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A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species

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Abstract The aims of this study were to test the effects of a mycorrhiza helper bacterium (MHB), *Pseudomonas monteilii* strain HR13 on the mycorrhization of (1) an Australian *Acacia*, *A. holosericea*, by several ectomycorrhizal fungi or one endomycorrhizal fungus *Glomus intraradices*, and (2) several Australian *Acacia* species by *Pisolithus alba* strain IR100 under glasshouse conditions. Bacterial inoculant HR13 significantly promoted ectomycorrhizal colonization for all the *Acacia* species, from 45.8% (*A. mangium*) to 70.3% (*A. auriculiformis*). A stimulating effect of HR13 on the ectomycorrhizal establishment was recorded with all the fungal isolates (strains of *Pisolithus* and *Scleroderma*). The same effect of bacteria on the frequency of endomycorrhizal colonization of *A. holosericea* seedlings by *G. intraradices* with vesicles and hyphae frequencies was recorded. The stimulation of saprophytic fungal growth by MHB is usually the main mechanism that could explain this bacterial effect on mycorrhizal establishment. MHB could stimulate the production of phenolic compounds such as hypaphorine and increase the aggressiveness of the fungal symbiont. However, no significant effect of MHB on fungal growth was recorded with *Scleroderma* isolates under axenic conditions but positive bacterial effects were observed with *Pisolithus* strains. From a practical viewpoint, it appears that MHB could stimulate the mycorrhizal colonization of Australian *Acacia* species with ectomycorrhizal or endomycorrhizal fungi, and could also facilitate controlled mycorrhization in nursery practices where *Acacia* species are grown for forestation purposes.

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Introduction

Excessive industrial exploitation, clearing for agricultural purposes and collection of firewood have led to a dramatic deforestation during recent decades in West Africa (Piéri 1991). One of the most critical issues of deforestation is the risk of erosion and landslides, which reduce soil fertility and water catchment quality. Reforestation is now a priority in all sahelian and soudano-sahelian countries. Among different tree species tested in reforestation programs, *Acacia* species have been used successfully on degraded lands (Galiana et al. 1990, 1994, 1996). *Acacia* is the largest mimosoid genus and includes nearly 1,200 species (Pedley 1986). *Acacia* species are abundant in savanas and arid regions of Australia, Africa, India and the Americas. Some of them prevent wind and rain erosion, control sand dunes, are sources of wood and provide fodder for livestock (Giffard 1975; Cossalter 1986). The ability of *Acacia* species to grow on low-N soils depends on their biological property of forming root symbiosis with rhizobial bacteria. Much of the nitrogen provided by the rhizobial symbiosis is returned to the soil through leaf fall and the resulting humus improves the fertility of the soil and its physical properties (Zakra 1994). Fast-growing leguminous trees belonging to the *Acacia* genus, brought from Australia and introduced to Western Africa, appear to be well adapted to sahelian and soudano-sahelian climatic conditions (Cornet and Diem 1982; Cossalter 1986). In addition to rhizobial symbiosis, this tree species can form endomycorrhiza and/or ectomycorrhiza (De La Cruz and Garcia 1991). It has been shown that controlled rhizobial or mycorrhizal symbiosis could significantly improve the growth of Australian acacias (Cornet and Diem 1982; Galiana et al. 1994; Duponnois et al. 2000). Moreover, it has been demonstrated that some soil bacteria, which have been named mycorrhiza helper bacteria (MHB), could enhance the

Table 1 Origins of the tested ectomycorrhizal fungi

Species and herbarium ID	Host tree	Region	Collectors
Pisolithus sp. SL2	*Acacia mangium* Willd.	Diagana, Senegal	R. Duponnois
Pisolithus alba COI007 (Martin et al. 2002)	*A. mangium*	Casamance, Senegal	R. Duponnois
P. alba COI024 (Martin et al. 2002)	*A. holosericea* A. Cunn. ex G. Don	Casamance, Senegal	R. Duponnois
Pisolithus sp. COI032	*Eucalyptus camaldulensis* Dehnh	Sine Saloum, Senegal	R. Duponnois
P. alba IR 100 (Martin et al. 2002)	*A. mangium*	Casamance, Senegal	R. Duponnois and A.M. Bâ[a]
Pisolithus tinctorius (Pers.) Coker & Couch GEMAS	*A. mangium*	Malaysia	F. Lapeyrie[b]
Scleroderma dictyosporum pat. IR109	*Afzelia africana* Sm. ex Pers.	Burkina Faso	A.M. Bâ[a]
Scleroderma verrucosum Bull. (Pers.) sensu Grev.IR500	*A. africana*	Burkina Faso	K. Sanon[c]

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development of the mycorrhizal symbiosis (Garbaye 1994). This bacterial-growth-promoting effect has been shown with different host plants, including herbaceous species (Meyer and Linderman 1986; Paula et al. 1992; Von Alten et al. 1993; Requena et al. 1997) and tree species (Duponnois et al. 1993; Rozycki et al. 1994; Dunstan et al. 1998). The MHB effect has been particularly well examined in symbioses between conifers and *Laccaria bicolor* S-238 under a wide variety of experimental conditions (Duponnois and Garbaye 1991a, b). MHBs have been found to inhibit mycorrhiza formation by fungi other than *Laccaria* spp. (Garbaye and Duponnois 1992), to improve the persistence of selected ectomycorrhizal fungi inoculated on seedlings in nursery soil (Duponnois et al. 1993), and to be effective in stimulating mycorrhizal development at a very low rate [10 bacteria $(cm^3\ soil)^{-1}$] (Frey-Klett et al. 1997, 1999).

The MHB effect on ectomycorrhizal and endomycorrhizal symbioses has been investigated with a limited number of plant species having mono-mycorrhizal associations with endo- or ectomycorrhizal fungi. In Australia, plants with ectomycorrhizal associations usually also have some endomycorrhizal structures in their roots. Australian plants recorded to have this dual symbiotic fungal association include major species used in plantation belonging to the genera *Casuarina* and *Allocasuarina* (Casuarinaceae), *Eucalyptus*, *Melaleuca* (Myrtaceae) and *Acacia* (Mimosaceae). Recently, it has been shown that endo- and ectomycorrhizal fungi could co-exist without competition on the same root system of *Acacia holosericea* A. Cunn. ex G. Don seedlings (Founoune et al. 2002a).

The aims of this study were to explore the effects of a MHB, *Pseudomonas monteilii* (Elomari et al. 1997) strain HR13, on mycorrhiza formation of *A. holosericea* A. Cunn. ex G. Don (by several ectomycorrhizal fungi or one endomycorrhizal fungus *Glomus intraradices* (Schenck and Smith 1982) and on ectomycorrhiza development of several Australian *Acacia* species by *Pisolithus alba* (Martin et al. 2002) strain IR100 under glasshouse conditions.

Materials and methods

Plant species

Seeds of Australian *Acacia* species (*A. auriculiformis* Cunn ex. Benth, *A. eriopoda* Maiden, *A. holosericea* A. Cunn. ex G. Don, *A. mangium* Willd and *A. platycarpa* F. Muell.) were surface sterilized with 95% concentrated 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 1–2 cm long.

Mycorrhizal fungi and MHB bacterial isolate

The origins of the mycorrhizal fungi are indicated in Table 1. They were maintained in Petri dishes on MMN agar medium at 25°C (Marx 1969). The ectomycorrhizal fungal inoculum was prepared according to Duponnois and Garbaye (1991c). Glass jars (1 l) 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 fungal plugs taken from the margin of the fungal colonies and incubated for 6 weeks at 28°C in the dark.

The arbuscular mycorrhizal fungus *G. intraradices* Schenk & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was cultivated on leek (*Allium porrum* L.) for 12 weeks under greenhouse conditions on Terragreen substrate. This calcined clay (particle size average 5 mm), Oil-Dri US-special Ty/IIIR (Oil-Dri Company, Chicago, Ill.) is an attapulgite from Georgia used as substrate for propagation of mycorrhizal fungi (Plenchette et al. 1996). Before inoculation of the *Acacia* seedlings, the leek plants were uprooted, gently washed and the roots were cut into 0.5 cm long pieces bearing around 250 vesicles cm^{-1}. The morphology of these vesicles was very different from external spores. Internal spores were never observed with our strain under our cultivation conditions. Non-mycorrhizal leek roots, prepared as above, were used for the control treatment without endomycorrhizal inoculation.

The fluorescent pseudomonad, *P. monteilii* (Elomari et al. 1997) strain HR13, was isolated from the rhizosphere of 3-month-old *A. mangium* plants growing in a sandy soil in Senegal (Founoune et al. 2002b). It has been demonstrated in a previous study that this bacterial strain could stimulate ectomycorrhizal establishment between *P. alba* strain IR100 and *A. holosericea* (Founoune et al. 2002b). It was cultured on King's B medium (King et al. 1954) and cryopreserved at –80°C in 60% glycerol/tryptic soy broth (TSB)

medium (3 g l^{-1}) (1/1, v/v). Before inoculation, bacteria were grown in 0.3% TSB medium (Difco, Detroit, Mich.) for 3 days at 25°C on a rotary shaker, centrifuged (2,400 *g*, 20 min) and resuspended in 0.1 M $MgSO_4$. The final concentration of the bacterial culture was about 10^8 cfu/ml, estimated after enumeration on plate count agar media (King's B medium). This suspension was used as an inoculum.

Effect of *P. monteilii* HR13 on the mycorrhization of Australian *Acacia* species with mycorrhizal fungi under greenhouse conditions

Australian *Acacia* seedlings were grown in 1 l pots filled with a disinfected sandy soil 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 40 min at 140°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). For ectomycorrhizal inoculation, the soil was mixed with fungal inoculum (10/1; v/v). The treatments without fungus received an autoclaved mixture of moistened (MMN medium) vermiculite/peat moss at the same rate. For endomycorrhizal inoculation, one hole (1 cm ×5 cm) was made in each pot and filled with 1 g fresh leek root (mycorrhizal, or not for the control treatment without fungus). The holes were then covered with the same autoclaved soil. Immediately after planting, 5 ml bacterial suspensions were injected around the stem into each pot. The treatments without bacteria received 5 ml 0.1 M $MgSO_4$.

One set of experiments was carried out with one fungal isolate (*P. alba* IR100) and Australian *Acacia* species (experiment 1), another with *A. holosericea* and ectomycorrhizal fungal isolates (experiment 2) and the last experiment was performed with *A. holosericea* seedlings inoculated with *G. intraradices* (experiment 3). Plants were watered twice a week with tap water (pH 6.0) without fertilizer. The pots were arranged in a randomized complete block design with ten replicates per treatment. They were placed in a glasshouse during the hot season under natural light (daylight approximately 12 h, mean daytime temperature 30°C).

Effect of *P. monteilii* HR13 on growth of ectomycorrhizal fungi under axenic conditions (experiment 4)

The fungal isolates were grown in Petri dishes on MMN agar at 25°C in the dark for 2 weeks. Agar plugs (4 mm in diameter; 4 mm thick) were taken from the margin of the fungal colonies. Fungal isolates and *P. monteilii* HR13 were confronted using two-compartment dishes. One compartment was filled with 0.3% agar medium inoculated or not (control treatment) with the bacterial isolate and the fungal plugs were laid on the dry bottom of the dish of the other compartment. Gas diffusion from one side to the other was permitted as the wall separating the two compartments did not touch the lid of the dish. Plates were sealed with tape to avoid drying during incubation and placed at 25°C in an incubator in the dark for 1 week. Fungal growth measurements were made through the lid with a stereomicroscope (magnification ×40) and the mean radial growth in two perpendicular directions was calculated. For each *P. monteilii* HR13/fungal isolate combination, there were three dishes, each with three mycelial plugs (three replicates). The data were statistically compared to the control treatments without bacteria using Student's *t*-test ($P < 0.05$).

Quantitative evaluation

After 4 month's growth, *Acacia* plants 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. Ectomycorrhizal colonization (number of ectomycorrhizal short roots/total number of short roots ×100) was determined under a stereomicroscope at 40× magnification on a random sample of at least 100 short roots per root system. To quantify the internal colonization of arbuscular mycorrhizal fungus along the root systems, the roots were cleared and stained according to the method of Phillips and Hayman (1970). The root pieces were placed on a slide for microscopic observation at 250× magnification (Brundrett et al. 1985). About 100 1-cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed in terms of fraction of root length with internal fungal structures (vesicles or hyphae).

Some nodules were observed along the root systems of the *Acacia* seedlings despite disinfection of the soil and the seed surface. Root nodules were counted and their dry weight (65°C, 1 week) determined. The dry weight of roots was then measured (65°C, 1 week).

Statistical analysis

All data were subjected to a one-way analysis of variance and the mean values were compared using Student's *t*-test ($P < 0.05$). For percentage mycorrhizal infection, data were transformed by Arc sin $\sqrt{x}$.

Results

Inoculation experiment with Australian *Acacia* species (experiment 1)

P. monteilii HR13 inoculated alone had no significant effect on growth and development of contaminant rhizobia in any of the Australian *Acacia* species tested (Table 2). After 4 months growth, the ectomycorrhizal isolate *P. alba* IR100 had colonized all the Australian *Acacia* species tested in the glasshouse experiment. Ectomycorrhizal colonization ranged from 20.1% (*A. mangium*) to 45.2% (*A. auriculiformis*) (Table 3). Bacterial inoculant HR13 significantly promoted ectomycorrhizal colonization for all species tested, from 45.8% (*A. mangium*) to 70.3% (*A. auriculiformis*). Ectomycorrhizal fungal inoculation without bacteria only significantly increased shoot biomass of *A. auriculiformis* and *A. platycarpa* and root biomass of *A. auriculiformis*, *A. holosericea* and *A. mangium* (Table 2). When HR13 was co-inoculated with IR100, plant growth of each *Acacia* species was significantly different from the control without bacteria and fungus (Table 2). With *A. holosericea* and *A. mangium*, the positive fungal effect on root growth was significantly enhanced by HR13 (Table 2).

Nodules were observed in all the treatments. Fungal inoculant significantly increased the number and the total weight of nodules per plant for *A. auriculiformis* and *A. eriopoda*, whereas these measurements (number and biomass) were significantly higher with all *Acacia* species when HR13 was co-inoculated with the fungus (Table 2). The number and total biomass of nodules per plant correlated significantly with the mycorrhizal rates (r^2 =0.38 and 0.41, respectively).

Table 2 Effect of *Pseudomonas monteilii* strain HR13 and/or the ectomycorrhizal fungus *P. alba* strain IR 100 on mycorrhiza formation, rhizobial development and growth of five Australian *Acacia* species after 4 months culture under glasshouse conditions

Acacia species	Microbial treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Number of nodules per plant	Total nodule weight/plant (mg)	Ectomycorrhizal colonization (%)
A. auriculiformis	Control	1,507 a*	1,097 a	8.3 a	2.9 a	0.0 a
	HR13	1,643 a	838 a	8.1 a	2.3 a	0.0 a
	IR100	2,603 b	1,830 b	17.7 b	55.2 b	45.2 b
	HR13 + IR100	2,356 b	1,836 b	36.6 c	42.3 b	70.3 c
A. eriopoda	Control	1,128 a	298 a	1.8 a	1.6 a	0.0 a
	HR13	1,082 a	323 ab	2.3 a	2.1 a	0.0 a
	IR100	1,074 a	424 ab	9.8 b	10.1 b	20.6 b
	HR13 + IR100	1,340 a	492 b	6.2 b	14.2 b	48.2 c
A. holosericea	Control	1,310 a	523 a	15.8 a	3.2 a	0.0 a
	HR13	1,244 a	488 a	10.0 a	3.1 a	0.0 a
	IR100	1,515 a	762 b	11.2 a	8.1 a	25.2 b
	HR13 + IR100	2,337 b	997 c	24.5 b	35.6 b	54.2 c
A. mangium	Control	1,112 a	598 a	5.2 a	3.1 a	0.0 a
	HR13	1,126 a	506 a	4.8 a	2.9 a	0.0 a
	IR100	1,194 ab	1,070 b	10.2 ab	8.2 a	20.1 b
	HR13 + IR100	1,735 b	1,697 c	16.5 b	30.2 b	45.8 c
A. platycarpa	Control	860 a	660 a	1.7 a	1.3 a	0.0 a
	HR13	840 a	580 a	2.6 a	1.6 a	0.0 a
	IR100	1,510 b	732 ab	4.5 a	2.5 a	31.6 b
	HR13 + IR100	1,933 b	1,087 b	16.8 b	8.3 b	46.5 c

* For each Australian *Acacia* species, values of the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P < 0.05$)

Table 3 Effect of ectomycorrhizal fungi with or without *P. monteilii* strain HR13 on mycorrhiza formation, rhizobial development and growth of *A. holosericea* after 4 months culture under glasshouse conditions

Microbial treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Number of nodules per plant	Total nodule weight/plant (mg)	Ectomycorrhizal colonization (%)
Pisolithus sp. SL2	1,501 a*	436 a	12.7 a	26.1 a	48.3 a
Pisolithus sp. SL2 + HR13	1,599 a	546 a	22.6 b	49.3 b	67.3 b
P. alba COI007	1,462 a	522 a	24.6 a	58.1 a	43.8 a
P. alba COI007 + HR13	1,514 a	746 a	44.1 b	78.3 b	60.6 b
P. alba COI024	1,310 a	470 a	14.8 a	26.1 a	10.8 a
P. alba COI024 + HR13	1,348 a	578 a	22.6 b	34.2 a	26.8 b
Pisolithus sp. COI032	1,444 a	446 a	11.9 a	18.1 a	15.0 a
Pisolithus sp. COI032 + HR13	1,594 a	578 a	21.2 b	49.2 b	41.7 b
P. alba IR 100	1,515 a	762 a	11.2 a	8.1 a	25.2 a
P. alba IR 100 + HR13	2,337 b	997 b	24.5 b	35.6 b	54.2 b
P. tinctorius GEMAS	1,541 a	454 a	15.1 a	34.3 a	43.1 a
P. tinctorius GEMAS + HR13	2,279 b	509 a	24.7 b	42.5 a	69.1 b
S. dictyosporum IR109	1,382 a	494 a	25.6 a	52.2 a	53.4 a
S. dictyosporum IR109 + HR13	1,552 a	520 a	34.6 b	58.3 a	74.6 b
Scleroderma verrucosum IR500	1,634 a	454 a	17.6 a	33.1 a	13.8 a
S. verrucosum IR500 + HR13	1,826 a	573 a	24.4 b	43.2 a	22.2 b
Control	650 a	390 a	8.1 a	11.2 a	0.0
HR13	620 a	330 a	6.2 a	10.8 a	0.0

* For each fungal isolate, values of the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P < 0.05$)

Inoculation experiment of *A. holosericea* (Australian Acacia) with mycorrhizal fungi (experiments 2 and 3)

A. holosericea seedlings were colonized by all the fungal isolates (Tables 4 and 5). Ectomycorrhizal colonization ranged from 10.8% (*P. alba* COI024) to 53.4% (*Scleroderma dictyosporum* IR109). A stimulating effect of HR13 on ectomycorrhizal establishment was recorded with all the fungal isolates (Table 3). A similar bacterial effect on endomycorrhizal colonization of *A. holosericea* seedlings by *G. intraradices* was recorded (Table 4).

Bacterial inoculant alone had no effect on plant growth. In contrast, all the fungal strains inoculated alone significantly stimulated shoot and root growth ($P<0.05$) (Tables 3 and 4). For shoot biomass, the highest promoting effect was measured with *Scleroderma verru-*

Table 4 Effect of *P. monteilii* strain HR13 and/or *Glomus intraradices* on mycorrhiza formation, rhizobial development and growth of *A. holosericea* after 4 months culture under glasshouse conditions

	Control	HR13	*G. intraradices*	*G. intraradices* + HR13
Shoot biomass mg dry weight)	748 a*	944 a	1,834 b	2,994 c
Root biomass (mg dry weight)	312 a	297 a	546 b	889 c
Number of nodules per plant	6.0 a	9.6 a	15.4 b	21.1 b
Total nodule weight/plant (mg)	2.1 a	4.1 a	13.3 b	18.5 b
Endomycorrhizal colonization				
Hypha (%)	0.0	0.0	24.1 a	91.2 b
Vesicle (%)	0.0	0.0	49.6 a	61.6 b

* Values of the same row followed by the same letter are not significantly different according to the one-way analysis of variance (P <0.05)

Table 5 Effect of *P. monteilii* strain HR13 on the radial growth (mm) of ectomycorrhizal fungi after gaseous interaction in axenic conditions. For each fungal isolate, data followed by the same letter are not significantly different according to Student's *t*-test (P <0.05)

Fungal isolate	Control	+ HR13
Pisolithus sp. SL2	11.3 a	13.2 b
P. alba COI007	12.0 a	14.1 b
P. alba COI024	11.5 a	12.9 b
Pisolithus sp. COI032	10.5 a	12.3 b
P. alba IR100	11.6 a	13.2 b
P. tinctorius GEMAS	11.9 a	13.6 b
S. dictyosporum IR109	11.5 a	11.2 a
S. verrucosum IR500	12.5 a	13.1 a

cosum IR500 (+60.2%) for ectomycorrhizal fungi (Table 4) and +59.2% with *G. intraradices* (Table 4).

No effect of *P. monteilii* HR13 on number and biomass of nodules per plant was recorded (Tables 3 and 4). With the exception of *P. alba* IR100, all the fungal isolates inoculated alone significantly enhanced the biomass of nodules per plant. A stimulating effect on the number of nodules per plant was recorded with *S. dictyosporum* IR109, *S. verrucosum* IR500, *P. alba* COI007, *P. alba* COI 024, *Pisolithus tinctorius* GEMAS and *G. intraradices*, whereas the ectomycorrhizal fungal isolates did not affect nodule biomass. The dual inoculation significantly increased the number of nodules per plant inoculated with ectomycorrhizal symbionts whereas nodule biomass was not significantly modified in *P. alba* COI024, *P. tinctorius* GEMAS, *S. dictyosporum* IR109, *S. verrucosum* IR500 and *G. intraradices* treatments (Tables 3 and 4). Rhizobial symbiosis expressed as number and biomass of nodules per plant correlated significantly with ectomycorrhizal and endomycorrhizal colonization (r^2 =0.21 and 0.19, respectively).

Interaction between *P. monteilii* HR13 and ectomycorrhizal isolates (experiment 4)

In axenic conditions, *P. monteilii* HR 13 significantly increased the radial growth of *Pisolithus* isolates whereas no effects were recorded with *S. dictyosporum* and *S. verrucosum* (Table 5).

Discussion

The main results of this study show that (1) mycorrhizal symbiosis clearly benefits growth of Australian *Acacia* species and rhizobial symbiosis development, and (2) these symbiotic effects are stimulated by the bacterial strain *P. monteilii* HR13. It is well known that many N_2-fixing trees and shrubs are especially dependent on mycorrhizas to absorb mineral nutrients required for plant growth and efficient N_2 fixation (Cornet and Diem 1982; Cornet et al. 1982; Bâ et al. 1996; Duponnois et al. 2001). For example, it has been found that *A. holosericea* growth could be greatly enhanced by *G. mosseae* (Cornet and Diem 1982), *G. fasciculatum* (Senghor 1998) and *G. aggregatum* (Duponnois et al. 2001). Similar positive effects have been obtained with *G. intraradices* in this study. In contrast, the importance of ectomycorrhizas for Australian *Acacia* has rarely been assessed. It has been previously demonstrated that *A. holosericea* responded to inoculation with one isolate of *P. alba* that strongly stimulated the plant growth (Founoune et al. 2002b). This study shows that two isolates of *S. verrucosum* and *S. dictyosporum* can form ectomycorrhiza with *A. holosericea* and that these fungi strongly stimulate plant growth under glasshouse conditions. According to our knowledge, there are no references in the literature indicating *Scleroderma* effects on *A. holosericea* plant growth. Moreover, these fungi are indigenous to West Africa and are associated with African tropical species (Sanon et al. 1997). This result could be of great importance for survival and growth in West Africa of Australian *Acacia* species introduced to this area for the first time.

Up to now, it has been reported that *A. holosericea*, *A. mangium* and *A. auriculiformis* are colonized effectively by ectomycorrhizal fungi (Duponnois and Bâ 1999; Duponnois et al. 2000; Founoune et al. 2002b) but there are no published reports indicating that *A. eriopoda* and *A. platycarpa* are ectomycorrhiza-dependent. This study also shows the compatibility of all these Australian *Acacia* species with West African ectomycorrhizal fungi. This result suggests that these tree species can form ectomycorrhiza with a large range of fungi.

A high percentage of ectomycorrhizal infection was correlated with the total number and biomass of nodules per root system. The main explanation for this bacterial contamination was that the irrigation tap water possibly contained indigenous rhizobia. Nodule formation and

functioning are dependent on mycorrhizal formation (Reddell and Warren 1986). This beneficial effect is generally attributed to the improvement of P uptake, which enhances nodulation and N_2 fixation (Cornet and Diem 1982).

Bacterial inoculant *P. monteilii* HR13 significantly increased ectomycorrhizal colonization of root systems with all the fungal symbionts and all Australian *Acacia* species, and consequently the number and biomass of rhizobial nodules. This beneficial effect has already been described in a previous study with *A. holosericea* and *P. alba* COI007 (Founoune et al. 2002b). Our results support the conclusions made by Garbaye (1994) that the MHB effect is not plant specific. Garbaye et al. (1992) have shown a MHB (*P. fluorescens* isolate BBc6)-promoted mycorrhiza formation with *Laccaria laccata* Scop ex Fr with four conifer species, Norway spruce [*Picea abies* (L.) Karst.], Austrian pine (*Pinus nigra* Arnold), Scots pine (*Pinus sylvestris* L.) and Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco]), and an angiosperm, pedunculate oak (*Quercus robur* L.). In contrast, it was demonstrated that the MHB effect was fungus specific. The intrageneric fungal specificity of MHB was demonstrated with the Douglas fir-*L. laccata* S238 symbiotic combination (Garbaye and Duponnois 1992) and observed in other studies (Duponnois et al. 1993; Dunstan et al. 1998). However, these conclusions are not supported by our results. Although *P. monteilii* HR13 was isolated from *P. alba*, it stimulated mycorrhiza development of *A. holosericea* with two species of *Scleroderma* and, more surprisingly, with the arbuscular mycorrhizal fungus *G. intraradices*. No significant correlation between the effect of *P. monteilii* HR13 on fungal growth in vitro and mycorrhizal formation has been found (r^2 =0.11). The main proposed mechanism for the MHB effect was that MHB stimulate hyphal growth in the rhizosphere. However, no positive bacterial effect has been recorded in the in vitro experiment on hyphal growth of *Scleroderma* isolates. Moreover, this bacterial isolate acted positively on the fungal growth of *G. intraradices* in the roots. Among mechanisms underlying the MHB effect, Garbaye (1994) has listed several hypotheses, such as the effect of MHB on the receptivity of the root. MHB could stimulate the production of phenolic compounds such as hypaphorine and increase the aggressiveness of the fungal symbiont.

From a practical point of view, it appears that *P. monteilii* could increase the mycorrhizal establishment of Australian *Acacia* species with ectomycorrhizal or endomycorrhizal fungi. It is well known that it is an advantage for the introduced fungus to be massively associated with the host plant during planting out in order to be in a strong competitive positive position against the other symbiotic fungi at the planting site. Inoculation with MHB could be beneficial to controlled mycorrhization practice in West Africa.

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