biological nitrogen fixation. N mineral added inhibited the nodulation, although spontaneous nodulation had occurred in *A. colubrina* and *M. bimucronata*. Rhizobia inoculation enhanced the number of nodules, nitrogenase activity and leghemoglobin content of these two species. Thus, the extent of rhizobial and mycorrhizal symbiosis in these species under nursery conditions can affect growth and consequently the post-planting success.

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1. Introduction

The Mimoseae tribe (Leguminosae–Mimosoideae) comprises common species in lowland tropical rainforests, especially near rivers and lakes. Among these, three species have been sampled in various floristic surveys of riparian forests in Brazil, namely *Anadenanthera colubrina* (Vell.) Brenan (Nilsson, 1989; Silva et al., 1992; Vilela et al., 1995; Bernacci et al.,

1998; Dias et al., 1998; Sampaio et al., 2000), *Mimosa bimucronata* (DC) O. Kuntze (Metzger et al., 1998) and *Parapiptadenia rigida* (Benth) Brenan (Nilsson, 1989; Dias et al., 1998).

Most genera of the tribe Mimoseae can nodulate and fix nitrogen, although there are some important exceptions (Sprent, 2001). Furthermore they can establish mutualistic symbiosis with arbuscular mycorrhizal fungi (AMF), which may result in reciprocal transfer of P from the fungus to the plant in exchange for carbon from the plant to the fungus (Ezawa et al., 2002). AMF are of widespread occurrence (Trufem, 1990) and may represent the natural

* Corresponding author. Tel.: +55-19-3526-4200;
fax: +55-19-3534-0009.
E-mail address: cpatreze@cena.usp.br (C.M. Patreze).

status of most tropical plant species (Siqueira et al., 1998). AMF colonization in *Anadenathera*, *Mimosa* and *Parapitadenia* were reported (Trufem, 1990; Carneiro et al., 1998; Siqueira, 1998; Frioni et al., 1999; Burity et al., 2000).

Dual inoculation of legumes with rhizobia and AMF can increase plant growth (Mosse et al., 1976; Redente and Reeves, 1981; Abd-Alla et al., 2000). In Brazil, Franco and Faria (1997) have lead studies of nodulated and mycorrhizal legume trees to revegetate poor or depleted soils with the goal of restoring their fertility, but little information is available about symbiotic relationships of dual inoculation in native Brazilian legume trees.

Information on nodulation in *A. colubrina* (Mendonça and Schiavinato, 1996), *M. bimucronata* (Sprent, 2001) and *P. rigida* (Corby, 1988) is available, but there is no information about responses to dual inoculation or the ecophysiology of such inoculated plants in nursery conditions.

The aim of our study was to investigate nitrogen-fixing and arbuscular mycorrhizal symbioses of three Mimoseae species that occur in Brazilian riparian forest. We submitted *A. colubrina*, *M. bimucronata* and *P. rigida* to mineral fertilization and inoculation with rhizobia and AMF in nursery conditions in order to aid the choice of species to recover riparian forests or drastically disturbed lands.

2. Materials and methods

The experiment was conducted in a glasshouse at the Paulista Estadual University (UNESP-SP), Brazil, in 4 L plastic bags containing unsterilized soil and vermiculite (2:1) (Table 1). The soil was collected from the riparian forest of Corumbataí, SP, Brazil (22°20'S and 47°40'W, 604 m a.s.l.). Seeds of *A. colubrina*, *P. rigida* and *M. bimucronata* were surface sterilized and germinated directly on substrate. *M. bimucronata* required dormancy break treatment with imbibitions in boiling water for 30 s.

Two rhizobia strains were obtained from *Anadenanthera peregrina* (L.) Speng root nodules and two strains were isolated from *M. bimucronata* nodules. The rhizobial colonies were grown on yeast extract mannitol (YEM) agar at 28 °C (Vincent, 1970) and stored in the Rhizobial Bank of UNESP Rio Claro, SP,

Table 1
Chemical analysis of substrate used in the experiment before the applications of mineral nutrients and inoculations

| N | P Resina | MO | pH | CaCl ₂ | K | Ca | Mg | H + Al | Al | SB | CTC | V | B | Cu | Fe | Mn | Zn | S |
|-------|------------------------|-----------------------|-----|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| (ppm) | (mg dm ⁻³) | (g dm ⁻³) | | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (mmol _c dm ⁻³) | (%) | (mg dm ⁻³) | (mg dm ⁻³) | (mg dm ⁻³) | (mg dm ⁻³) | (mg dm ⁻³) | (mg dm ⁻³) |
| 1000 | 7 | 6 | 5.5 | 1 | 12 | 4 | 10 | 7 | 17 | 27 | 63 | 0.01 | 0.7 | 23 | 8.7 | 0.4 | 9 | |

Brazil with numbers IBRC 199 and 200 (*A. peregrina*) and IBRC 201 and 204 (*M. bimucronata*). Aerial parts of these plants were pressed and catalogued in the Bioscience Institute Herbarium, UNESP, marked as HRCB 34330 and 34501, respectively.

For rhizobial inoculation, *A. colubrina* and *P. rigida* seeds were left to soak in a turbid suspension (100 ml) of mixture of IBRC 199 and 200 and *M. bimucronata* seeds of IBRC 201 and 204, for 1 h. Reinoculation with 10 ml of the same mixtures were made near the roots of seedlings 30 days later.

For mycorrhizal inoculation, pieces of *A. peregrina* var. *falcata* roots ± 1 cm in length, collected from Corumbataí cerrado reserve (22°15'S and 47°00'W, 810 m a.s.l.) were mixed on each pot surface (0.4 g) near seedlings 28 days after sowing.

All pots received the following basal nutrients prior to sowing (in mg kg⁻¹ substrate): K (60), CaCO₃ (80), MgCO₃ (40), S (30), B (1), Zn (2), Cu (2), Fe (4), Mn (20), Mo (4) and nitrogen and phosphorus addition, at three different levels: NP with N (40) and P₂O₅ (80); P with P₂O₅ (80) and 3.8 mM of N as a start dose; N only with N (40), using NH₄NO₃. These lots varied in function of inoculations with rhizobia (r), mycorrhiza (m) or both (rm). So, our experiment followed seven treatments, with 10 replicates: NP, P, P + r, P + rm, N, N + m, N + rm. All plants were grown in a greenhouse under natural daylight in seven randomized blocks. Additional nutrients (10 ml of solution) were added to the surface of each pot every 30 days, according to fertilization treatments above.

Height of 10 plants per treatment was recorded at 2-week intervals from 30 days after sowing until 120 days, then five plants per treatment until the end of the experiment (255 days after sowing). Five plants per treatment were harvested at 120 and at 255 days, for leaf area (CI-202 Area Meter, CID Inc.), dry weight of roots, stems and leaves and chemical analyses of tissue (shoot) and substrate.

Nitrogenase activity and leghemoglobin content from all nodules of two plants per treatment were evaluated in both harvests. Nitrogenase activity was measured by the closed acetylene reduction activity (ARA; Hardy et al., 1968) and leghemoglobin content by Becana et al. (1986) at 540 nm. Fresh nodules were sieved to separate those larger than 4 mm, between 2 and 4 mm and less than 2 mm of

diameter. They were then counted and dried. Nodule morphology was classified according to Sprent (2001).

Roots were stained (Philips and Hayman, 1970) and the percentage of AMF infected roots of two plants per treatment was estimated using the gridline intersect method (Giovannetti and Mosse, 1980) under a stereomicroscope (40 \times).

Data were analyzed separately using ANOVA. Dunn's test was applied to compare means at $P \leq 0.05$ using the program BioEstat 2.0 (Ayres et al., 2000), except for P and N contents that were analyzed on combined samples (per treatment). Principal component analysis (PCA) was performed for all data by Pcord program, version 4.0, for Windows. Variables were log transformed or arc square root transformed in order to normalize variance. Correlation coefficients $r < 0.05$ in the first axis were eliminated from the analysis.

3. Results

The growth parameters were positively affected by full mineral fertilization (NP) in the three species. Mineral deficiency treatments limited plant growth, except *M. bimucronata*, where mineral nitrogen deficiency was probably corrected by biological fixation with rhizobia. This species presented the best growth, as measured by height, dry biomass and leaf area, at 255 days after sowing (Table 2).

The highest values exhibited for root colonization by AMF (Table 2) were 70.3, 14 and 58.6% average of infected roots by *M. bimucronata*, *A. colubrina* and *P. rigida*, respectively. Typical hyphae and vesicles were observed in the preparations of all species. Native-born fungi were able to infect these three host plants. AMF inoculated treatments promoted similar values of infection of roots with those uninoculated under the same fertilization treatment. These results indicated that the AMF inoculation did not enhance the mycorrhizal colonization.

Addition of soluble P reduced AMF colonization in *A. colubrina* and *M. bimucronata* regardless of fungal inoculation. In *P. rigida* the opposite was found to occur (Table 2).

A variation in resource allocation patterns was observed among species, as well as within species

Table 2

Response of *A. colubrina*, *M. bimucronata* and *P. rigida* to mineral fertilization and inoculation treatments at 255 days after sowing

| Treatments | Leaf (cm ²) | Height ^a (cm) | Biomass (g) | AMF ^b (%) | N (mg g ⁻¹) | | P (mg g ⁻¹) | |
|-----------------------|-------------------------|--------------------------|-------------|----------------------|-------------------------|-----------|-------------------------|-----------|
| | | | | | Tissue | Substrate | Tissue | Substrate |
| <i>A. colubrina</i> | | | | | | | | |
| NP | 259.2 aA | 94 aB | 35.3 aA | 0.0 | 19.0 | 0.5 | 2.9 | 0.056 |
| P | 25.3 bcB | 51.6 cB | 5.7 bB | 1.5 | 17.5 | 0.7 | 5.6 | 0.108 |
| P + r | 59.5 bcB | 54.8 bcB | 7.1 bB | 4.5 | 15.5 | 0.5 | 4.2 | 0.084 |
| P + rm | 18.8 cB | 55.8 bcB | 5.4 bB | 2.0 | 14.5 | 0.7 | 3.8 | 0.088 |
| N | 192.1 aA | 71.4 abB | 11.2 abA | 13.5 | 18.0 | 0.5 | 0.8 | 0.004 |
| N + m | 128.4 abA | 71 abcB | 5.6 bB | 14.0 | 22.0 | 0.7 | 0.7 | 0.002 |
| N + rm | 195.2 aA | 76.2 abB | 7.2 bB | 10.8 | 18.5 | 0.7 | 0.7 | 0.003 |
| <i>M. bimucronata</i> | | | | | | | | |
| NP | 530.9 aA | 138.2 abA | 46.7 aA | 46.6 | 15.5 | 0.7 | 1.9 | 0.051 |
| P | 295.4 abA | 161.8 aA | 40.8 aA | 23.2 | 10.5 | 0.5 | 1.6 | 0.085 |
| P + r | 452.1 aA | 143.2 abA | 55.6 aA | 12.8 | 17.0 | 0.7 | 1.6 | 0.054 |
| P + rm | 225.2 abA | 127.4 abcA | 38.2 abA | 17.3 | 16.5 | 0.7 | 1.5 | 0.06 |
| N | 158.7 abcA | 100.8 cA | 15.1 cA | 0.3 | 17.0 | 0.7 | 0.5 | 0.003 |
| N + m | 131.3 bA | 127.4 abcA | 17.4 bcA | 68.4 | 15.0 | 0.7 | 0.4 | 0.001 |
| N + rm | 61 cB | 114.8 bcA | 15.0 cA | 70.3 | 16.5 | 0.7 | 0.4 | 0.001 |
| <i>P. rigida</i> | | | | | | | | |
| NP | 197.1 aA | 40.6 aC | 18.02 aB | 25.4 | 23.0 | 0.5 | 2.6 | 0.034 |
| P | 53.5 aB | 28.4 abB | 3.7 cdB | 47.0 | 19.0 | 0.5 | 6 | 0.064 |
| P + r | 53.2 aB | 24.2 bcC | 2.1 dC | 45.6 | 19.5 | 0.5 | 4.4 | 0.073 |
| P + rm | 29.8 aB | 24 bcC | 2.5 dB | 58.6 | 22.0 | 0.5 | 6.5 | 0.097 |
| N | 142.6 aA | 37.2 abC | 8.5 bA | 20.9 | 23.5 | 0.7 | 0.9 | 0.004 |
| N + m | 71.8 aA | 23.2 bcC | 4.3 cdB | 22.4 | 27.0 | 0.5 | 0.8 | 0.001 |
| N + rm | 72.9 bA | 34.8 abC | 6.1 bcB | 32.0 | 23.5 | 0.7 | 0.8 | 0.001 |

Mean of five plants per treatment. N and P content in tissue were measured in the shoot (leaf plus stem). Means followed by the same letter (small in treatments within one species and capital in different species for the same treatments) are not different by ANOVA, test Dunn at $P < 0.05$ level of significance. No such analyses were indicated for AMF, N and P of shoots.

^a Mean of 10 plants per treatment.

^b Mean of two plants per treatment.

through time, at 120 and 255 days of planting (Fig. 1). *A. colubrina* and *M. bimucronata* plants had more biomass of stem and leaves while *P. rigida* produced more roots at 120 days after sowing. At 255 days, *A. colubrina* and *P. rigida* increased biomass allocation to roots and *M. bimucronata* continued allocating stem and leaves biomass (Fig. 1).

P contents in tissue (shoot) and on substrate were low in treatments without P addition, and there was no difference in N contents (in tissue and on substrate) between treatments (Table 2).

Nodules of three species were classified morphologically as indeterminate, according to Sprent (2001), of the astragaloid (caesalpinoid) type of Corby (1988) (Fig. 2). Nodulation with native soil rhizobia occurred in *A. colubrina* and *M. bimucronata* but not in *P. rigida*

(Tables 3 and 4). Added mineral N inhibited nodulation in *A. colubrina* and *P. rigida*. There were few nodules in these treatments for *M. bimucronata* (Tables 3 and 4). All nodules were reddish inside (see *M. bimucronata* nodules in Fig. 2F).

Dry weight of *A. colubrina* and *P. rigida* nodules was not statistically different among all the treatments (Table 4). Although *M. bimucronata* nodule dry weight did not differ among treatments with P addition, rhizobia inoculated treatments (P + r and P + rm) had significantly higher numbers of nodules, nitrogenase activity (ARA) and leghemoglobin contents 255 days after sowing (Table 4).

ARA was relatively low in *P. rigida*, 128.5 and 292.2 μmol of ethylene per plant per hour at P + r and P + rm treatments, respectively. Leghemoglobin

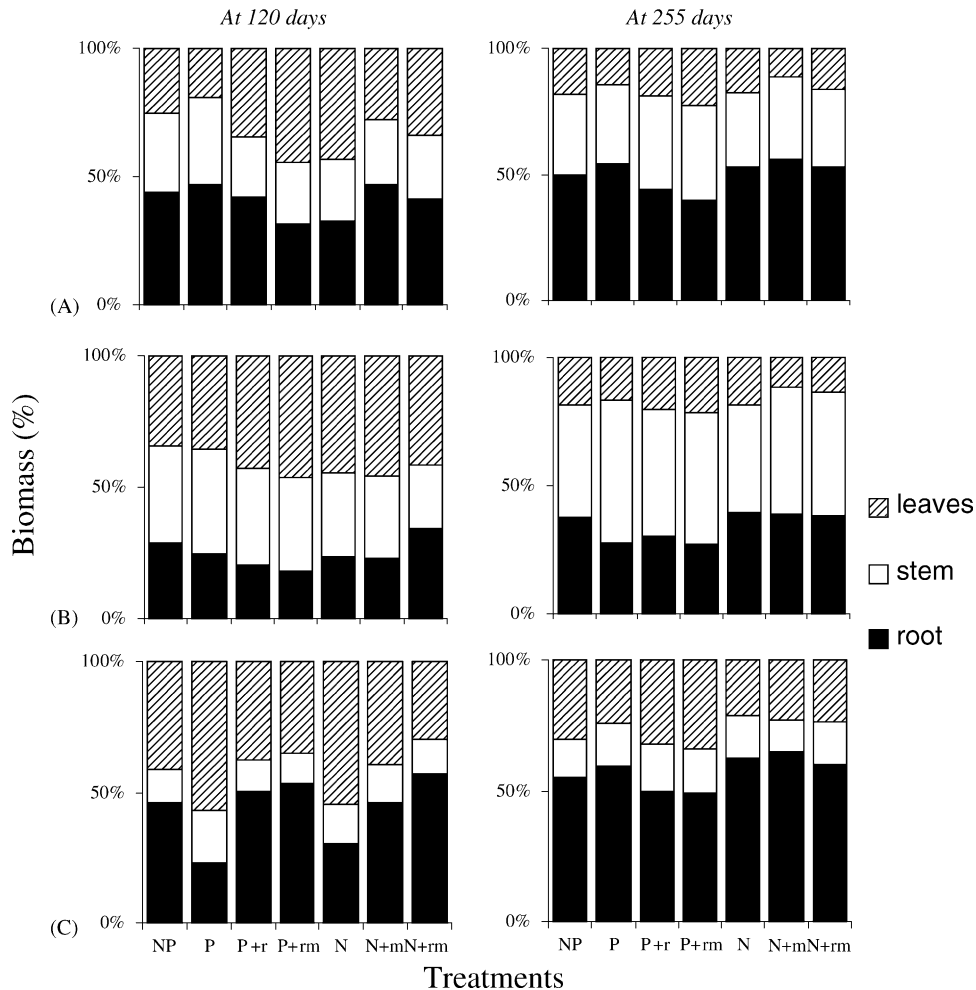


Fig. 1. Average biomass production (%) of roots, stems and leaves in *A. colubrina* (A), *M. bimucronata* (B) and *P. rigida* (C) plants cultivated with fertilization (NP, P and N) and inoculation of rhizobia (r), mycorrhiza (m) and both (rm) at 120 and at 255 days after sowing.

content was not assessed in this species because of insufficient nodule material.

We performed the PCA analysis of treatments and all data studied in order to see roughly, if the treatments would form groups and which factor(s) might explain the grouping. The PCA analysis simplifies the interpretation of complex results. Results of nutrient contents of tissues (shoots) and substrate were included in this analysis and each species was treated separately. The PCA plots (Fig. 3) showed that PC1 + PC2 axis explained 77.59, 88.47 and 77.79% of total for *A. colubrina*, *M. bimucronata* and *P. rigida*, respectively.

Mineral fertilization and nodulation influenced grouping in all three species. Treatments with mineral P added were grouping on the left and treatments with N formed a group to the right of the axis. Then, PC1 can be seen as representing the nitrogen fixation capacity of *A. colubrina* and *M. bimucronata*. In *P. rigida* the axis PC1 can be attributed only to mineral fertilization, since the nodulation was negligible. Because the vectors representing data nodulation pointed to the left, the treatments on the left responded more positively to rhizobial inoculation. Vectors representing AMF colonization and K level pointed to the right of origin for *A. colubrina* and

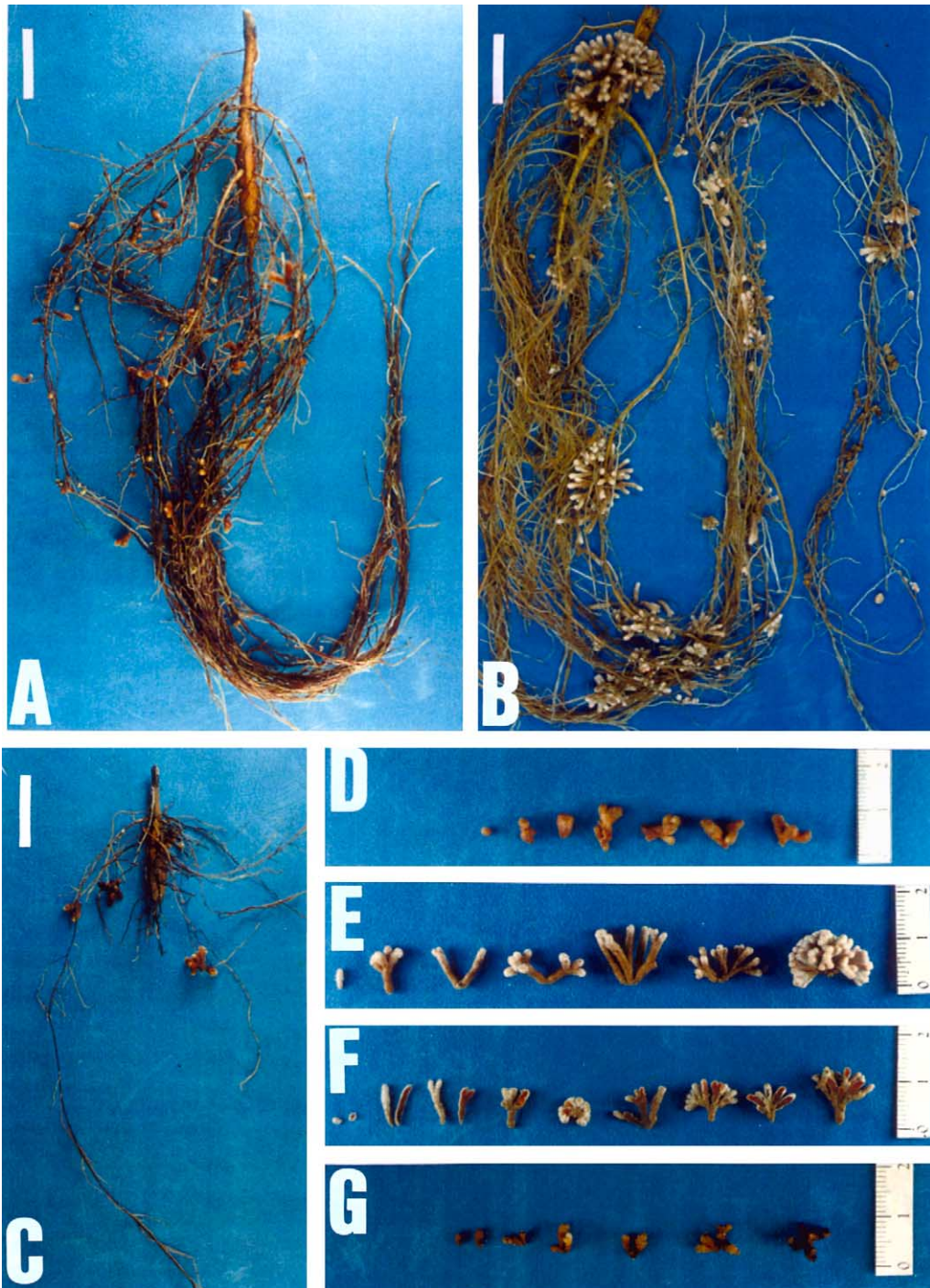


Fig. 2. Nodulated roots at 120 days: (A) *A. colubrina* (2 cm); (B) *M. bimucronata* (2 cm); (C) *P. rigida* (2 cm). Nodules morphology: (D) *A. colubrina*; (E) *M. bimucronata*; (F) *M. bimucronata* nodule slices showing internal reddish hue due to leghemoglobin; (G) *P. rigida*.

Table 3

Nodule number per diameter size, larger than 4 mm, between 2 and 4 mm and smaller than 2 mm, ARA and leghemoglobin content of *A. colubrina*, *M. bimucronata* and *P. rigida* at 120 days

| Treatments | Mean number of nodules | | | | ARA ^a | LegHb ^b |
|-----------------------|------------------------|-----------------|------|----------|------------------|--------------------|
| | >4 | Between 2 and 4 | <2 | Total | | |
| <i>A. colubrina</i> | | | | | | |
| NP | 0.8 | 3.6 | 1 | 5.4 b | 0.027 | n.d. |
| P | 2.2 | 3.4 | 0.6 | 6.2 b | 0.025 | n.d. |
| P + r | 2.6 | 42.8 | 63.6 | 109 a | 0.092 | 0.642 |
| P + rm | 0.8 | 4 | 7.4 | 12.2 b | 0.027 | n.d. |
| <i>M. bimucronata</i> | | | | | | |
| NP | 33 | 118.2 | 99.4 | 250.6 a | 10.545 | 0.381 |
| P | 73.4 | 117 | 64.4 | 254.8 a | 10.764 | 0.347 |
| P + r | 17.2 | 66.8 | 47 | 131 ab | 4.258 | 0.822 |
| P + rm | 18 | 41.4 | 12.6 | 72 abc | 8.112 | 0.714 |
| N | 1 | 7.2 | 18.4 | 26.6 bcd | 0.67 | n.d. |
| N + m | 0 | 0.8 | 2 | 2.8 d | n.d. | n.d. |
| N + rm | 0 | 0 | 7.8 | 7.8 cd | n.d. | n.d. |
| <i>P. rigida</i> | | | | | | |
| P + r | 0.8 | 2.2 | 5.6 | 8.6 a | 0.040 | n.d. |
| P + rm | 0 | 0.6 | 1.4 | 2 b | n.d. | n.d. |

Means followed by the same letter in the column are not significantly different by ANOVA, Dunn test at $P < 0.05$.

^a Acetylene reduction activity in $\times 10^{-2}$ $\mu\text{mol C}_2\text{H}_4$ per plant h^{-1} .

^b Leghemoglobin content in mg Hb g^{-1} fresh nodule.

Table 4

Nodule number per diameter size larger than 4 mm, between 2 and 4 mm and smaller than 2 mm, ARA, leghemoglobin content and nodule dry weight of *A. colubrina*, *M. bimucronata* and *P. rigida* at 255 days

| Treatments | Mean number of nodules | | | | ARA ^a | LegHb ^b | Nodule dry weight | | | |
|-----------------------|------------------------|-----------------|--------|----------|------------------|--------------------|-------------------|-----------------|------|--------|
| | >4 | Between 2 and 4 | <2 | Total | | | >4 | Between 2 and 4 | <2 | Total |
| <i>A. colubrina</i> | | | | | | | | | | |
| NP | 3 | 7.2 | 9.6 | 19.8 b | 151.9 | | 0.08 | 0.04 | 0.01 | 0.13 a |
| P | 5.6 | 9.2 | 25.2 | 40 b | 1098.8 | 0.483 | 0.16 | 0.03 | 0.01 | 0.2 a |
| P + r | 12.8 | 36.2 | 75.8 | 124.8 a | 2022.2 | 0.450 | 0.19 | 0.18 | 0.05 | 0.42 a |
| P + rm | 8.8 | 11.8 | 9 | 29.6 b | 677.9 | 0.481 | 0.23 | 0.09 | 0 | 0.32 a |
| <i>M. bimucronata</i> | | | | | | | | | | |
| NP | 99.2 | 310.6 | 278.6 | 688.4 b | 1633.2 | 0.533 | 0.92 | 0.9 | 0.25 | 2.07 b |
| P | 149.2 | 245.6 | 155.4 | 550.2 b | 13907.3 | 0.512 | 2.95 | 1.07 | 0.18 | 4.2 a |
| P + r | 145.8 | 473.4 | 836.2 | 1455.4 a | 66160.6 | 1.023 | 2.35 | 1.94 | 0.76 | 5.05 a |
| P + rm | 41 | 366.8 | 1844.4 | 2252.2 a | 80416.3 | 0.892 | 1.06 | 1.34 | 1.61 | 4.02 a |
| N | 2 | 30.2 | 160.8 | 193 bc | 377.3 | 0.455 | 0 | 0.05 | 0.11 | 0.15 c |
| N + m | 0.2 | 7.8 | 133.4 | 141.4 c | 1251.5 | n.d. | 0 | 0.02 | 0.03 | 0.04 c |
| N + rm | 0 | 0.8 | 37 | 37.8 c | n.d. | n.d. | 0 | 0 | 0.01 | 0.01 c |
| <i>P. rigida</i> | | | | | | | | | | |
| P + r | 0 | 0.6 | 5.2 | 5.8 a | 128.5 | n.d. | 0 | 0 | 0 | 0 a |
| P + rm | 1 | 2.2 | 10 | 13.2 a | 292.2 | n.d. | 0.01 | 0 | 0.01 | 0.02 a |

Means followed by the same letter in the column are not significantly different by ANOVA, test Dunn at $P < 0.05$.

^a Acetylene reduction activity in $\times 10^{-2}$ $\mu\text{mol C}_2\text{H}_4$ per plant h^{-1} .

^b Leghemoglobin content in mg Hb g^{-1} nodule fresh.

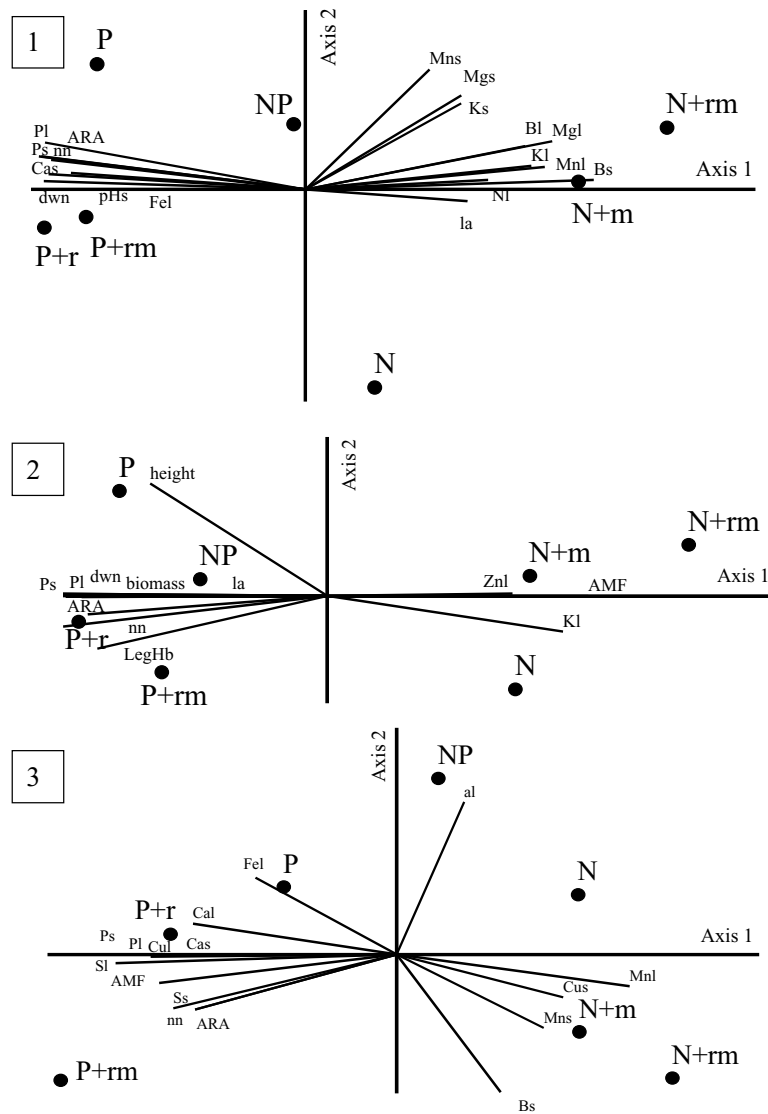


Fig. 3. Principal component analysis of mineral fertilization treatments (NP, P and N) with inoculation of rhizobia (r), mycorrhiza (m) or both (rm). Leaf area (LA), height, biomass, nodule number (NN), dry weight nodules (DWN), acetylene reduction activity (ARA), leghemoglobin content (LegHb) and mycorrhizal percentage (AMF) were performed for *A. colubrina* (1), *M. bimucronata* (2) and *P. rigida* (3). s: substrate; l: leaf; macroelements: N, P, K, Ca, S; microelements: B, Mn, Zn, Cu and Fe.

M. bimucronata plants. This fact indicates that the treatments with N added (without P) has higher levels of AMF colonization and K contents. In contrast, the vector representing AMF colonization in *P. rigida* pointed to the left of origin.

Mn and B were positively correlated with mycorrhizal treatments in *A. colubrina*, Zn in *M. bimucronata*, and S, Cu and Fe in *P. rigida* (Fig. 3).

4. Discussion

The results showed that *M. bimucronata* plants grew better than *A. colubrina* and *P. rigida* and also had the highest values for root colonization by AMF. Also, *M. bimucronata* was profusely nodulated, particularly when inoculated with rhizobia. Native rhizobia were able to promote high levels of infection in this species

probably because the soil used in the experiment was collected near to nodulated *M. bimucronata* plant populations. This species is a typical pioneer and produces small seeds, about 105,000 seeds kg^{-1} (Lorenzi, 1998). Some authors have suggested that small-seeded species have seedlings with a high relative growth rate (Gross and Smith, 1991; Siqueira et al., 1998). The small seeds and therefore the low cotyledon reserves can have an influence on the initial biomass allocation of seedlings to leaves and stems in this species as *Acacia cochliantha* (Cervantes et al., 1998). *A. colubrina* and *P. rigida* had larger seeds, about 15,600 and 38,600 seeds kg^{-1} (Lorenzi, 1992), respectively.

In this experiment we did not see an effect of AMF inoculation on root colonization and P uptake. Probably the fertilization treatments influenced both inoculated and native fungi in a similar way. *A. colubrina* and *M. bimucronata* plants had low AMF colonization in P fertilizer treatments. This result is consistent with the suggestion that nutritionally adequate or high P supply tends to reduce colonization (Smith and Read, 1997; Siqueira et al., 1998) with the magnitude of the effect varying between plant species and also being sensitive to change in environmental parameters (Smith and Read, 1997). In contrast, Paron et al. (1997) observed beneficial growth responses in *Trema micrantha* (L.) Blume inoculated by AMF, with addition of 100 mg kg^{-1} of P. A similar response seems to have occurred in *P. rigida* in our experiment, whereas a high percentage of mycorrhizal colonization in P fertilizer treatments was noted. The substrate P level, in P treatments for this species resulted in about 64–97 mg dm^{-3} (see Table 2). Burity et al. (2000) noted that addition of P level enhanced AMF root colonization in *M. caesalpinifolia* in both 20 and 40 kg ha^{-1} of P_2O_5 . Also, P addition (50–200 mg dm^{-3}) did not affect the colonization of *Dalbergia nigra* (Chaves et al., 1995). These variable responses to P level in different species leads to requirements of more experiments on legume trees to recognize the optimal P level to fungi colonization for each species of fungi and host plant.

In *A. colubrina*, AMF colonization was relatively low (0–14%). Carneiro et al. (1998) found 20–49% for *A. falcata* and 1–19% for *A. peregrina* in nursery conditions. Apart from nutritional factors, this low infection rate may be associated with specificity

between host plant and fungi. On the other hand, our values of mycorrhizal colonization of *M. bimucronata* (12.8–70.3%) and *P. rigida* (20.9–58.6%) were similar to those reported by Siqueira (1998), who found AMF colonization in eight pioneer species to be generally high (mean of 60%), and by Frioni et al. (1999) in root segments of *Mimosa* spp. (60%) and *P. rigida* (50%) in the field. However, the uptake of P by AMF in N treatments (without P added) was not sufficient to sustain good growth of plants.

Information on the association of host plants with specific AMF is ambiguous; while some species of AMF have a wide distribution among host plants, others have been found in rhizospheres of a single host plant (Carrenho et al., 2002). In *P. rigida*, for instance, Trufem (1990) observed the occurrence of only two *Glomus* species.

Nutrient contents by PCA analysis showed positive correlations between the percentage of K, Mn, B, S, Zn, Cu and Fe, and AMF. Carneiro et al. (1996) observed higher contents of S and Mn in mycorrhizal *Cassia rosa* plants. According to Redente and Reeves (1981), plants colonized by mycorrhiza can have higher concentrations of Zn and Cu.

A. colubrina and *P. rigida* were inoculated with rhizobia isolated from *A. peregrina*. This may have limited the infection or the satisfactory development of nodules, particularly in *P. rigida*, where nodulation was negligible.

The high capacity for nodulation in *A. colubrina* and *M. bimucronata* plants with mineral P added could be related to nutrition, if P is an essential element to enhance biological nitrogen fixation or it might be due the N absence, and therefore unable to inhibit nodulation and nitrogen fixation. The increase of mineral nitrogen diminished both the number of nodules and nitrogenase activity, as measured by ARA, in *Sesbania rostrata*, although a starter dose of 30 kg N ha^{-1} had positive effects (Becker et al., 1991). Mendonça and Schiavinato (1996) found higher dry mass of *A. colubrina* and *A. peregrina* in soil with 20 mg of $(\text{NH}_4)_2\text{SO}_4$ than without N. More research is needed to find the adequate N dose in different species with different sizes of seed and N content.

Frioni et al. (1998) suggested that environmental constraints such as P deficiency could explain the failure in nodulation of *P. rigida*. Nitrogenase activity (ARA) for this species was 24,200 μmol

ethylene $\text{h}^{-1} \text{g}^{-1}$ fresh nodule h^{-1} , which is considered to be low compared with crop legumes. In contrast, *M. bimucronata* was found here to have ARA of about $8.0 \times 10^4 \mu\text{mol}$ ethylene $\text{h}^{-1} \text{g}^{-1}$ fresh nodule h^{-1} at 255 days of sowing. Although low values of ARA that we found in *P. rigida* and *A. colubrina* can be due to many factors, such as P deficiency or ineffective rhizobia, we believe that high rates of N_2 fixation are less essential for perennial than annual species, according to Sprent (1994). We also need to consider the size and morphology of nodules when discussing their activity and potential efficiency. In spite of having found some nodules larger than 4 mm in size with elevated values of dry weight in uninoculated treatments of *A. colubrina* and *M. bimucronata*, these treatments did not show high ARA or leghemoglobin content at the time of measurement. Also, indeterminate nodules have meristematic regions and the volume of effective tissue per nodule is smaller than in determinate ones. As a result, nitrogenase activity of rhizobia may be underestimated in indeterminate nodules.

When trees are being grown in nurseries, the methods of cultivation and the extent of rhizobial and mycorrhizal symbiosis can affect post-planting success, particularly when the trees are destined for disturbed lands or regeneration of riparian forest. Because of this, we suggest that *M. bimucronata* may be more adapted to N-limited environments, mainly when inoculated with specific rhizobia and mycorrhizal fungi. Although physiological aspects of seedling growth remain to be studied in detail and dual inoculation with rhizobia and mycorrhizal fungi is not a common practice, we also suggest dual inoculation in this species in the nursery and subsequent use to reforest riparian forests. Dual inoculation also gives good results in *A. colubrina* and *P. rigida* when specific mycorrhizal fungi are used.

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