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Growth performance and nodulation response of *Acacia mangium* co-inoculated with *Bradyrhizobium* sp. and *Pisolithus tinctorius*

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Abstract
A study was conducted to find out if co-inoculation with N_2-fixing *Bradyrhizobium* sp. and P-solubilizing ectomycorrhizal *Pisolithus tinctorius* can provide the synergistic effects for the growth of *Acacia mangium* under both N and P deficient conditions. Total dry matter production, and N and P contents were significantly higher in seedlings that received dual inoculation in the absence of both mineral N and soluble P than in uninoculated control seedlings that received similar nutrient supply. Individual inoculation of *Bradyrhizobium* or *P. tinctorius* failed to enhance seedling growth under both N and P deficiency. Seedlings that received dual inoculation also performed better than uninoculated control seedlings that received both nutrients in the available form and seedlings that received *Bradyrhizobium* and soluble P. However, presence of *P. tinctorius* did not have a stimulatory effect on nodule formation and functioning. Percentage dry matter allocated to nodules, specific nodule number (number of nodules formed g^{-1} of total dry matter produced) and specific nitrogenase activity (mmoles ethylene produced $h^{-1}g^{-1}$ of nodule dry weight) were significantly lower in seedlings that received dual inoculation when compared to seedlings that received *Bradyrhizobium* and soluble P. Relatively poor formation of nodules in seedlings that received dual inoculation could be due to competition for carbon between the plant and microbial symbionts in this tripartite symbiotic association. Future studies should focus on carbon partitioning between these two microbial symbionts during their association with the host plant. Also, studies are required to identify the fungal compounds that may be involved in restriction or regulation of nodule growth during ectomycorrhiza formation.

Keywords: *Acacia mangium*, *Bradyrhizobium* sp., *Pisolithus tinctorius*, tripartite symbiosis, nodulation

1. Introduction

As low N and P availabilities are typical of forest ecosystems (Attiwill and Adams, 1993; Helmisaari, 1990), integration of leguminous trees that can form symbiotic associations with both rhizobia and mycorrhizal fungi into agro forestry and silvo-pastoral systems has been suggested as a possible solution to the problems faced in reforestation (Marques et al., 2001). *Acacia mangium* is a leguminous tree that can form symbiotic association with both N_2-fixing *Bradyrhizobium* sp. and P-solubilizing ectomycorrhizal *Pisolithus tinctorius* (Pers) Coker and Couch. Individual inoculations of *Bradyrhizobium* (Fremont et al., 1999; Prin et al., 2003) and *P. tinctorius* (Jayakumar and Tan, 2005) have been reported to enhance the growth of *A. mangium* when compared to uninoculated seedlings.

Dual inoculation with arbuscular mycorrhizal (AM) fungi and *Rhizobium* significantly enhanced root nodulation in field crops (El Ghandour et al., 1996; Ianson and Linderman, 1993; Rahman and Parsons, 1997) and woody legumes (André et al., 2003; Marques et al., 2001) more than inoculation with either mycorrhizal fungi or *Rhizobium*. Similarly, dual inoculation of *A. mangium* with *P. albus* and *Bradyrhizobium* enhanced the growth of the seedlings (Duponnois et al., 2002). On the contrary, it has been reported that co-inoculation of *A. mangium* with AM fungi (*Glomus intraradices*) negatively affected nodule formation by *Bradyrhizobium* (Weber et al., 2005). Similarly, Bâ et al. (1994) found that inoculation of *P. tinctorius* to *A. holosericea* seedlings prior to inoculation with *Bradyrhizobium* resulted in the inhibition of nodule formation. Hence, the present study was undertaken to find out if co-inoculation with *Bradyrhizobium* and *P. tinctorius* can provide the synergistic effects for growth of

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A. mangium seedlings under both N and P deficient conditions, and also to find out the nodulation response of *A. mangium* seedlings to co-inoculation.

2. Materials and Methods

Culture maintenance and inoculum production

Bradyrhizobium (Strain WAS 9) was stored on modified Yeast extract-mannitol (YM) agar plates at 4°C and used as the stock culture. Ten-day-old cultures on YM agar plates incubated at 27°C were used as mother cultures. *Bradyrhizobium* cultures on plates were transferred to sterile distilled water with 0.5% glucose and adjusted to a final concentration of 10^9 cells ml^{-1} by measuring the optical density of the suspension at 650 nm as described by Cooper (1979) and Hoben and Somasegaran (1982). *Pisolithus tinctorius* (Isolate P53) was cultured on modified Melin-Nokran's (MMN) (Marx, 1969) agar plates, stored at 4°C, and sub-cultured every 2 months. Twenty-day-old colonies on MMN agar plates incubated at 27°C were used as mother cultures. Vegetative mycelial inoculum was prepared according to the procedures described by Marx and Bryan (1975).

Pot culture experiment

Acacia mangium seeds were surface sterilized in 95% sulphuric acid for 30 min, rinsed with sterile distilled water, and germinated on 1% water agar at 25°C in the dark. One 14-d-old healthy seedling was transferred into a plastic cup containing 200 g of sterilized, water-washed river sand and 50 g of peat moss. Uninoculated control 1 seedlings were grown in the absence of mineral N and soluble P while uninoculated control 2 seedlings were grown in the presence of both mineral N and soluble P. Treatment 1 and 2 seedlings were inoculated with N_2-fixing *Bradyrhizobium* (WAS 9) in the absence and presence of soluble P, respectively. Mineral N was not given to treatment one and two seedlings. Treatment 3 and 4 seedlings were inoculated with P-solubilizing ectomycorrhizal *P. tinctorius* (P53) in the absence and presence of mineral N, respectively. Soluble P was not given to treatment three and four seedlings. Treatment 5 seedlings were inoculated with both *Bradyrhizobium* and *P. tinctorius* in the absence of mineral N and soluble P. Each seedling that did not receive soluble P was given 56 mg of mussori rock phosphate at the time of transplantation. 20.4 mg of NH_4NO_3 (mineral N) and/or 2.1 mg of KH_2PO_4 (soluble P) were given in solution at 15 d interval to each seedling according to the treatment conditions. *Bradyrhizobium*-inoculated seedlings received 2 ml of suspension of *Bradyrhizobium* with 10^9 cells ml^{-1}. *Pisolithus tinctorius* inoculation was done by mixing 40 g of vermiculite-peat moss-vegetative mycelium mixture to the rooting medium. Seedlings that were not inoculated with *P. tinctorius* received a mixture of moistened vermiculite-peat moss without any fungal mycelium. Fifteen replicate plants were set up for each treatment and uninoculated control. All the plants were supplied with 30 ml of sterile N-free and P-free nutrient solution, pH 6.8 (modified from Broughton and Dilworth, 1971) once in 15 d. The seedlings were watered with sterile distilled water and the irrigation regime was varied as required to maintain moisture conducive to seedling growth.

Pre- and post-harvest analysis

The chlorophyll fluorescence parameters, i.e. minimum fluorescence (Fo), maximum fluorescence (Fm), and variable fluorescence (Fv), and the fluorescence ratios, Fv/Fm, Fv/Fo and Fm/Fo were determined for 3 consecutive days before harvesting. A portion (diameter = 0.5 cm) of the fully developed phyllode was covered with a plastic clip at 10.00 am in the morning to avoid exposure to light and left for 1 h. The chlorophyll fluorescence in that portion, immediately on exposure to light, was measured using a Plant Efficiency Analyzer (Model - PEA MK2, Hansatech, England). The plants were harvested after 4 months and the N_2-fixing efficiency of the nodules was analyzed on the entire root system by measuring the Acetylene Reduction Activity according to the procedure described by Turner and Gibson (1980). Although Acetylene Reduction Activity measured in closed vessels does not represent the true nitrogenase activity (Chang et al., 1994; Minchin et al., 1983), it can be appropriate, however, in assays for comparative purposes (Becana et al., 1986; Irigoyen et al., 1992). The number of nodules per plant was recorded and the plant materials were dried in an oven with air circulation at 60°C for 72 h and dry weights were recorded. The dried samples were digested (Novasamzky et al., 1983) and N (Novasamzky et al., 1974) and P contents (Allen, 1989) were analyzed. Statistical analyses were performed using SPSS program. A multiple range analysis was used to test for significant differences between treatments using Duncan's procedure at $P \leq 0.05$.

3. Results and Discussion

Total dry weight, total N and P contents, photosynthetic efficiency and nodulation response of 4-month-old *A. mangium* seedlings co-inoculated with *Bradyrhizobium* and *P. tinctorius* in absence of mineral N and soluble P are presented in Tables 1, 2 and 3.

Seedlings that received dual inoculation and grown in the absence of N and soluble P showed significantly higher dry matter accumulation, and N and P contents when compared to uninoculated control seedlings grown under similar conditions (Table 1). Individual inoculation of *Bradyrhizobium* or *P. tinctorius* failed to enhance seedling growth under both N and P deficiency. Synergistic effects of

Table 1. Total dry weight, and N and P contents of 4-month-old *A. mangium* seedlings co-inoculated with *Bradyrhizobium* and *P. tinctorius* in the absence of mineral N and soluble P. Values are means ± SE (n=15). Values followed by same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. RP, Mussori rock phosphate; MN, Mineral N (NH_4NO_3); SP, Soluble P (KH_2PO_4); Brady, *Bradyrhizobium* WAS9; Pt, *P. tinctorius* P53.

Treatments	Seedling dry weight (g)	Seedling N content (mg)	Seedling P content (mg)	Shoot N concentration (%)	Shoot P concentration (%)
Uninoculated control 1 (RP)	0.38 ± 0.03a	5.1 ± 0.5a	0.09 ± 0.01a	1.94 ± 0.07ab	0.027 ± 0.0005a
Uninoculated control 2 (MN, SP)	1.03 ± 0.05b	25.6 ± 1.9b	0.37 ± 0.02b	2.66 ± 0.12d	0.040 ± 0.0004d
Treatment 1 (Brady, RP)	0.37 ± 0.03a	5.7 ± 0.6a	0.09 ± 0.01a	1.82 ± 0.05a	0.027 ± 0.0003a
Treatment 2 (Brady, SP)	1.12 ± 0.04b	23.8 ± 0.9b	0.43 ± 0.02b	2.29 ± 0.02c	0.043 ± 0.0004e
Treatment 3 (Pt, RP)	0.42 ± 0.03a	7.6 ± 0.6a	0.14 ± 0.01a	2.12 ± 0.04bc	0.044 ± 0.0004e
Treatment 4 (Pt, RP, MN)	3.01 ± 0.09c	57.5 ± 1.6d	0.91 ± 0.03c	2.13 ± 0.05bc	0.031 ± 0.0006b
Treatment 5 (Brady, Pt, RP)	2.82 ± 0.09c	53.1 ± 1.9c	0.89 ± 0.03c	2.16 ± 0.01c	0.036 ± 0.0004c

Table 2. Photosynthetic efficiency of 4-month-old *A. mangium* seedlings co-inoculated with *Bradyrhizobium* and *P. tinctorius* in the absence of mineral N and soluble P. Values are means ± SE (n=15). Values followed by same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. ND, Not detected; for other abbreviations, see Table 1.

Treatments	Fv/Fm[a]	Fv/Fo[a]	Fm/Fo[a]
Uninoculated control 1 (RP)	ND	ND	ND
Uninoculated control 2 (MN, SP)	0.833 ± 0.004b	5.1 ± 0.11b	6.1 ± 0.11b
Treatment 1 (Brady, RP)	ND	ND	ND
Treatment 2 (Brady, SP)	0.835 ± 0.005bc	5.2 ± 0.13bc	6.2 ± 0.13bc
Treatment 3 (Pt, RP)	0.814 ± 0.008a	4.5 ± 0.22a	5.5 ± 0.22a
Treatment 4 (Pt, RP, MN)	0.845 ± 0.002cd	5.5 ± 0.07cd	6.5 ± 0.07cd
Treatment 5 (Brady, Pt, RP)	0.850 ± 0.001d	5.7 ± 0.05d	6.7 ± 0.05d

[a]Higher value means higher photosynthetic efficiency.

Table 3. Nodulation and N_2 fixation in 4-month-old *A. mangium* seedlings co-inoculated with *Bradyrhizobium* and *P. tinctorius* in the absence of mineral N and soluble P. Values are means ± SE (n=15). Values followed by same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. ND, Not detected; for other abbreviations, see Table 1.

Treatments	Nodule dry weight (g)	Number of nodules	Total nitrogenase activity[a]	Dry matter allocated to nodules (%)	Specific nodule number[b]	Specific nitrogenase activity[c]
Uninoculated control 1 (RP)	0.002 ± 0.001a	3 ± 1a	ND	0.71 ± 0.29a	8 ± 3ab	ND
Uninoculated control 2 (MN, SP)	0.010 ± 0.003a	10 ± 2bc	ND	0.99 ± 0.30a	10 ± 2ab	ND
Treatment 1 (Brady, RP)	0.003 ± 0.001a	5 ± 1ab	ND	1.05 ± 0.50a	15 ± 7bc	ND
Treatment 2 (Brady, SP)	0.086 ± 0.004b	54 ± 3d	0.40 ± 0.01a	7.78 ± 0.34c	50 ± 3d	4.6 ± 0.3b
Treatment 3 (Pt, RP)	0.003 ± 0.001a	4 ± 1ab	ND	0.76 ± 0.24a	12 ± 3abc	ND
Treatment 4 (Pt, RP, MN)	0.010 ± 0.002a	11 ± 2c	ND	0.33 ± 0.06a	4 ± 1a	ND
Treatment 5 (Brady, Pt, RP)	0.150 ± 0.006c	59 ± 3d	0.42 ± 0.04a	5.36 ± 0.22b	21 ± 1c	2.9 ± 0.3a

[a]Expressed as mmoles ethylene produced h^{-1} $plant^{-1}$; [b]expressed as number of nodules formed g^{-1} of total dry matter produced; [c]expressed as mmoles ethylene produced h^{-1} g^{-1} of nodule dry weight.

inoculation of legumes with AM mycorrhiza and rhizobia in low P soils on the whole plant growth are well documented (Azimi et al., 1980; Brown et al., 1988; El Ghandour et al., 1996; Subba Rao et al., 1986). Similar effects using ectomycorrhizal *P. tinctorius* and *Bradyrhizobium* under both N and P deficient conditions are clearly demonstrated from the present study. Seedlings that received dual inoculation also performed well when compared to uninoculated control seedlings that received both nutrients in the available form and seedlings that received *Bradyrhizobium* and soluble P (Table 1). Seedlings that received dual inoculation had higher Fv/Fm, Fv/Fo and Fm/Fo ratios when compared to other seedlings (Table 2) indicating higher photosynthetic quantum yield (Babani and Lichtenthaler, 1996). Additionally, seedlings that received dual inoculation showed significantly higher nodule dry matter when compared to seedlings that received *Bradyrhizobium* and soluble P (Table 3). Legumes

inoculated with both rhizobia and mycorrhizal fungi benefit from P uptake and have greater nodule mass leading to higher N, P and dry matter accumulation than legumes inoculated only by rhizobia (Barea and Azcon-Aguilar, 1983; Robson et al., 1981).

Although seedlings that received dual inoculation had a significantly higher nodule dry matter when compared to seedlings that received *Bradyrhizobium* and soluble P, there was no significant increase in the number of nodules formed and nodule activity (Table 3). The responsiveness of nodule dry weight per plant than of nodule number and activity indicates that co-inoculation with *P. tinctorius* increased nodule dry matter by stimulating host plant growth rather than by exerting specific effects on rhizobial growth and survival or on nodule formation and functioning. Moreover, the percentage of total dry matter allocated to nodules, number of nodules formed g^{-1} of total dry matter produced and N_2-fixing efficiency of the nodules were significantly lower in seedlings that received dual inoculation when compared to seedlings that received *Bradyrhizobium* and soluble P. Poor nodulation and N_2 fixation in soybean plants when inoculated with both AM fungi and *Rhizobium* has been reported earlier (Bethenfalvay et al., 1985; Brown and Bethenfalvay, 1987). Similarly, poor nodulation in *A. mangium* seedlings upon inoculation with AM *Glomus intraradices* (Weber et al., 2005) and in *A. holosericea* seedlings upon inoculation with ectomycorrhizal *P. tinctorius* (Bâ et al., 1994) have been reported earlier. Development of nodules and fungal hyphae depends on the supply of carbon by the host plant (Cooper, 1984; Ho and Trappe, 1973). Several studies have shown that there is a competition for carbon between plants, mycorrhiza and bacteria in symbiotic N_2-fixing systems (Bayne et al., 1984; Bethenfalvay et al., 1985; Michelsen and Sprent, 1994; Reinhard et al., 1992). These studies indicate that relatively poor nodule formation in *A. mangium* seedlings that received dual inoculation could be due to the competition for carbon between the plant and microbial symbionts.

Alternatively, it has been reported that mycorrhizal roots have a depressive effect on the rhizosphere bacterial population when compared to non-mycorrhizal ones (Ames et al., 1984; Meyer and Linderman, 1986), which indicates that the compounds produced by the fungus during ectomycorrhiza formation can have a direct inhibitory effect on the nodulating bacteria. Ectomycorrhizal fungi secrete organic acids, especially oxalic acid and citric acid, which significantly reduce the pH of the rhizosphere soil (Arocena and Glowa, 2000; Griffiths et al., 1994; Wallander, 2000). Although gross concentrations of organic anions in the soil solution may appear insufficient to cause a significant reduction in the pH, higher concentrations are likely to be present in microenvironments surrounding fungal hyphae (Drever and Stillings, 1997). It has been reported that individual organic acids in the soil solution exceeds millimolar concentrations (Fox and Comerford, 1990; Stevenson, 1967), with extremely high concentrations in the vicinity of certain plants and fungal hyphae (Cromack et al., 1979; Gardener et al., 1983). Reduction in rhizosphere pH due to production of organic acids by the ectomycorrhizal fungi could be another reason for the poor formation of nodules as the bacterial multiplication in the soil and the nodulation process are very sensitive to low pH (Whelan and Alexander, 1986; Wolff et al., 1993). Poor formation of nodules could also be due to morphological and biochemical changes in the roots due to mycorrhizal colonization (Martin and Hilbert, 1991). There is little or no information available on the direct interactions between these two microsymbionts in the rhizosphere and their processes in the host system. Studies are required to identify the fungal compounds that may be involved in restriction or regulation of nodule growth during ectomycorrhiza formation.

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REFERENCES

Allen, S.E. 1989. Analysis of vegetation and other organic materials. In: *Chemical Analysis Of Ecological Materials*. Blackwell Scientific Publication, Oxford, UK, pp. 46–61.

Ames, R.N., Reid, C.P.P., and Ingham, E.R. 1984. Rhizosphere bacterial population responses to root colonization by vesicular arbuscular mycorrhizal fungus. *New Phytologist* **96**: 555–563.

André, S., Neyra, M., and Duponnois, R. 2003. Arbuscular mycorrhizal symbiosis changes the colonization pattern of *Acacia tortilis* spp. Raddiana rhizosphere by two strains of rhizobia. *Microbial Ecology* **45**: 137–144.

Arocena, J.M. and Glowa, K.R. 2000. Mineral weathering in ectomycorrhizosphere of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) as revealed by soil solution composition. *Forest Ecology and Management* **133**: 61–70.

Attiwill, P.M. and Adams, M.A. 1993. Nutrient cycling in forests. *New Phytologist* **124**: 561–582.

Azimi, S., Gianinazzi-Pearson, V., and Gianinazzi, S. 1980. Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhiza and *Rhizobium* in soybeans. *Canadian Journal of Botany* **58**: 2200–2205.

Bâ, A.M., Balaji, B., and Piché, Y. 1994. Effect of time of inoculation on *in vitro* ectomycorrhizal colonization and nodule initiation in *Acacia holosericea* seedlings. *Mycorrhiza* **4**: 109–119.

Babani, F. and Lichtenthaler, H.K. 1996. Light-induced and age-dependant development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO_2 assimilation and different

kinds of chlorophyll fluorescence ratios. *Journal of Plant Physiology* **148**: 555–566.

Barea, J.M. and Azcon-Aguilar, C. 1983. Mycorrhizas and their significance in nodulating nitrogen fixing plants. *Advances in Agronomy* **36**: 1–54.

Bayne, H.G., Brown, M.S., and Bethenfalvay, G.J. 1984. Defoliation effects on mycorrhizal colonization, nitrogen fixation, and photosynthesis in the *Glycine-Glomus-Rhizobium* symbiosis. *Physiologia Plantarum* **62**: 576–580.

Becana, M., Aparicio-Tejo, P., Peña, P., Aguirreolea, J., and Sánchez-Díaz, M. 1986. N_2 fixation (C_2H_2-reducing activity) and leghemoglobin content during nitrate and water stress induced senescence of *Medicago sativa* root nodules. *Journal of Experimental Botany* **37**: 597–605.

Bethenfalvay, G.J., Brown, M.S., and Stafford, A. 1985. The *Glycine-Glomus-Rhizobium* symbiosis. II. Antagonistic effects between mycorrhizal colonization and nodulation. *Plant Physiology* **79**: 1054–1058.

Broughton, W.J. and Dilworth, M.J. 1971. Control of leghaemoglobin synthesis in snake bean. *Journal of Biochemistry* **125**: 1075–1080.

Brown, M.S. and Bethenfalvay, G.J. 1987. The *Glycine-Glomus-Rhizobium* symbiosis. VI. Photosynthesis in nodulated, mycorrhizal, or N- and P-fertilized soy bean plants. *Plant Physiology* **85**: 120–123.

Brown, M.S., Thamsurakul, S., and Bethenfalvay, G.J. 1988. The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Phosphorus-use efficiency of CO_2 and N_2 fixation in mycorrhizal soybean. *Physiologia Plantarum* **74**: 159–163.

Chang, Y.K., Barraquio, W., and Knowles, R. 1994. N_2-fixing pseudomonads and related soil bacteria. *FEMS Microbiology Review* **13**: 95–118.

Cooper, J.E. 1979. Rapid method for counting antibiotic-resistance *Rhizobia* in soils. *Soil Biology and Biochemistry* **11**: 433–435.

Cooper, K.M. 1984. Physiology of VA-mycorrhizal associations. In: *VA Mycorrhizae*. Powell, C.L. and Bagyaraj, D.J., eds. Chemical Rubber Co. Press, Boca Ratan, Fla, pp. 155–203.

Cromack, K., Sollins, P., Graustein, W.C., Speidel, K., Todd, A.W., Spycher, G., Li, C.Y., and Todd, R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of hypogeous fungus, *Hysterangium crassum*. *Soil Biology and Biochemistry* **11**: 463–468.

Drever, J.I. and Stillings, L.L. 1997. The role of organic acids in mineral weathering. *Colloids and Surfaces A: Physiochemical and Engineering Aspects* **120**: 167–181.

Duponnois, R., Founoune, H., and Lesueur, D. 2002. Influence of the controlled dual ectomycorrhizal and rhizobial symbiosis on the growth of *Acacia mangium* provenances, the indigenous symbiotic microflora and the structure of plant parasitic nematode communities. *Geoderma* **109**: 85–102.

El Ghandour, I.A., El Sharawy, M.A.O., and Abdel Moniem, E.M. 1996. Impact of vesicular arbuscular mycorrhizal fungi and *Rhizobium* on the growth and P, N and Fe uptake by faba-bean. *Fertilizer Research* **43**: 43–48.

Fox, T.R. and Comerford, N.B. 1990. Low-molecular-organic acids in selected forest soils of the south eastern USA. *Soil Science Society of America Journal* **54**: 1139–1144.

Fremont, M., Prin, Y., Chauviére, M., Diem, H.G., Pwee, K.H., and Tan, T.K. 1999. A comparison of *Bradyrhizobium* strains using molecular, cultural and field studies. *Plant Science* **141**: 81–91.

Gardener, W.K., Barber, D.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L.: III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant and Soil* **70**: 107–204.

Griffiths, R.P., Baham, J.E., and Caldwell, B.A. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biology and Biochemistry* **26**: 331–337.

Helmisaari, H.S. 1990. Temporal variation in nutrient concentrations of *Pinus sylvestris* needles. *Scandinavian Journal of Forest Research* **5**: 177–193.

Ho, I. and Trappe, J.M. 1973. Translocation of ^{14}C from *Festuca* plants to their endomycorrhizal fungi. *Nature* **244**: 30–41.

Hoben, H.J. and Somasegaran, P. 1982. Comparison of the pour, spread and drop plate methods for enumeration of *Rhizobium* sp. in inoculants made from pre-sterilized peat. *Applied and Environmental Microbiology* **44**: 1246–1247.

Ianson, D.C. and Linderman, R.G. 1993. Variation in the response on nodulating pigeonpea (*Cajanus cajan*) to different isolates of mycorrhizal fungi. *Symbiosis* **15**: 105–119.

Irigoyen, J.J., Emerich, D.W., and Sánchez-Díaz, M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum* **84**: 67–72.

Jayakumar, P. and Tan, T.K. 2005. Phosphorus solubilization by ectomycorrhizal *Pisolithus tinctorius* in pure culture and in association with *Acacia mangium*. *Symbiosis* **39**: 125–130.

Marques, M.S., Pagano, M., and Scotti, M.R.M.M.L. 2001. Dual inoculation of a woody legume (*Centrolobium tomentosum*) with rhizobia and mycorrhizal fungi in south-eastern Brazil. *Agroforestry Systems* **52**: 107–117.

Martin, F.M. and Hilbert, J.L. 1991. Morphological, biochemical and molecular changes during ectomycorrhiza development. *Experientia* **47**: 321–331.

Marx, D.H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153–163.

Marx, D.H. and Bryan, W.C. 1975. Growth and mycorrhizal development of loblolly pine seedlings in fumigated soil infected with fungal symbiont *Pisolithus tinctorius*. *Forest Science* **21**: 245–254.

Meyer, J.R. and Linderman, R.G. 1986. Selective influence of populations of rhizosphere bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. *Soil Biology and Biochemistry* **18**: 191–196.

Michelsen, A. and Sprent, J. 1994. The influence of vesicular arbuscular mycorrhizal fungi on the nitrogen fixation of nursery grown Ethiopian acacias estimated by the ^{15}N natural abundance method. *Plant and Soil* **160**: 249–257.

Minchin, F.R., Witty, J.F., and Müller, M. 1983. A major error in the acetylene reduction assay: decreases in nodular nitrogenase activity under assay conditions. *Journal of Experimental Botany* **34**: 641–649.

Novozamsky, Houba, V.J.G, van Eck, R., and van Vark, W. 1983. A novel digestion technique for multi-element plant analysis. *Communications in Soil Science and Plant Analysis* **14**: 239–249.

Novozamsky, van Eck, R., van Schouwenburgn, J.Ch., and Walinga, I. 1974. Total nitrogen determination in plant material by means of indophenol blue method. *Netherland Journal of Agricultural Sciences* **22**: 3–5.

Prin, Y., Galiana, A., Le Roux, C., Méléard, B., Razafimaharo, V., Ducousso, M., and Chaix, G. 2003. Molecular tracing of *Bradyrhizobium* strains helps to correctly interpret *Acacia*

mangium response to inoculation in a reforestation experiment in Madagascar. *Biology and Fertility of Soils* **37**: 64–69.

Rahman, M.K. and Parsons, J.W. 1997. Effects of inoculation with *Glomus mossae*, *Azorhizobium caulinodans* and rock phosphate on the growth of and nitrogen and phosphorus accumulation in *Sesbania rostrata*. *Biology and Fertility of Soils* **25**: 47–52.

Reinhard, S., Martin, P., and Marschner, H. 1992. Interactions in the tripartite symbiosis of pea (*Pisum sativum* L.), *Glomus*, and *Rhizobium* under nonlimiting phosphorus supply. *Plant Physiology* **141**: 7–11.

Robson, A.D., O'Hara, G.W., and Abbott, L.K. 1981. Involvement of phosphorus in nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Plant Physiology* **8**: 427–436.

Stevenson, F.J. 1967. Organic acids in soil. In: *Soil Biochemistry* Volume 1. McLaren, A.D. and Peterson, G.H., eds. Marcel Dekker, New York, pp. 119–146.

Subba Rao, K.V., Tilak, B.R., and Singh, B.S. 1986. Dual inoculation with *Rhizobium* sp. and *Glomus fasciculatum* enhances nodulation, yield and nitrogen fixation in chick pea (*Cicer arietinum* Linn.). *Plant and Soil* **95**: 351–359.

Turner, G.L. and Gibson, A.H. 1980. Measurement of nitrogen fixation by indirect means. In: *Methods For Evaluating Biological Nitrogen Fixation*. Bergerson, F.J., ed. John Wiley and Sons, New York, pp. 111–138.

Wallander, H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. *Plant and Soil* **218**: 249–256.

Weber, J., Ducousso, M., Tham, F.Y., Nourissier-Mountou, S., Galiana, A., Prin, Y., and Lee, S.K. 2005. Co-inoculation of *Acacia mangium* with *Glomus intraradices* and *Bradyrhizobium* sp. in aeroponic culture. *Biology and Fertility of Soils* **41**: 233–239.

Whelan, A.M. and Alexander, M. 1986. Effects of low pH and high Al, Mn, and Fe levels on the survival of *Rhizobium trifolii* and the nodulation of subterranean clover. *Plant and Soil* **92**: 363–371.

Wolff, A.B., Singleton, P.W., Sidirelli, M., and Bohlool, B.B. 1993. Influence of acid soil on nodulation and interstrain competitiveness in relation to tannin concentrations in seeds and roots of *Phaseolus vulgaris*. *Soil Biology and Biochemistry* **25**: 715–721.