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Growth response of *Pterocarpus soyauxii* and *Lophira alata* seedlings to host soil mycorrhizal inocula in relation to land use types

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Deficiency in mycorrhizal inoculum in soils due to land use types (LUT) can be alleviated by quantity and quality inoculum addition. A bioassay was carried out to determine how host soil mycorrhizal inoculum influenced mycorrhizal colonization, carbon allocation and partitioning of seedlings of two native timber species of Cameroon humid forest. Seedlings of *Pterocarpus soyauxii* and *Lophira alata* were raised for six months on surface soils (0 - 20 cm) collected from early secondary forests and LUT derived from slash-and-burn agriculture and selective logging. Mycorrhizal inoculation effect (MIE) was derived. Seedlings were mainly colonized by members of the Glomaceae and Gigasporaceae, respectively, as shown by molecular typing. They generally performed poorly in soils with indigenous inoculum. But addition of soil inoculum from *P. soyauxii* trees favored nodulation, significantly increased mycorrhizal colonization and total biomass but decreased root-to-shoot ratios, resulting in large and positive MIE, irrespective of LUT. In contrast, host soil inoculum of *L. alata* did not affect fractional mycorrhizal colonization but significantly increased total biomass and resulted in high carbon allocation to roots in low and sometimes negative MIE. Therefore, seedlings' responses to mycorrhizal inoculum depend on host soil inoculum and that could be critical for successful rejuvenation of tropical trees.

Key words: Arbuscular, mycorrhiza-host, soil, inoculum-land use types, *Pterocarpus soyauxii*, *Lophira alata*, Cameroon.

INTRODUCTION

Current rates of deforestation and timber exploitation in the humid tropics suggest that sustained production of highly priced timbers will require alternative forest management scheme. Such design includes plantation forestry and artificial regeneration through quality seedlings in enrichment planting of logged-over forest management units or former agricultural farms. Recruitment and establishment of seedlings in natural forests and artificial woodlands are vital for maintaining forest structure and diversity. Virtually, seedlings of all rain forest trees are arbuscular mycorrhizal (AM) and many of them strongly depend on, and are highly responsive to

mycorrhizal fungi (Janos, 1980, 1996; Siqueira and Saggin-Junior, 2001; Onguene and Kuyper, 2005). However, the availability of mycorrhizal fungal inoculum might not always be sufficient and suitable to guarantee optimal seedling growth under the prevailing soil physical, chemical and biological conditions, thereby, suggesting that quality of mycorrhizal propagules in substrates or soils may determine seedling's fitness. In particular, land use practices may affect the abundance of mycorrhizal fungal inoculum. Michelsen (1992) noted scarce native mycorrhizal fungi in nurseries in East Africa for most grown tree species. Degraded tropical savannas of Venezuela also lacked enough autochthonous mycorrhizal inoculum to enhance seedling survival and growth (Cuenca et al., 1998). In general, agricultural practices did not negatively affect quantity and quality of

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mycorrhizal fungal propagules (Onguene, 2000; Sieverding, 1991). On the opposite, logging practices such as mechanical clearing, creation of forest roads, skid trails and landings, erosion and compaction have been shown to always be detrimental to mycorrhizal inoculum (Alexander et al., 1992; Carpenter et al., 2001, Musoko et al., 1994; Onguene and Kuyper, 2005). Consequently, growth of seedlings could be enhanced by addition of ample mycorrhizal fungal propagules to nursery substrates. Influence of mycorrhizal inoculum on seedlings' growth of distinct host plants has been little investigated in the tropics. This may be partly due to production constraints of large quantities of efficient and effective mycorrhizal inocula for field application. This impediment is likely to be partly alleviated if small quantities of soil could harbor adequate mycorrhizal inoculum to enhance growth and establishment of seedlings in soils deprived of effective mycorrhizal fungi. Such low-input technology, successfully applied in African nurseries for exotic, ecto-mycorrhizal pines inoculation with natural soil and humus from established plantations (Mikola, 1973), could also be valuable for silviculture of indigenous tree species, irrespective of whether they form ecto-mycorrhiza or arbuscular mycorrhiza. A consortia of indigenous AM fungi differently and significantly increased mycorrhizal root colonization and plant biomass of five fodder crops (Gaur and Adholeya, 2002). Both *Dicorynia guianensis* and *Eperua falcata* seedlings differed in mycorrhizal response as a combination of high seed mass, phosphorus reserve and quality of mycorrhizal inoculum (De Grandcourt et al., 2004).

Thus, preferential associations rather than ecological specificity *sensu stricto* may be more common in AM symbiosis. AM fungi may have different colonization strategies, varying both with the fungus and host plants (Bever, 2002). Mycorrhizal colonization effect may also vary with successional status of host tree species (Siqueira and Saggin-Junior, 2001) and seed reserves (Muthukumar and Udaiyan, 2000). In tropical rain forests, there may be an overwhelming set of interactions between host plants and mycorrhizal fungi (Kiers et al., 2000; Schmitt et al., 2001; Lovelock et al., 2003). The aims of this investigation were to determine how varied mycorrhizal colonization, above- and below-ground biomass of seedlings of two major native timber species of Cameroon humid forests grown in soils with different inherent inoculum potential of AM fungi caused by slash-and-burn agriculture and selective logging, and to assess their responsiveness to inoculation with host soil mycorrhizal inoculum taken from the root zones of con-specific tree species.

MATERIALS AND METHODS

Site and vegetation description

The soils used for the bioassays belonged to the Ebom series. Ebom (3°06'N; 10°44'E) is a rural community, on a rolling land-

scape (350 - 500 m a.s.l.), located 114 km east of Kribi (2°57'N; 9°55'E), in the southern coastal zone of Cameroon. Annual rainfall varies between 1500 and 2000 mm with two maxima in May and October; average monthly temperatures fluctuate between 23 and 27°C. Relative humidity is generally high, above 80% (Waterloo et al., 2000). Soils are moderately heavy clayey (60 - 80% clay), strongly acid (pH water 4.7), with low to medium organic matter content and very poor in available phosphorus (0.005 µg P in H₂O ml⁻¹ soil). They are classified in the FAO system as Xanthic Ferralsol (Van Gemerden and Hazeu, 1999). Within the Ebom area, forests are under intense human influence of both fallow-rotational shifting cultivation and selective logging (Van Gemerden and Hazeu, 1999; Onguene and Kuyper, 2001).

Five land use types (LUT) were selected, viz. food crop fields, fallows of *Chromolaena odorata* (Asteraceae), thereafter referred to as fallow, skid trails, bare landings and landings recolonised by *Musanga cecropioides* (Moraceae), thereafter, referred to as re-vegetated landings. In addition, early secondary forest stands were also included.

These stands are very dense due to the abundance of climbers, young saplings, juveniles and undergrowth vegetation. Fields of food crops were chosen after slashing the undergrowth vegetation, felling the existing trees, removing surface debris and burning dried biomass. Well-vegetated fallows with shrubs of 3 - 5 m high of about three to four years old were selected. Fields and fallow are LUT derived from agricultural practices, while skid trails and both types of landings are LUT resulting from selective logging; re-vegetated landings constitute the first phase of forest recovery following selective logging practices.

Timber species

Two native, large tree species, which provide highly valued timber and which are among the most frequently harvested trees nationwide, were chosen: *Pterocarpus soyauxii* (Fabaceae) and *Lophira alata* (Ochnaceae), locally known as Padouk and Azobe, respectively. *P. soyauxii* (Taub.) is commercially popular, being harvested for exports in Gabon, Equatorial Guinea and Cameroon where it is well-renowned for the local wood transformation industry. Its seeds are flat, circular (diameter about 1.5 - 2 cm) and papery (0.1 g). Seedlings develop a finely branched whitish root system devoid of root hairs but with numerous large pinkish nodules. *L. alata* (Banks ex Gaertn.) is harvested in Ghana, Gabon, Equatorial Guinea and Cameroon. Its seeds are bulging and elongated, weighing about 1.0 g. Seedlings grow rapidly and produce abundant dark red roots which are coarsely branched and devoid of root hairs.

Collection of soil samples and host soil mycorrhizal inoculum

Surface soils cores (0 - 20 cm) were collected from three random and independent spots in each land use type, bulked into about 20 kg composite of soil and fine root samples.

The host soil mycorrhizal soil inoculum used for inoculum addition was a mixture of fresh soil and fine root samples (0 - 10 cm) taken at four cardinal points around and in the vicinity of the stem base of five widely spaced mature seed-bearer trees of *P. soyauxii* and *L. alata*. These inocula were thereafter referred to as *Pterocarpus* and *Lophira* inoculum, respectively.

Collected soil samples and mycorrhizal inocula were placed separately, in air-filled polyethylene bags, taken to Kribi and kept under greenhouse benches to preserve soil from direct sun heat. Abundance of each type of mycorrhizal propagules in soils of all LUT and early secondary forest was determined by three methods (Table 1). Attempt to identify the spore types from host soil inoculum from *Pterocarpus* and *Lophira* trees in trap culture was carried out by DNA extraction from a pool of spores using DNeasy Plant

Table 1. Variation in inoculum potential of indigenous arbuscular mycorrhizal fungi in relation to land use types and changes in nodule number before and after addition of soil inoculum from *P. soyauxii* seed-bearer trees.

Land use types	Spore number ¹	MPN ²	Root colonization ³	Nodule number ⁴	
				Indigenous inoculum	Tree-specific inoculum
Food crop fields	28	29	31	1	3
Fallow	44	55	39	1	2
Secondary forest	21	16	23	1	2
Skid trails	9	0	16	0	2
Bare landing	5	0	3	0	2
Re-vegetated landing	8	6	25	2	3
Some AM spore species identified from the root zones of soil inocula of both tree species				<i>G. constrictum</i>	<i>Gi. decipiens</i>
				<i>G. manihotis</i>	<i>Gi. margarita</i>
				<i>G. coronatum</i>	<i>Gi. gigantae</i>
				<i>Glomus</i> sp	<i>Sc. nigra</i>

¹Number of spores. g⁻¹ dry soil assessed by the wet decanting and sugar centrifugation method (Onguene, 2000). ²Number of infective propagules. g⁻¹ dry soil assessed by a four-fold dilution series with five replicates a local variety of cowpea (*Vigna unguiculata*) (Fabaceae) as bait plant (porter, 1979). ³Root colonization (percent mycorrhizal root length colonized) of a local variety of cowpea in intact soil cores, replicated five times and grown for 30 days under greenhouse conditions in Kribi, South Cameroon. ⁴Nodule numbers in roots of seedlings of *P. soyauxii* defined as: 1: 1 - 5 nodules per seedling; 2: 6 - 10 nodules per seedling; 3: >10 nodules per seedling. G: *Glomus*; Gi: *Gigaspora*; Sc: *Scutellospora*. Fallow refers to fallow of *C. odorata*; Re-vegetated landing refers to bare landing re-colonized by the pioneer tree *M. cecropioides*.

Mini Kit (QIAGEN), followed by PCR amplification of partial LSU rDNA region, cloning, restriction fragment length polymorphism analysis and sequence alignment and phylogenetic analysis (Ngonkeu, 2003, 2009).

Mycorrhizal inoculation

Three days after field collection of soil and inoculum samples, half of potted bags were thoroughly mixed with 50 g (d.w. basis) portions kg⁻¹ soil of either *Pterocarpus* or *Lophira* inoculum. Non-inoculated unsterile soils received similar amounts of steam sterilized host soil inoculum and 50 ml kg⁻¹ soil of filtered soil inoculum leaching to insure similar microbial activity (Hetrick et al., 1988). Steam sterilization was achieved at 100°C for 1 h. The sterilized soil was left to stand for five days on greenhouse benches before use.

For each plant species, there were 36 experimental units composed of six land use types (LUT), two inoculation treatments and three replicates. In addition, a triplicate control was prepared to assess growth and mycorrhizal colonization effect of host rhizospheric soil inoculum. Plastic bags were placed on greenhouse benches in a randomized complete block design.

Seed collection, treatment and plant nurturing

Seeds were collected around seed-bearer mother trees in Ebom. Bulging and firm *L. alata* seeds were soaked overnight in cool water before manual scarification; flat seeds of *P. soyauxii* did not require a pre-treatment. Seeds of both plants were sterilized in 70% alcohol for 1 min, rinsed three times with sterile water and placed in washed and sterilized sea sand.

One one-week-old pregerminated seed was then placed in five kg (dry weight basis) portions of potted soils. Plants were grown without nutrient amendment. Water was added as needed to maintain potted soils at water holding capacity. Plants were raised under natural light in a shaded house in Kribi.

Assessment of seedling growth and mycorrhizal colonization

Six months after transplanting, shoots were separated from the root systems, dried at 70°C for 72 and 24 h, respectively. Then, shoot and root dry weights were taken; total biomass and root-to-shoot ratio were derived. Nodules on roots of *P. soyauxii* were counted and grouped in three categories: category 1: 1 - 5 nodules, 2: 6 - 10; 3: above 10.

The response to host soil mycorrhizal inoculation was assessed as mycorrhizal inoculation effect (MIE). MIE were calculated as follows:

$$\text{MIE} = 100 \times a(1 - b/a)$$

where a and b were the average total biomass of seedlings grown in soil with host soil and indigenous inoculum added, respectively (Bagyaraj, 1994; Muyanziza et al., 1997). Fractional colonization by AM fungi was estimated by scoring the presence or absence of mycorrhizal fungal structures in at least 100 intersection points between root fragments and gridlines of a Petri dish, under a dissecting microscope at 40x (Giovannetti and Mosse, 1980), after clearing, bleaching (only of roots of *L. alata*), staining with acid fuchsin and destaining in a lacto-glycerol solution (Onguene and Kuyper, 2001). 10 to 20 short root fragments were randomly chosen, mounted under glass and cover glass, gently squashed and examined under a photonic microscope at 25 – 40X to confirm mycorrhizal structures such as arbuscules, vesicles, hyphal coils, internal hyphae and/or auxiliary bodies attached to external hyphae.

Statistical analyses

Statistical analyses were performed using the SAS package (SAS Inc., 2004). All data were tested for normality and homogeneity of variances. As variances were unequal, fractional colonization data were arc sin square root transformed and total biomass dry weight data was square root transformed. A two-way analysis of variance (ANOVA) with land use types and mycorrhizal inoculum as

Table 2. General linear model procedure of arc sin transformed fractional mycorrhizal colonization, square root transformed total biomass and root-to-shoot ratio (RIS ratio) of six months-old *Pterocarpus soyauxii* and *Lophira alata* seedlings in relation to land use types (LUT) and addition of host-tree specific inoculums.

Sources of variation	Fractional colonization			Total biomass		R/S ratio	
	Df	F	P	F	p	F	P
<i>P. soyauxii</i>							
LUT	5	10.4	<0.0001 **	2.84	0.0176*	2.52	0.057ns
Inoculum	1	68.5	<0.0001 **	29.1	<0.00001**	11.7	0.00023**
LUT x Inoculum	5	1.34	0.281ns	1.20	0.375ns	2.40	0.0684ns
<i>L. alata</i>							
LUT	5	4.14	0.0075**	24.7	<0.0001**	4.15	0.0074**
Inoculum	1	1.96	0.175ns	9.04	0.0061**	7.57	0.0111*
LUT x Inoculum	5	0.53	0.749ns	6.70	0.0005**	0.58	0.716ns

ns: non significant at 0.05% level of significance; * and **: significant and highly significant at 0.05% level of significance, respectively.

independent variables was performed using the general linear model.

Average means were separated by Duncan's multiple range tests. Pearson's correlation coefficients were calculated between fractional mycorrhizal colonization, total biomass of seedlings and root-to-shoot ratio. Mycorrhizal inoculation effect (MIE) was based on average total biomass of seedlings with indigenous inoculum and seedlings with host soil mycorrhizal inoculum added, thus, no statistical tests of MIE were executed.

RESULTS

P. soyauxii

Identification of spores from both *Pterocarpus* soil inocula showed mostly *Glomus* species including *Glomus constrictum*, *Glomus manihotis*, *Glomus coronatum* and the most abundant small size and hyaline *Glomus* sp (Table 1). In the presence of indigenous inoculum, few nodules were observed in soils from all land use types (LUT) but the re-vegetated landings, *Pterocarpus* soil inoculum increased the number of nodules in soils from all LUT; the highest nodule number was recorded in soils from food crop fields and re-vegetated landings (Table 1). Mycorrhizal colonization of roots of *P. soyauxii* seedlings consistently showed abundant intra-radical hyphae, various shaped vesicles, few arbuscules and hyphal coils; a small number of auxiliary bodies on extra-radical hyphae were also observed. Fractional mycorrhizal colonization (FMC) of roots of *P. soyauxii* seedlings was very highly significantly affected by LUT and by inoculum addition but not by their interaction (Table 2). Before inoculation, FMC was the highest in soils from re-vegetated landings and food crop fields. It substantially increased after addition of *Pterocarpus* inoculum in soils from all LUT; it was still the lowest in soils of bare landing though (Table 4).

FMC was positively and very highly correlated to total biomass: $r = 0.875$, $n = 36$; $P < 0.0001$.

Total biomass was significantly affected by LUT and very highly significantly affected by addition of *Pterocarpus* inoculum but not by their interaction (Table 2). In soils taken under *P. soyauxii* seed-bearer trees, total biomass averaged 11.6 g per seedling. In the presence of indigenous inoculum, total biomass was lower in soils from all LUT; the lowest total biomass was observed in soils from bare landings. Addition of *Pterocarpus* inoculum significantly increased total biomass in soils from all LUT but not from the re-vegetated landings; fallow soils yielded the highest total biomass with similar level of magnitude as the control (Figure 1A).

There was no correlation between total biomass and root-to-shoot ratio. Root-to-shoot ratio of *P. soyauxii* seedlings was very highly significantly affected by addition of *Pterocarpus* inoculum but neither by LUT nor by their interaction (Table 2). Mycorrhizal inoculation generally decreased root-to-shoot ratio with the exception of seedlings grown in soils from food crop fields and re-vegetated landings (Table 3). Root-to shoot ratio was negatively correlated with FMC: $r = 0.374$; $n = 36$; $P < 0.0248$. Mycorrhizal inoculation effect (MIE) on *P. soyauxii* seedlings was large and positive, irrespective of LUT; the highest MIE was recorded in soils from bare landing and the lowest in soils from re-vegetated landings (Table 4).

L. alata

Lophira soil inoculum was dominated by AM fungi belonging to Gigasporaceae family, particularly, *Gigaspora decipiens*, *Gigaspora margarita*, *Gigaspora gigantea* and *Scutellospora nigra* (Table 1). Mycorrhizal colonization of roots *L. alata* seedlings was inconsistently extra-radical with large and thick-walled hyphae, frequently carrying numerous spiny auxiliary cells; internal colonization was low without arbuscules, vesicles and

Table 3. Variation in root-to-shoot ratio (RIS ratio) of six months-old seedlings of *P. soyauxii* and *L. alata* seedlings in relation to land use types (LUT) and addition of host-tree specific inoculum.

Land use types/ Tree species/ Mycorrhizal inoculum	<i>P. soyauxii</i>		<i>L. alata</i>	
	Indigenous	Tree-specific	Indigenous	Tree-specific
Food crop fields	0.667	0.461	0.319	0.385
Fallow	0.534	0.545	0.347	0.380
Secondary forest	0.902	0.666	0.332	0.406
Skid trails	0.726	0.321	0.681	0.554
Bare landing	0.728	0.439	0.663	0.672
Re-vegetated landing	0.471	0.478	0.529	0.567

Table 4. Fractional mycorrhizal colonization (FMC) after addition of *Pterocarpus* and *Lophira* inocula and mycorrhizal inoculation effect (MIE) based on total biomass of six months-old seedlings of *P. soyauxii* and *L. alata* seedlings in relation to land use types.

Tree species	<i>P. soyauxii</i>		<i>L. alata</i>	
	FMC	MIE	FMC	MIE
Land use types				
Food crop fields	52.7b	+55	34.0 ns	+18
Fallow	69.4 a	+79	12.7 ns	- 42
Secondary forest	58.4 ab	+56	16.7 ns	+24
Skid trails	54.0b	+41	26.7 ns	+14
Bare landing	26.3 c	+81	2.00 ns	+20
Re-vegetated landing	72.7 a	+23	36.7 ns	-31

Different letters indicate significant differences at $p < 0.05$. ns: no significant differences at $p < 0.05$. Fallow refers to fallow of *C. odorata*; Re-vegetated landing refers to bare landing re-colonized by the pioneer tree *M. cecropioides*.

hyphal coils.

Fractional mycorrhizal colonization of *L. alata* was very highly significantly affected only by LUT but neither by inoculum addition nor by their interaction (Table 2). The highest fractional mycorrhizal colonization (FMC) was recorded from re-vegetated landings and was of the same level of magnitude as that of food crop fields. However, it was the lowest in soils from bare landings, low in fallow soils, intermediate in soils from successional forest and skid trails (Table 4). FMC was not correlated with total biomass.

Total biomass of *L. alata* seedlings was very highly significantly influenced by both independent variables and their interaction (Table 2). In soils taken under *L. alata* seed-bearer trees, total biomass was 12.2 g per seedling. In the presence of indigenous inoculum, total biomass of *L. alata* seedlings was significantly lower in soils from skid trails, bare and re-vegetated landings than in soils from fields and fallow. Addition of *Lophira* inoculum significantly increased total biomass in soils from all LUT but fallow and re-vegetated landings (Figure 1B). Total biomass was negatively correlated with root-to-shoot ratio: $r = 0.466$; $n = 36$; $P < 0.0041$.

Root-to-shoot ratio of *L. alata* seedlings was very highly affected only by inoculum but neither by LUT nor by their

interaction (Table 2). In soils from most LUT, but not skid trails, root-to-shoot ratio was increased after addition of *Lophira* soil inoculum (Table 3). Mycorrhizal inoculation effect on *L. alata* seedlings was generally low and positive; it was however negative in soils from fallow and re-vegetated landings (Table 4). There was no correlation between root-to-shoot ratio and FMC.

DISCUSSION

Initial evidence of the effect of host soil mycorrhizal colonization of seedlings of both tree species was provided by dissimilar morphological characteristics of colonizing AM fungi. Mycorrhizal colonization of roots of *P. soyauxii* seedlings showed abundant inter- and intra-hyphal mycelia, profuse vesicles, few arbuscules and hyphal coils, typical of the *Arum-type* mycorrhizas (Smith and Read, 1997). The presence of variously shaped vesicles in roots of *P. soyauxii* seedlings strongly indicates mutual associations with AM fungi of the Glomacean family. On the opposite, the presence of numerous spiny auxiliary cells on external hyphae attached to roots of *L. alata* seedlings and the large size of internal hyphae points out to colonization mainly by members of the Gigasporaceae. In another investigation, seedlings of both *Dicorynia guianensis* and *Eperua falcate* exhibited the *Paris-type* AM colonization (De Grandcourt et al., 2004).

AM fungi may differ in their colonization strategies depending both on the type of fungal propagules and the host plants. Isolates of *Glomus* and *Acaulospora* were both able to colonize host plants from spores, colonized root fragments and extra radical hyphae while those of *Gigaspora* and *Scutellospora* colonized only from colonized root fragments, to a lesser extent, though (Klironomos and Hart, 2002). Regulating mechanisms of particularly effective host-mycorrhizal fungus interactions remain to be clarified, though. Attempt to identify spores from tree-specific inocula used in this study clearly indicates that different AM fungal taxa could preferentially associate with different tree species and elicit enhanced growth. Seedling performance of common *Pulsatilla* species grown with native AM fungal communities from grassland compared with forest inoculum was more

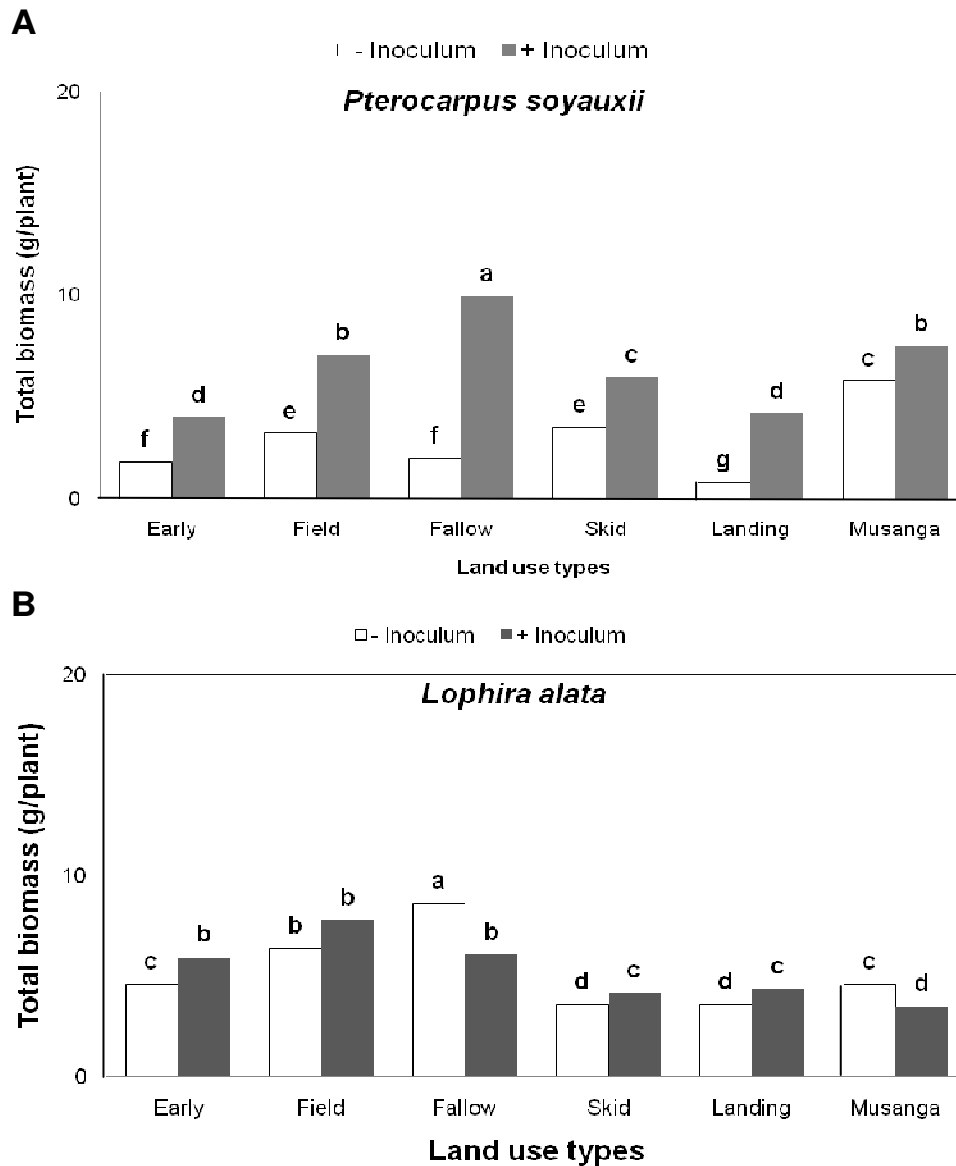


Figure 1. Total biomass of six months-old seedlings of *P. soyauxii* (A) and *L. alata* (B) in relation to land use types (LUT) and addition of host-tree specific inoculum. Different letters indicate significant differences at $p < 0.05$

vigorous than that of rare *Pulsatilla* species (Moora et al., 2004). A large degree of selectivity of the effect of mycorrhizal colonization has also been recently noted on tropical tree species. In Guyana rain forest, seedlings of *D. guianensis* and of *E. falcate* (both members of the Caesalpiniaceae) grown in soil with roots of mature trees of the former trees species had dissimilar growth response, with increased and decreased growth of the first and the second, respectively (De Grandcourt et al., 2004). If preferential associations of AM fungi with various tree species are widespread in tropical rain forests, it could to some extent contribute regeneration attempts of indigenous timber species even to the explanation for the lack of success in artificial in the

presence of large quantity of mycorrhizal inoculum.

Our results demonstrate that addition of host tree-specific inoculum, just like addition of grass inoculum, can yield substantially larger seedlings of timber species (Onguene and Kuyper, 2005). *Pterocarpus* and *Lophira* soil inocula significantly increased carbon build up and allocation of seedlings but to different extent. Similar results have been recorded by earlier workers (Michelsen, 1993; Gaur and Adholeya, 2002; Gerhing, 2003; De Grandcourt et al., 2004; Moora et al., 2004). Various factors could influence mycorrhizal responses of seedlings, such as light demand and seed size. A positive correlation between mycorrhizal colonization and growth performance was recorded in seedlings of *D. guianensis* raised

under 14% of full sunlight, showing a greater mycorrhizal efficiency (Béreau et al., 2000). Responses in growth and biomass allocation to AM colonization were higher under small gap light intensities and resulted in larger plants with small carbon allocation to roots (Gerhing, 2003). In this study, total biomass of seedlings of *P. soyauxii* grown under full light was positively and highly correlated with fractional mycorrhizal colonization, conversely to *L. alata*. Differences between tree species in dependency on and responsiveness to AM fungi has been attributed to various characteristics. Janos (1996) proposed that early successional species are generally less dependent on arbuscular mycorrhizas than late successional and climax forest tree species. *L. alata* is considered a pioneer tree that may have expanded in the "Forêt littorale" during the 18th and 19th centuries following forest clearings and man-induced fallowing; conversely, *P. soyauxii* is a climax tree species of Atlantic Biafrean moist forests of south Cameroon (Letouzey, 1968). Thus, the higher growth response of *P. soyauxii* seedlings than that of *L. alata* seedlings is consistent with the successional hypothesis on mycorrhizas.

Seedlings of small-seeded pioneer species also were more dependent on AM inocula for initial survival and growth (Kiers et al., 2000). However, mycorrhizal dependency of pioneer, shade tolerant and light-demanding Brazilian woody species varied from highly to very highly mycorrhizal dependent (Siquiera and Saggin-Junior, 2001).

Root features have also been implicated as a determinant of mycorrhizal dependency and responsiveness (Baylis, 1975). Incidence of root mass may be more important than other root features such as root diameter, root density, root hair incidence and root hair length (Manjunath and Habte, 1991). Species with a lower root production are predicted to be highly mycorrhizal dependent. In this study, six months-old seedlings of *P. soyauxii* and *L. alata* did not markedly differ in carbon allocation to roots and likewise lacked root hairs. After soil inoculation, root-to-shoot ratio of *P. soyauxii* seedlings significantly decreased in all land use types but fallow and re-vegetated landings, conversely to *L. alata* seedlings, with the exception of soils from skid trails. Decrease in carbon allocation to roots of *P. soyauxii* seedlings also suggests the efficiency of *Pterocarpus* soil inoculum.

Both groups of seedlings also markedly differ in growth rate after addition of quality inoculum. Seedlings of *P. soyauxii* grew very rapidly following tree-specific inoculation while those of *L. alata* did not (Onguene, 2000). Janos (1980) suggested that non-mycotrophic and facultatively mycotrophic species have lighter seeds than obligately mycorrhizal species. Our data do not conform to this hypothesis, as light-seeded seedlings of *P. soyauxii* were more responsive to tree-specific addition than heavy-seeded seedlings of *L. alata*. The high phosphorus requirements of a nodulating legume might

make such species more mycotrophic, independent of seed size. However, in an earlier study with seedlings of three timber species (Onguene and Kuyper, 2005), it was observed that the non-nodulating legume *Distemonanthus benthamianus* (Caesalpiniaceae) with heavy seeds had a larger mycorrhizal inoculation effect than *Terminalia superba* (Combretaceae), thus, confirming the nutrient effect stirring up mycorrhizal demands. By contrast, seedlings of the large-seeded Caesalp tree species *E. falcate* seemed less responsive to mycorrhizas (Baraloto, 2001).

Seedlings of both timber species differed in specific mycorrhizal inoculation effect. In general, MIE was large and positive for *P. soyauxii* seedlings. For *L. alata* seedlings, MIE was low and negative in soils from fallow and revegetated landings. Fallow soils showed high mycorrhizal inoculum potential across various sites in South Cameroon (Onguene, 2000). However, both seedlings varied in growth in soils from agricultural practices. Poor growth of *P. soyauxii* contrasted with relatively good growth of *L. alata* seedlings in these soils, suggestive of possible difference in light demand. If indeed *L. alata* is an early light-demanding species, then, it can readily establish on former agricultural land, conversely to *P. soyauxii*. Both seedlings grew poorly on soils from skid trails and bare landing, to different extent, though, owing to the negative impacts of heavy machinery on physical and chemical soil surface properties in addition to reduction in mycorrhizal propagules (Table 1). A substantial increase in bulk density and soil compaction was also recorded along skid trails and landings, which might be partially responsible for the reduced performance of seedlings on soils of forestry practices. Earlier authors also reported negative effects of logging practices (Nadian et al., 1996). We regularly observed seedlings of both species on bare landings, but they all died within a few years. However, in our experiment, *P. soyauxii* seedlings grew very well in soils from re-vegetated landings. These logged-over sites are rapidly invaded by the early successional, facultative mycorrhizal tree, *Mussanga cecropioides* (Onguene and Kuyper, 2001) and generally show a progressive build up in surface soil horizon and organic matter. It is also possible that contaminating debris from various logging operations could enrich such re-vegetated landings by mixing of different soils horizons.

In conclusion, our results demonstrate that seedlings of timber species do not always form effective mycorrhizal associations with all kinds of arbuscular mycorrhizal fungal communities, confirming the hypothesis that the quality of the mycorrhizal propagules in soils determines seedling fitness. For sustainable timber management and conservation, there is a need for the assessment of inoculum quality for the effective and rapid mycorrhization of seedlings of important timber species. This aspect of practical mycorrhization should be considered for successful rejuvenation of forests.

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REFERENCES

- Alexander I, Ahmad N, See LS (1992). The role of mycorrhizas in the regeneration of some Malaysian forest trees. *Phil. Trans. R Soc. Lond. ser., B* 335: 379-388.
- Bagyaraj DJ, (1994). Vesicular-arbuscular mycorrhiza: application in agriculture. In: J.R. Norris, D.Read and A.K. Varma (eds.), *Techniques for mycorrhizal research* London, Academic Press, pp. 819-833.
- Baraloto C (2001). Tradeoffs between neotropical tree seedling traits performance in contrasting environments. PhD thesis. Ann. Arbor, MI, USA: University of Michigan.
- Baylis GTS (1975). The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: F.E. Sanders, B. Mosse and P.B. Tinker (eds), *Endomycorrhizas*, London, Academic Press, pp. 373-389.
- Béreau M, Barigah TS, Louisanna E, Garbaye J (2000). Effects of endomycorrhizal development and light regimes on the growth of *Dicotylnia guianensis* AmshoT seedlings. *Ann. For. Sci.*, 57: 725 - 733.
- Bever JD (2002). Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant Soil*, 244: 281-290.
- Carpenter FL, Mayorga SP, Quintero EG, Schroeder M (2001). Land-use and erosion of a Costa Rican ultisol affect soil chemistry, mycorrhizal fungi and early regeneration. *For. Ecol. Manage.*, 144: 1-17.
- Cuenca G, De Andrade Z, Escalante G (1998). Arbuscular mycorrhizae in the rehabilitation of fragile degraded tropical lands. *Biol. Fertil. Soils*, 26: 107-111.
- De Grandcourt A, Epron D, Montpioo P, Louisanna E, Béreau M, Garbaye J, Guehl IM (2004). Contrasting responses to mycorrhizal inoculation and phosphorus availability in seedlings of two tropical rain forest tree species. *New Phytol.*, 16: 865-875.
- Gaur A, Adholeya A (2002). Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol. Fertil. Soils*, 35: 214-218.
- Gerhing CA (2003). Growth responses to arbuscular mycorrhizas by rain forest seedlings vary with light intensity and tree species. *Plant Ecol.*, 167: 127-159.
- Giovannetti G, Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489-500.
- Hetrick RAD, Wilson GT, Kitt DG, Schwab AP (1988). Effects of mycorrhizal contribution to growth of big bluestem grass in non-sterile soil. *Soil Biol. Biochem.*, 20: 501-507.
- Janos DP (1980). Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, 61: 151-162.
- Janos DP (1996). Mycorrhizas, succession and rehabilitation of deforested lands in the humid tropics. in: J.C. Frankland, N. Magan and G.M. Gadd (eds), *Fungi and environmental change*, Cambridge University Press, Cambridge, pp. 129-161.
- Kiers ET, Lovelock CE, Krueger EL, Herre EA (2000). Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecot. Lett.*, 3: 106-113.
- Klironomos IN, Hart MM (2002). Colonization of roots of arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza*, 12: 181-184.
- Letouzey R (1968). *Etude phytogéographique du Cameroun*. P. Lechevalier, Paris, p. 511.
- Lovelock CE, Andersen K, Morton R (2003). Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment *Oecologia*, 135: 268-279
- Manjunath A, Habte M (1991). Categories of vesicular-arbuscular mycorrhizal dependency of host species. *Mycorrhiza*, 1: 3-12.
- Michelsen A (1992). Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. *For. Ecot. Manage.*, 48: 335-344.
- Michelsen A (1993). Growth improvement of Ethiopian acacias by addition of vesicular arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soil. *For. Ecot. Manage.*, 59: 193-206.
- Mikola P (1973). Applications of mycorrhizal symbiosis in forestry practice. in: C. G. Marks and T.T. Kozłowski (eds), *Ectomycorrhizae - their ecology and physiology*, New York, Academic Press, pp. 383-411.
- Moorra M, Opil M, Sen R, Zobel M (2004). Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. *Funct. Ecot.*, 18: 554-562
- Musoko M, Last FT, Masan PA (1994). Populations of spores of vesicular-arbuscular mycorrhizal fungi in undisturbed soils of secondary semi-deciduous moist tropical forest in Cameroon. *For. Ecot. Manage.*, 63: 359-377.
- Muthukumar T, Udaiyan K (2000). The role of seed reserves in arbuscular mycorrhizal formation and growth of *Leuceana leucocephala* (Lam.) de Wit and *Zea mays* L. *Mycorrhiza*, 9(6): 323-330
- Muyanziza E, Kehri HK, Bagyaraj DJ (1997). Agricultural intensification, soil biodiversity and agro-ecosystem function in the tropics: the role of mycorrhiza in crops and trees. *Appl. Soil Ecol.*, 6: 77-85.
- Nadian H, Smith SE, Alston AM, Murray RS (1996). The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonization. *Plant Soil*, 182: 39-49.
- Ngonkeu MEL (2003). Biodiversité et potentiel des mycorrhizes à arbuscules de certaines zones agro-écologiques du Cameroun. Thèse de Doctorat 3^e cycle. Université de Yaoundé I Cameroun, p. 258.
- Ngonkeu MEL (2009). Tolérance de certaines variétés de maïs aux sols à toxicité aluminique et manganique du Cameroun et diversité moléculaire et fonctionnelle des mycorrhizes à arbuscules. Thèse de Doctorat Ph.D Université de Yaoundé I Cameroun, p. 224.
- Onguene AN (2000). Diversity and dynamics of mycorrhizal associations in tropical rain forests with different disturbance regimes in south Cameroon. Ph.D thesis, Wageningen University, Tropenbos Documents Series, 3: 167
- Onguene NA, Kuyper THW (2001). Mycorrhizal associations in the rain forest of south Cameroon. *For. Ecol. Manage.*, 140: 277-287
- Onguene NA, Kuyper THW (2005). Growth responses of three native timber species to soils with different arbuscular mycorrhizal inoculum potentials in South Cameroon. Indigenous inoculum and effect of addition of grass inoculum. *For. Ecol. Manage.*, 210: 283-290
- SAS Institute Inc. (2004). *SAS/STAT users' guide*, V.9.13, SAS Campus Drive, NC, USA.
- Sieverding E (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Eschborn, Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.
- Siqueira JO, Saggin-Junior OJ (2001). Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian woody species. *Mycorrhiza*, 9(6): 245-255.
- Smith SE, Read DI (1997). *Mycorrhizal symbiosis*, 2nd ed. London, Academic Press.
- Van Gernerden BS, Hazeu GW (1999). Landscape ecological survey (1:100 000) of the Bipindi - Akom il - Lolodorf region, Southwest Cameroon. Tropenbos Cameroon Documents, 1: 1-164.
- Waterloo MI, Ntonga JC, Dolman AJ, Ayangma AB (2000). Impact of shifting cultivation and selective logging on the hydrology and erosion of rain forest land in South Cameroon. Tropenbos-Cameroon Documents 3. Tropenbos-Cameroon, Kribi.