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Full Length Research Paper

A new process to promote the use of controlled mycorrhization practice in forest nurseries

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The aims of this study were to test a new mycorrhizal inoculation process using a "catalyser" of the mycorrhizal establishment (termite mounds of *Macrotermes subhyalinus*) to minimize the requested volume of fungal inoculum added to the cultural substrate. The effects of the termite mound were explored on mycorrhiza formation between an Australian Acacia, *Acacia holosericea* and an ectomycorrhizal fungus or an arbuscular mycorrhizal fungus using a two-step cultural system. The first step of this cultural practice was the inoculation of *A. holosericea* seedlings in small soil volumes (5 L plastic containers planted with 100 pre-germinated seeds) whereas the second one allowed the development of these mycorrhized plants in larger soil volumes (1 L pots planted with one seedling). Termite mound amendment significantly enhanced the mycorrhizal formation from both types of fungal isolates. This stimulating effect could probably be attributed to the introduction *via* the termite mound of a bacterial group (that is, fluorescent pseudomonads) that could act as Mycorrhiza Helper Bacteria (MHB). Since it is possible to reduce the requested fungal inoculum in controlled mycorrhization practice using *M. subhyalinus* mound powders, this biotechnological process could be useful in re-afforestation of tropical regions by lowering the requested fungal inoculum quantities and reducing the financial costs of controlled mycorrhization in forest nurseries.

Key words: Termitaria, fluorescent pseudomonads, controlled mycorrhization, Acacia holosericea.

INTRODUCTION

Mycorrhizal fungi are ubiquitous components of most ecosystems throughout the world and are considered key ecological factors in governing the cycles of major plant nutrients and in sustaining the vegetation cover (Requena et al., 2001; Schreiner et al., 2003). Two major morphological forms of mycorrhizas are usually distinguished namely arbuscular mycorrhizas (AM) and ectomycorrhizas (ECMs). AM symbiosis is the most widespread mycorrhizal association and is found in average on 80 to 90% of land plants in natural, agricultural and forest ecosystems (Brundrett, 2002). ECMs are observed on trees, on woody shrubs and on a small number of herbaceous plants (Dickie et al., 2004), either gymnosperms or angiosperms and usually result from the association between Homobasidiomycetes and about 20 families of mainly woody plants (Smith and Read, 1997).

Mycorrhizal fungi enhance the uptake of low mobility

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minerals such as phosphorus and micronutrients (Smith and Read, 1997) and water absorption (Georges et al., 1992; Bandou et al., 2006). They also improve plant health by providing protection against pathogens (Dehne, 1982; Morin et al., 1999) and heavy metal pollution (Chen et al., 2003; Ortega et al., 2004). Hyphae of mycorrhizal fungi also play a role in the formation and stability of soil aggregates (Wright and Upadhyaya, 1998; Caravaca et al., 2002) and contributes to the composition of plant community structures (van der Heijden et al., 1998; Simard and Durall, 2004). Hence, these symbiotic microorganisms have been found to be essential components of sustainable soil-plant systems (van der Heijden et al., 1998; Dunabeita et al., 2004).

In Mediterranean and tropical ecosystems, desertification has occurred following several decades of scarce and irregular rainfall and over exploitation of natural resources (Francis and Thornes, 1990). It mainly results from degradation of natural plant communities (population structure, succession pattern and species diversity) and of physico-chemical and biological soil properties (nutrient availability, microbial activity, soil structure, etc.) (Marx, 1991; Requena et al., 2001; Smith et al., 2005). These disturbances generally induce a loss or a reduction of mycorrhizal propagules in the soil and, consequently, decrease the mycorrhizal potential in the degraded areas (Mc Lellan et al., 1995; Dickie and Reich, 2005). Because of their main ecological functions, it has been suggested that the absence or diminution of mycosymbiont propagules altered the artificial or natural processes of revegetation (Marx, 1980; Requena et al., 2001).

To overcome this problem, it has already been shown that mycorrhizal inoculation of seedlings was very efficient in establishing plants at outplanting on disturbed soils (Estaun et al., 1997; Duponnois et al., 2007). Controlled mycorrhization is based on the use of mycorrhizal strains best suited to host plant species that rapidly colonize their root systems and are well adapted to the environmental conditions of the planting site (Perry et al., 1987). But this practice requires high quantities of mycorrhizal fungal inoculum. Hence this biotechnological process is generally associated with large financial investment that limits its use in forest nurseries, especially in developing countries.

In order to reduce the requested quantities of fungal inoculum, it has been previously suggested that some rhizosphere bacteria (Mycorrhiza Helper Bacteria) could improve the establishment of the fungal inoculant and consequently, minimize the volume of fungal inoculum added into the soil (Garbaye and Duponnois, 1992). More recently, it has been demonstrated that the termite mounds of *Macrotermes subhyalinus* (a litter-forager termiter commonly found in tropical areas) were inhabited by a specific microflora that enhances the ectomycorrhizal fungal development (Duponnois et al., 2006). Thus, *M. subhyalinus* mound amendment could be a tool to improve the efficiency of controlled mycorrhization in forest nursery and to minimize the technical and financial investment of this biotechnology.

The aims of this study were to test a new mycorrhizal inoculation process using a “catalyser” of the mycorrhizal establishment (termite mounds of *M. subhyalinus*) to minimize the requested volume of fungal inoculum added to the cultural substrate. The effects of the termite mound on mycorrhiza formation were explored between an Australian Acacia, *Acacia holosericea* A. Cunn. ex G. Don (a fast growing leguminous tree species frequently used in afforestation programs in West Africa) and an ectomycorrhizal fungus (*Pisolithus albus* IR 100 (Cooke & Massee); Martin et al., 2001) or an arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenk Smith; Schenck and Smith, 1982).

MATERIALS AND METHODS

Analysis of the termite mounds of *M. subhyalinus*

Termite mounds of *Macrothermes subhyalinus* were sampled in a shrubby savanna, 50 km north of Ouagadougou in Burkina Faso. They were crushed and passed through a 2 mm sieve before use. The chemical and microbiological characteristics have been determined in a previous study (Table 1) (Duponnois et al., 2005). Briefly, the NH_4^+ and NO_3^- contents were measured according to the method of Bremner (1965), available phosphorus was determined according to Olsen et al. (1954). The content of ergosterol was determined using the method of Grant and West (1986). The fumigation–extraction method was used to estimate the microbial biomass (Amato and Ladd, 1988). The enumeration of colony-forming units was carried out on King’s B agar medium for the fluorescent pseudomonads (King et al., 1954) and on actinomycete isolation agar medium (Difco Laboratories, Detroit, MI) for the actinomycetes. Fluorescent pseudomonads mostly belong to the *Pseudomonas monteillii* species (Duponnois et al., 2006).

Fungal inoculum

The ectomycorrhizal fungus *P. albus* IR100 was routinely maintained on modified Melin-Norkrans (MMN) agar medium at 25°C (Marx, 1969). The ectomycorrhizal fungal inoculum was prepared as follow: One litre glass jars were filled with 600 ml of a mixture of vermiculite and peat moss (4:1; v:v) and autoclaved (120°C, 20 min). The substrate was then moistened to field capacity with 300 ml liquid MMN medium, the jars were sealed and autoclaved at 120°C for 20 min. After cooling, the substrate was inoculated with 10 fungal plugs taken from the margin of fungal colonies. The glass jars were placed at 25°C in the dark for 3 months. An autoclaved mixture of moistened (MMN medium) vermiculite/peat moss was used for the control without ectomycorrhizal inoculation.

The AM fungus *G. intraradices* (DAOM 181 602, Ottawa Agricultural Herbarium) was propagated on millet (*Pennisetum typhoïdes* cv. IKMV 8201) for 12 weeks in a glasshouse on an autoclaved sandy soil (120°C, 60 min).

AM fungal inoculum consisted in a mixture of rhizosphere soil containing spores, hyphae and mycorrhizal roots cut into 1 to 3 mm long pieces bearing around 250 vesicles cm^{-1}. Non-mycorrhizal millet roots, prepared as above, were used for the control without AM inoculation.

Table 1. Biological and chemical characteristics of *Macrotermes subhyalinus* mound powder.

NH_4^+ (µg N g^{-1} of dry mound powder)	9.4
NO_3^- (µg N g^{-1} of dry mound powder)	3408.9
Available P (µg g^{-1} of dry mound powder)	3.5
Microbial biomass (µg C g^{-1} of dry mound powder)	22.5
Fluorescent pseudomonads (x 10^2 CFU g^{-1} of dry mound powder)	79.3
Actinomycetes (x 10^2 CFU g^{-1} of dry mound powder)	39.5
Ergosterol (µg g^{-1} of dry mound powder)	0.316

Experimental design

Seeds of *A. holosericea* were surface-sterilized with 95% sulfuric acid for 60 min. The acid solution was then decanted and the seeds rinsed and imbibed for 12 h in sterile distilled water. Seeds were then transferred aseptically to Petri dishes filled with 1% (w/v) agar/water medium. These plates were incubated at 25°C in the dark. The germinating seeds were used when rootlets were 1 to 2 cm long.

The sandy soil used in this experiment was collected in a stand of *A. holosericea* located east of Dakar. After sampling, the soil was crushed, passed through a 2-mm sieve and autoclaved for 60 min at 120°C to eliminate the native microflora. After autoclaving, its physico-chemical characteristics were as follows: pH (H_2O) 5.3; 3.6% clay; 0.0% fine silt; 0.8% coarse silt; 55.5% fine sand; 39.4% coarse sand; 0.17% carbon; 0.02% nitrogen; 8.5 C/N; 39 ppm total P and 4.8 ppm soluble P (Olsen). This soil was mixed with 0, 1, 5 and 10% (v/v) of *M. subhyalinus* mound powder. Ectomycorrhizal and AM inoculation were performed by mixing the soils with fungal inoculum (10/1; v/v) or with autoclaved mixture of moistened vermiculite/peat moss at the same rate (ectomycorrhizal control) or with non-mycorrhizal millet roots and their rhizosphere soil (AM control).

Plastic containers (30 × 30 × 5 cm) filled with soil mixtures were planted with 100 pre-germinated seeds each of *A. holosericea*. Seedlings were kept in a glasshouse under natural light (daylight approximately 12 h, mean daytime temperature 30°C) and daily watered without fertilizer. Three replicates per treatment were arranged in a complete randomized design.

After one month's culture, fifteen plants per container were uprooted and transferred into 1 L pot (one seedling per pot) filled with the same sterilized soil but without termite mound powder amendment and fungal inoculation. Seedlings were kept in a glasshouse in the same conditions as before and daily watered without fertilizer. The pots were arranged in a randomized complete block design. After 3-month's culture, five *A. holosericea* plants randomly chosen in each treatment were uprooted and the root systems gently washed. The oven dry weight (1 week at 65°C) of the shoot was measured. The root systems were cut into 1-cm root pieces and mixed. The percentage of ectomycorrhizal short roots ([number of ectomycorrhizal short roots / total number of short roots] x 100) was determined under a stereomicroscope at 40 x magnification on a random sample of at least 50 short roots per root system. The internal colonization of AM fungus along the root systems was quantified by clearing and staining the roots following the method of Phillips and Hayman (1970). The root pieces were placed on a slide for microscopic observation at 250 x magnification (Brundrett et al., 1985). The extent of mycorrhizal colonization was expressed in terms of fraction of root length with mycorrhizal internal structures (vesicles or hyphae): (length of colonized root fragment / total length of root fragments) x 100. The dry weight of roots was then measured (1 week at 65°C). Beside this experiment, *A. holosericea* seedlings were grown in 1 l pots filled with the same disinfected soil as before. For ectomycorrhizal inoculation, the soil was mixed with *P. albus* IR100 fungal inoculum (10/1; v/v). Control (without fungus) received an autoclaved mixture of moistened (MMN medium) vermiculite/peat moss at the same rate. For AM inoculation, a hole (1 × 5 cm) was made in each pot and filled with 1 g fresh millet root (mycorrhizal or not for the control treatment without fungus). The holes were then covered with the same autoclaved soil. Plants were daily watered without fertilizer and arranged in randomized complete block design with eight replicates per treatment. They were kept in a glasshouse under natural light (daylight approximately 12 h, mean daytime temperature 30°C). After 4 month's culture, *A. holosericea* plants were uprooted and their shoot biomasses and mycorrhizal indexes were measured as described before.

Statistical analysis

Data were treated with one-way and two-way analysis of variance. Means were compared using the Newman and Keuls test ($p < 0.05$). The percentages of mycorrhizal colonization were transformed by arc sin (sqrt) before statistical analysis.

RESULTS

Effect of AM and ectomycorrhizal inoculation or termite mound powder amendment on *A. holosericea* growth and on mycorrhiza formation after 3 month's culture in 1 L pots

Mycorrhizal inoculation had significantly improved *A. holosericea* growth (shoot and root biomass) whereas no significant effects have been recorded with the different rates of termite mound amendment (Table 2). For AM and ectomycorrhizal inoculations, the highest shoot biomasses have been measured with the 10% fungal inoculum rate and the highest root biomass with the 5% inoculum rate and the 10% inoculum rate for AM inoculation and ectomycorrhizal inoculation, respectively (Table 2).

Effects of the AM fungal inoculation and the termite mound powder amendment on plant growth and mycorrhizal colonization of *A. holosericea* seedlings

For all termite mound amendment rates combined, there were significant differences between fungal inoculum

Table 2. Effect of AM and ectomycorrhizal mono-inoculation rates or termite mound powder amendment rates, separately performed, on *A. holosericea* growth and on mycorrhiza formation after 3 month's culture in 1 L pots.

Type of soil treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
AM inoculum rate (%)			
0	264 (43.8) [(1)a(2)]	60 (14.5)[a]	0
1	760 (109.5)[b]	148 (38.1)[ab]	30 (8.9)[a]
5	1156 (20.4)[c]	394 (75.1)[c]	28 (5.8)[a]
10	1174 (72.4)[c]	220 (49.7)[bc]	32 (10.2)[a]
Ectomycorrhizal inoculum rate (%)			
0	264 (14.5)[a]	60 (14.5)[a]	0
1	404 (28.7)[b]	182 (29.9)[b]	35 (3.4)[a]
5	586 (119.9)[b]	296 (77.6)[bc]	46 (0.9)[b]
10	1488 (220.2)[c]	416 (94.7)[c]	53 (3.4)[b]
Termite mound amendment rate (%)			
0	264 (14.5)[a]	60 (14.5)[a]	0
1	300 (62.1)[a]	58 (13.9)[a]	0
5	266 (66.1)[a]	64 (25)[a]	0
10	284 (57.8)[a]	68 (21.5)[a]	0

[(1)]Standard error of the mean. [(2)] Data in the same column and for each factor followed by the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$).

Table 3. Effect of AM inoculation and termite mound powder amendment rates on *A. holosericea* growth and on mycorrhiza formation after 3 month's culture in 1 L pots.

Factor [(A)]	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
Fungal inoculum rate (%)			
0	278.5 (26.8) [(1)a(2)]	62.5 (8.9)[a]	0
1	1215.1 (89.1)[b]	350.5 (31.9)[b]	59.5 (5.1)[b]
5	1580 (73.1)[c]	520.1 (34.1)[d]	47.5 (5.6)[a]
10	1550 (88.8)[c]	444.1 (43.3)[c]	60.7 (4.9)[b]
Termite mound amendment rate (%)			
0	838.5 (90.9)[a]	205.5 (36.2)[a]	18.0 (4.4)[a]
1	1261.5 (146.8)[b]	429.0 (55.1)[b]	47.5 (6.8)[b]
5	1218.5 (136.9)[b]	378.2 (48.2)[b]	49.2 (7.1)[b]
10	1305.5 (156.2)[b]	364.5 (48.2)[b]	53.1 (7.4)[b]
Fungal inoculum rate (FIR)	S	S	S
Termite mound amendment rate (TAR)	S	S	S
FIR x TAR	NS	S	S

S: Significant ($p < 0.05$), NS: not significant ($p < 0.05$). [(1)] Standard error of the mean. [(2)] Data in the same column and for each factor followed by the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$). [(A)] Values are means of 20 replicates for fungal inoculum and termite mound amendment rates. Fungal inoculum rate factor is for all termite mound amendment rate treatments combined; the termite amendment factor is for all fungal inoculum rate treatments combined.

treatments for the shoot and root biomasses andmycorrhizal colonization of *A. holosericea* seedlings (Table 3). Compared to the control, the highest bio-masses were found with the 10 and 5% fungal inoculum rates and the highest mycorrhizal colonizations with the 1 and 10% fungal inoculum rates (Table 3). For all fungal inoculum rates combined, the growth and mycorrhizal colonization were significantly higher in the termite powder inoculated soils compared to the control (Table 3). Significant interactions between the fungal inoculations

Table 4. Effect of ectomycorrhizal inoculation and termite mound powder amendment rates on *A. holosericea* growth and on mycorrhiza formation after 3 month's culture in 1 L pots.

Factor [(A)]	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
Fungal inoculum rate (%)			
0	278.5 (26.8) [(1)a(2)]	62.5 (8.9)[a]	0
1	830.5 (101.9)[b]	341.5 (29.4)[c]	54.2 (3.2)[a]
5	771.0 (79.8)[b]	261.5 (25.7)[b]	54.6 (2.7)[a]
10	813.5 (104.9)[b]	277.5 (31.9)[b]	50.4 (2.4)[a]
Termite mound amendment rate (%)			
0	658.5 (122.5)[b]	288.5 (42.1)[a]	33.5 (4.8)[a]
1	482.5 (44.9)[a]	208.0 (23.6)[a]	39.6 (5.4)[b]
5	830.1 (101.3)[c]	268.1 (35.4)[a]	42.6 (6.3)[b]
10	722.5 (93.6)[bc]	228.0 (35.3)[a]	43.6 (6.3)[b]
Fungal inoculum rate (FIR)	S	S	S
Termite mound amendment rate (TAR)	S	NS	S
FIR x TAR	S	S	S

S: Significant ($p < 0.05$), NS: not significant ($p < 0.05$). [(1)] Standard error of the mean. [(2)] Data in the same column and for each factor followed by the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$). [(A)] Values are means of 20 replicates for fungal inoculum and termite mound amendment rates. Fungal inoculum rate factor is for all termite mound amendment rate treatments combined; the termite amendment factor is for all fungal inoculum rate treatments combined.

and the termite mound amendments were found for the root biomass and mycorrhizal colonization (Table 3).

Effects of the ectomycorrhizal fungal inoculation and the termite mound powder amendment on plant growth and mycorrhizal colonization of *A. holosericea* seedlings

For all termite mound amendment rates combined, the ectomycorrhizal inoculation significantly increased the growth of *A. holosericea* seedlings (Table 4). The root growth was significantly higher when the plants were inoculated at the rate of 1% (Table 4).

The soil amendment with termite mound powder had no significant effect on the root growth whereas it significantly increased the seedling mycorrhizal colonization (Table 4). Compared to the control (soil without mound powder), the shoot growth was significantly lower in the 1% termite mound amendment and higher in the 5% termite mound powder (Table 4). Significant interactions between the fungal inoculations and the termite mound amendments were recorded for the plant growth and mycorrhizal colonization (Table 4).

Effects of fungal inoculation and termite mound amendment on the mycorrhizal colonization of *A. holosericea* seedlings

Using the conventional process of controlled mycorrhization and after 4 months growth, AM and ectomycorrhizal fungal inoculations significantly increased shoot biomass (+64.7 and +50.9%, respectively) and root biomass (+42.8 and +48.2%, respectively) (Table 5). The mycorrhizal colonization rates of *A. holosericea* seedlings were of 59.3% for the AM treatment and of 35.6% for the ectomycorrhizal treatment (Table 5).

Relationships between termite mound amendment, fungal inoculation and plant growth

For ectomycorrhizal inoculation, growth responses of *A. holosericea* to ectomycorrhizal inoculum densities were significantly linked to the rates of termite mound amendments (Figure 1). At the 5% termite mound amendment, the highest shoot biomasses were found with the 1 and 5% fungal inoculum densities whereas at the 10% termite mound amendment, *A. holosericea* growth was significantly higher than that in the other treatments, when the seedlings were inoculated with the 1% fungal inoculum density.

Termite mound amendment decreased the mycorrhizal effect on plant growth in the 10% fungal inoculum treatment (Figure 1). For AM inoculation treatments, the plant growth was significantly linked with the amounts of mycorrhizal propagules inoculated to the soil with termite powder for 1 and 5% fungal inoculum treatments (Figure 1). For the 10% inoculum fungal treatment, the termite mound had no significant influence on the effect of

Table 5. Effect of ectomycorrhizal or AM inoculations on growth of *A. holosericea* and on mycorrhiza formation after 4 month's culture under glasshouse conditions using the conventional process of controlled mycorrhization.

Treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
AM inoculation			
Uninoculated control	648.1[a (1)]	312.2[a]	0
Glomus intraradices	1834.1[b]	546.3[b]	59.3
Ectomycorrhizal inoculation			
Uninoculated control	550[a]	290[a]	0
Pisolithus albus IR100	1120.1[b]	560.2[b]	35.6

[(1)]For each type of fungal inoculation, data in the same column followed by the same letter are not significantly different according to the Newman Keuls test ($p < 0.05$).

mycorrhizal inoculation on the plant growth (Figure 1). At the 5 and 10% termite mound amendment, no significant differences were recorded between shoot biomasses within fungal treatments (Figure 1). The shoot biomass of *A. holosericea* seedlings conven-tionally inoculated with *P. albus* IR100 was reached to highest shoot biomass in the 1% ectomycorrhizal inoculum treatments with 5 and 10% termite powder amendments and in the 5% ectomycorrhizal inoculum treatment with 5% termite powder amendment. For AM inoculation, shoot growth measured on plants conventionaly inoculated with *G. intraradices*, reached to highest shoot biomass with all the fungal densities when the soil was mixed with 5 and 10% termite powder (Figure 1).

DISCUSSION AND CONCLUSION

The main objectives of this study were to test the effect of *Macrotermes subhyalinus* mound structure amendment on the formation of mycorrhizae between *A. holosericea* and *P. albus* (*P. albus* IR100) and *Glomus intraradices* using a two-step cultural system in order to minimize the amounts of fungal inocula added to the cultural substrate. The first step of this cultural practice was the inoculation of *A. holosericea* seedlings whereas the second one allowed the development of these mycorrhized plants in larger soil volumes.

In this present study, termite mound amendment significantly enhanced the formation and performance on the plant growth of both types of fungal isolates. The termite mound amendment had no effect on the plant growth and, consequently, did not enhance the number of fungal infection sites (short roots). Hence this stimulating effect could be attributed to the introduction *via* the termite mound of a bacterial group (that is, fluorescent pseudomonads) that could act as Mycorrhiza Helper Bacteria (MHB) (Duponnois and Plenchette, 2003).

Termite mounds (*Isoptera*) are ubiquitous features of tropical ecosystems, more particularly in savanna environments. Translocations of large amounts of soil from various depths of the soil profile result from termite activities (Holt and Lepage, 2000). These structures strongly influence their environment and have a considerable impact on soil physical and chemical properties (Black and Okwakol, 1997; Holt and Lepage, 2000) that explains the termite role as ecosystem engineers.

Previous microbiological studies of termite mounds have been carried out to compare the microbial communities in grass-, litter- and soil-feeding termite mounds (Duponnois et al., 2005) and fluorescent pseudomonads have only been detected in *M. subhyalinus* mound powder. The phylogenetic analysis performed on these fluorescent pseudomonads showed that these bacteria mostly belonged to *Pseudomonas monteillii* species (Duponnois et al., 2006). It has been demonstrated that an isolate of this pseudomonad species (isolate HR13) could stimulate ectomycorrhizal formation between *A. holosericea* and different fungal isolates (*Scleroderma dictyosporum*, *S. verrucosum*, *P. albus* and *P. tinctorius*) (Founoune et al., 2002; Duponnois and Plenchette, 2003) as well as AM establishment between *this Acacia* species and *G. intraradices* (Duponnois and Plenchette, 2003). More recently, it was shown that another isolate of *P. monteillii*, isolate KR9, stimulated ectomycorrhizal formation between *S. dictyosporum* IR412 and *A. holosericea* (Duponnois et al., 2006). Hence these bacterial strains present in *M. subhyalinus* mounds could be involved in the stimulation of ectomycorrhizal and AM establishment recorded in this present study.

The MHB effects on mycorrhiza formation has been usually observed by inoculating about 10^8 CFU per litre of cultural substrate (Duponnois and Plenchette, 2003; Duponnois et al., 2006). In this present study, the amounts of termite powder added to the soil at the lowest rate (1%, v/v) induced a significant enhancement of mycorrhizal formation. Previous studies have shown that a MHB could exert its positive effect on mycorrhiza formation at a low density (less than 10^2 CFU g^{-1} of soil) in nursery conditions (Duponnois and Garbaye, 1992; Frey-Klett et al., 1999), Frey-Klett et al. (1999) highlighted the contrast between the low inoculum densities required for the beneficial MHB effect and those generally reported with PGPRs (Plant Growth Promoting

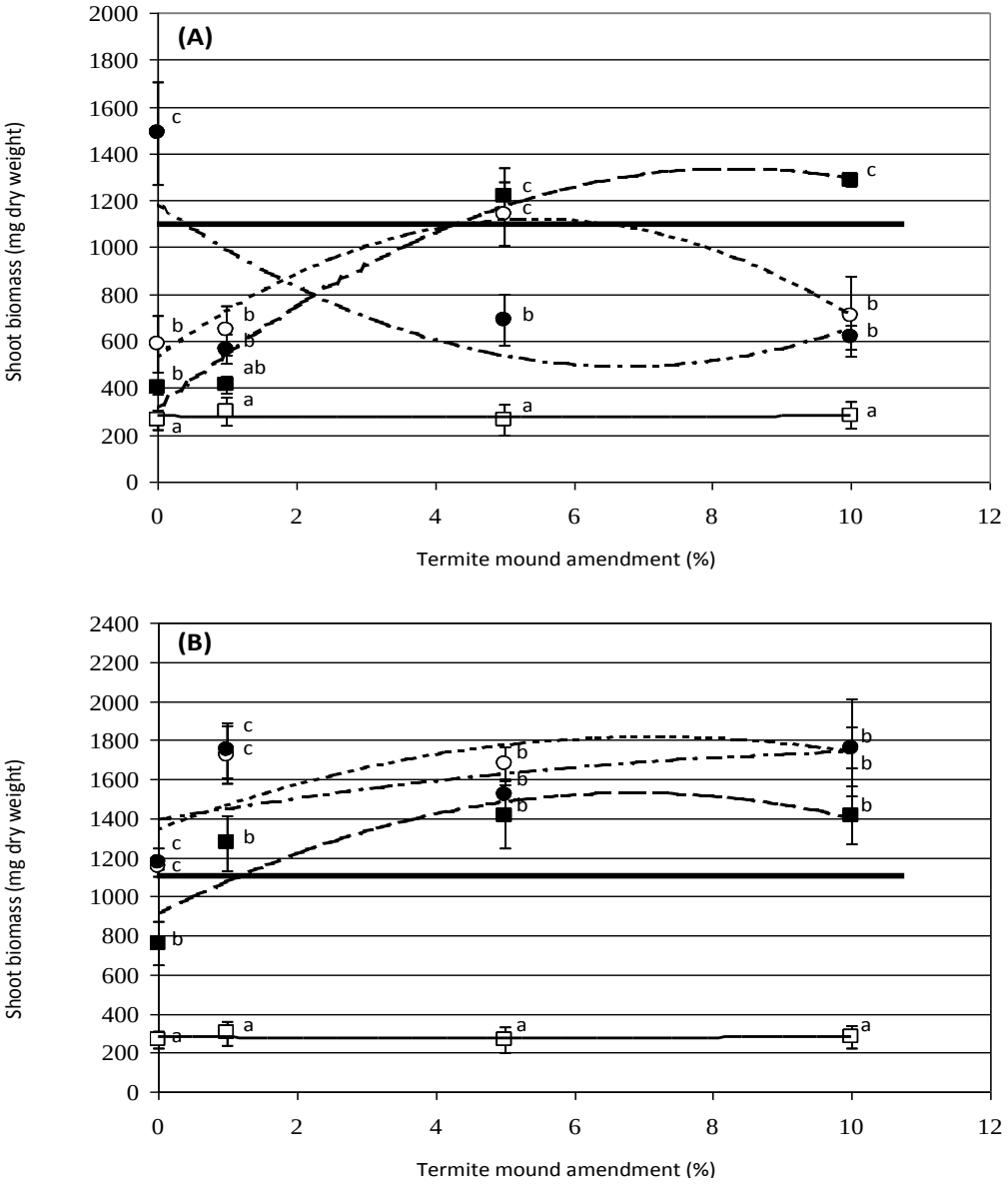

Figure 1. Effect of termite mound amendment on the growth response of *A. holosericea* seedlings (Shoot biomass) inoculated with different fungal inoculum densities after 4 month's culture in glasshouse conditions. (A) Inoculation with different ectomycorrhizal inoculum rates of *P. albus*; (B): Inoculation with different AM inoculum rates of *G. intraradices*. Error bars represent standard errors (n = 5). For each rate of termite mound amendment, data indexed by the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$). ▬ : Shoot biomass of *A. holosericea* seedlings inoculated with *P. albus* IR100 (A) or *G. intraradices* (B) using the conventional process of controlled mycorrhization (Table 4). *Ectomycorrhizal inoculation: Fungal inoculum rate (FIR) = 0% (v/v) (fitted curve is y = 280.05 – 2.627 x + 0.284 x^2; r = 0.026; p = 0.99);* Fungal inoculum rate (FIR) = 1% (v/v) (fitted curve is y = 312.3 + 245.7 x – 14.76 x^2; r = 0.94; p < 0.0001); Fungal inoculum rate (FIR) = 5% (v/v) (fitted curve is y = 529.7 + 216.2 x – 19.79 x^2; r = 0.61; p = 0.018); Fungal inoculum rate (FIR) = 10% (v/v) (fitted curve is y = 1181.1 + 203.9 x + 15.08 x^2; r = 0.56; p = 0.038)

Rhizobacteria) where a minimal inoculation dose of 10^5 CFU g^{-1} soil is required to detect the beneficial and plant protection efficiency. This PGPR effect is generally Linked with increasing inoculation doses (Bull et al., 1991; Raajmakers et al., 1995). In this present study, we found a significant interaction between the fungal doses and the rates of termite mound amendment. These results are not consistent with those of Frey-Klett et al.

(1999) where the success of bacterial inoculation was independent of the amount of fungus inoculum used. It has been suggested that a positive dose-response relationship (as recorded in this present study) results from a better colonization of the rhizosphere by the introduced microorganism (Raaijmakers et al., 1995) that facilitate the multiplication of the bacterial populations and its functional activities (that is, enzymes, antibiotics, etc) (Chin-A-Woeng et al., 1997). Thus all these results suggest that the use of termite mound powder such as *M. subhyalinus*, which natively contains some MHB, provides a beneficial inocul-um tool for the development of these bacteria by protecting the bacterial cells against adverse environmental factor.

In most of the fungal treatments reported in this present study, the stimulating effect of termite mound amendment on mycorrhizal formation was associated with an increase of plant growth suggesting that termite mound amendment did not interact with the symbiotic efficiency of mycorrhizas.

It has been previously reported that adding a mycorrhiza helper bacterial strain to the fungal inoculum decreases the requested amount of fungal inoculum while keeping the same mycorrhizal formation (Frey-Klett et al., 1999). Our study also shows that the dose of MHB added to the soil and their efficiency on mycorrhiza formation could be optimized through the use of a native fluorescent pseudomonad inoculum such as termite mounds.

One of the main problems encountered with the conventional controlled mycorrhization of forest planting stocks is the large quantity of fungal inoculum requested for the production of high quality mycorrhizal plants in nursery conditions. In tropical and Mediterranean areas, the mycorrhizal inoculum dose added per plant to the cultural substrate is generally of 0.1 L per litre of soil (ectomycorrhizal inoculation) and 1 g fresh weight of mycorrhizal root per litre of soil (AM inoculation) (Duponnois et al., 2007). With our process, these quantities are drastically lowered since, with only 45 ml of ectomycorrhizal inoculum or AM inoculum (mixture of spores, mycorrhizal roots and rhizosphere soil), about 100 mycorrhized *A. holosericea* plants can be produced with the same growth and mycorrhizal colonization as mycorrhized plants produced with the conventional controlled mycorrhization procedure. Therefore the inoculation cost could be highly minimized since termite mounds are commonly found in tropical ecosystems while requiring very low fungal amounts. This inoculation practice could be useful in reafforestation of tropical regions as it is well known that controlled mycorrhization is a beneficial tool for improving the survivaland productivity of tree species in degraded areas.

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