Chapter 15

EARLY GROWTH IMPROVEMENT ON ENDEMIC TREE SPECIES BY SOIL MYCORRHIZAL MANAGEMENT IN MADAGASCAR

H. Ramanankierana[1], R. Baohanta[1], J. Thioulouse[2], Y. Prin[3], H. Randriambanona[1], E. Baudoin[4], N. Rakotoarimanga[1], A. Galiana[3], E. Rajaonarimamy[1], M. Lebrun[4] and Robin Duponnois[4,5,*]

[1]Laboratoire de Microbiologie de l'Environnement. Centre National de Recherches sur l'Environnement. BP 1739 Antananarivo. Madagascar; [2]Université de Lyon, F-69000, Lyon ; Université Lyon 1 ; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France; [3]CIRAD. Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UM2, Campus International de Baillarguet, TA A-82/J, Montpellier, France; [4]IRD. Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UM2, Campus International de Baillarguet, TA A-82/J, Montpellier, France; [5]Laboratoire Ecologie & Environnement (Unité associée au CNRST, URAC 32). Faculté des Sciences Semlalia. Université Cadi Ayyad. Marrakech. Maroc

Abstract

Mycorrhizal fungi are ubiquitous components of most ecosystems throughout the world and are considered key ecological factors in (1) governing the cycles of major plant nutrients and (2) sustaining the vegetation cover. Two major forms of mycorrhizas are usually recognized: the arbuscular mycorrhizas (AM) and the ectomycorrhizas (ECM). The lack of mycorrhizal fungi on root systems is a leading cause of poor plant establishment and growth in a variety of forest landscapes. Numerous studies have shown that mycorrhizal fungi are

* E-mail address: robin.duponnois@ird.fr

able to improve the survival and early growth of various tree species in the field. Mycorrhizal association is estimated to occur in 95% of native undisturbed vegetation, whereas it occurs in less than 1% of vegetation from disturbed sites. Thereafter, mycorrhizal symbiosis has to be reestablished at these latter sites to benefit from the mycorrhizal effects on plant growth. This can be achieved by enhancing the mycorrhizal status of seedlings before they are transplanted to disturbed sites. It is necessary that nurseries produce tree seedlings associated with mycorrhizal fungi that are ecologically compatible with the tree species and the planting sites to make afforestation successful. According to these conditions that have to be taken into account, different methods of mycorrhizal inoculation have been identified to optimize fungal effects on plant growth. The main objective of this chapter was to describe some methods to obtain mycorrhizal seedlings at the nursery and to present some tree growth data resulting from the use of mycorrhization under such conditions in Madagascar.

Introduction

Land degradation is expanding around the world, and the (i) decline in soil fertility, and deterioration of soil physical and biological properties, and (ii) invasion by aggressive vegetation are serious concerns to forest regeneration. Particularly, tropical deforestation is of great concern worldwide for its impact on biological diversity and biochemical cycles, especially the global C cycle, which is known to affect climatic changes[1]. Trees play major ecological and functional roles within ecosystems. Also, they are regarded as a source of cash, savings and assets to the rural poor, and can help to meet the growing global demand for timber and other forest products[2]. However, forest cover continues to decrease over the world, and native species are particularly endangered especially on tropical ecosystems. Fostering of reforestation, formation of riparian woodlands and agroforestry programs have been undertaken to reverse this trend, especially in arid areas and deforested lands that have poor natural forest regeneration. Tree seedling mortality and development during the early growth stage are major factors influencing forest dynamics. Thus, research must aim at understanding how and why tree seedling either grow or die[3].

Among tropical forests, Madagascar's natural forest contains a diverse and highly endemic flora and fauna[4]. Composed by rainforest, and dry and spiny forests, Malagasy native forest cover was about 9.4 hectares in 2005, all of which was considered as highly exploited and endangered[5]. The recruitment processes are poorly known within these native forests, and they constitute a serious gap in our understanding of forest recovery processes and forest regeneration and conservation. Knowledge of seedling development and plant coexistence are not only important for our understanding of the forest recovery processes, but are also required for increasing the success and efficiency of restoration practices, and the performance of afforestation. In this way, it has been demonstrated that mycorrhizal fungi, an ubiquitous component of most ecosystems throughout the world, are an ecological key in improving seedling dynamics (development and mortality rate) by governing the cycle of major plant nutrients and by mitigating the attack of plant pathogens[6, 7, 8, 9]. Mycorrhizas constitute an important root symbiosis for approximately 92% of plant families and offer the potential to make

a significant contribution to natural regeneration of vegetation communities[10]. Two major forms of mycorrhizas are usually recognized: the arbuscular mycorrhizas (AMs) and the ectomycorrhizas (ECMs). Arbuscular mycorrhizal symbiosis is the most widespread mycorrhizal association type and is fundamental in optimizing plant fitness and soil quality[11]. Particularly, the AM symbioses improve the resilience of natural plant communities against environmental stresses[12]. Some studies in Africa and in Central America have shown that most plant species found in rainforests are endomycorrhizal[13]. However, the impact of AM fungi on growth of individual plant species varies depending on the AM fungal taxa involved[7, 14]. The ectomycorrhizal symbiosis is phylogenetically restricted, and has evolved separately in several lineages of land plants[10]. ECMs are clearly younger than the ancient AMs, and occur in the forests of cool-temperate and boreal latitudes[11]. They also occur in an ecologically and economically important minority of tropical tree species belonging to the families and sub-families of Fagaceae, Caesalpinioideae, Betulaceae, Diptericarpaceae, Leptospermoideae in the Myrtaceae, Phyllanthaceae, Gnetaceae, Sapotaceae, Papilionoideae, Proteaceae, Asteropeiaceae, Sarcolaenaceae, Casuarianaceae and Acacieae[15, 16, 17, 18, 19, 20, 21].

For many decades, the importance of mycorrhizal fungi to terrestrial ecosystems has been recognized, and their potential use in forestry has been explored. In Madagascarian forest ecosystems, it has been illustrated that some endemic trees are associated with a high diversity of ectomycorrhizal fungi[22, 23]. Also, a large part of endemic forest trees have evolved with, at least, one type of mycorrhizal structure[24] (Ducousso et al., 2008). In this chapter, we address the implications of mycorrhiza on the early growth of some Madagascarian, endemic tree seedlings with emphasis on the importance of mycorrhizal fungi diversity and some pioneer plant species.

Mycorrhizal status description of native forest tree species in the central and eastern parts of Madagascar

Although mycorrhizal structures are dominant within native tree and shrub species in the natural forests of Madagascar, little is known about the importance of these symbiotic structures on the regeneration strategies of forests or the ecological restoration of perturbed areas. The mycorrhizal status of dominant shrub and tree species within three Malagasy natural forest formations is indicated in Table 1. Surveyed sites were located along the eastern (Analalava and Ianjomara forest) and the central (Sclerophyllous forest of Arivonimamo) part of Madagascar. Analalava and Ianjomara forests are situated in well-preserved stands of coastal tropical rainforests. They are characterized by a high diversity of endemic trees. The sclerophyllous forest of Arivonimamo is mainly formed by a population of *Uapaca bojeri* with some shrub species of Sarcolaenaceae and Asteropeiaceae, two botanical families endemic to Madagascar.

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Mycorrhizal results were obtained by examining 30 randomly chosen root fragments of 10 mm length each using a light microscope, for each plant species. Roots were considered AM when intracellular arbuscules and/or hyphal coils and/or vesicles were observed. The degree of AM infection was assessed according to four classes: (i) nonmycorrhizal (termed "NM"), when no fragments presented any trace of AM infection; (ii) lightly infected [termed "(AM)"], when only one to three fragments presented AM intracellular structures; (iii) AM infected (termed "AM"), when four to 29 fragments presented AM structures, and (iv) heavily infected (termed "AM+"), when all 30 fragments presented abundant AM structures.

Only four tree species (*Mascarenhasia arborescens* and *Tabernaemontana coffeoïdes* in Analalava forest, *Landolphia sp* and *Voacanga thouarsii* in Ianjomara forest) were identified as nonmycorrhizal. These four nonmycorrhizal tree species belong to the botanical family of Apocynaceae. Among the 111 study plant species, 12 were lightly infected; 62 species presented typical, well-developed AM infections; 27 species were heavily infected, and 6 species were found with both AM and ECM. In the family of the Sarcolaenaceae, all the examined species had both ECM and AM. These results illustrated the massive occurrence of mycorrhizal structures within the Malagasy flora, particularly within the endemic flora. All 42 endemic species presented mycorrhizal structures. Moreover, results of table 1 showed that more than 95% of the examined species in the three different forest ecosystems were associated with mycorrhizal fungi.

Importance of mycorrhizal symbionts on seedling development under controlled conditions

In addition to the high diversity observed within the flora of Madagascar, Malagasy natural forests are well known by their high rate of endemicity[25]. However these native tree species which have economical and ecological value were rarely used by the national program of reforestation. This is because of the little knowledge on the conditions of early development of their seedlings. The success of an outplanted nursery –grown tree seedlings depends on their ability to rapidly access nutrients and water held within the soil matrix[26]. In nature, this process is enhanced by the formation of symbiotic mycorrhizal associations. However, on many disturbed sites (e.g., mine spoils or abandoned agricultural lands), suitable mycorrhizal fungi are lacking, and this might limit seedling establishment and growth[27]. In this part of the chapter, we describe research activities relative to the effect of soil symbiotic microorganisms, especially of mycorrhizal fungi on seedling development of Malagasy native tree species. These activities affected particularly some forest tree species for which socio-economical and/or ecological values have already been illustrated.

Effects of arbuscular mycorrhizal native strains on seedling development of Adansonia za (Jum & H. Perrier) H. Perrier

Among eight species of *Adansonia* (baobab) all over the world, six species (*A. grandidieri, A. madagascariensis, A. perrieri, Rubrostipa, A. suarezensis, A. za*) are endemic to Madagascar. Another species (*A. digitata*) develops in the western, central and Eastern part of Africa, and the last species (*A. gobossa*) is endemic to North-western Australia. Depending on the species, baobabs develop in a wide range of ecosystems, including arid zones and savannahs, as well as dry and wet forests.

Adansonia za constitutes a well known Baobab in the western part of Madagascar because of its different use in everyday life of Malagasy people in this region of the island. However, ecosystems of *A. za* have been highly disturbed by deforestation. Large parts of these ecosystems have been transformed to agriculture lands, especially to rice lands, which really threatens the population of that tree species. Moreover, seedlings of *A. za* have been rarely observed within these ecosystems, where baobab's populations are particularly constituted by adult trees. This species of baobab belongs to the *Longitubae* section which makes their seeds with water-impermeable coats[28]. Thus, severe treatments are needed to remove the physical dormancy to allow seed germination.

Controlled mycorhization of *A. za* was undertaken by Razafimiaramanana in 2010 by using *Glomus intraradices* as a reference mycorrhizal strain, and three native strains of arbuscular mycorrhizas (*Glomus* sp., *Scutellospora* sp. and *Entrophospora* sp.)[29]. They were isolated from a baobab ecosystem of Kirindy forest in the western part of Madagascar. After 6 months of culturing under greenhouse conditions, the native strain *Glomus* sp. stimulated the development of *A. za* seedlings more than the other strains did (Table 2). Compared to the control, shoot growth of plants inoculated with *Glomus* sp., *Glomus intraradices*, *Scutellospora* sp. or *Entrophospora* sp. was stimulated 4.6, 3.7, 1.9 or 2.4 times, respectively. Shoot and root dry weights of all inoculated plants were significantly higher than values in the control treatment. These results showed a high degree of mycorrhizal dependency of *A. za* seedlings, and particularly the importance of native strains on the development of mycorrhiza on this plant. Thus, the establishment program of *A. za* seedlings in these original areas and/or in others degraded soils requires a preliminary management of soil mycorrhizal communities. Under natural conditions, the germination of baobab seeds constitutes a limiting factor to plant regeneration[30]. In this case, the development of regeneration or multiplication technologies is an important option to increase seedling performance of Baobab, and to preserve this genetic resource of great economic and medicinal value.

Effects of arbuscular mycorrhiza native strains on seedling development of
Dalbergia trichocarpa Baker

Malagasy species of *Dalbergia* are characterized by an undeniable wood quality. As a result, they have a great socio-economical, environmental or commercial value all over the world. Among the 125 described species of *Dalbergia*, 42 out of the 48 found in Madagascar are endemic[31]. A large part of these endemic tree species is scarce due to its overexploitation in many natural forest regions of Madagascar. As an example, 52,000 tones of wood from 100,000 individual trees of rosewood (*Dalbergia* spp.) and ebony trees were logged in north-east of Madagascar[32]. During the last decade, illegal logging and export of rosewood was undertaken even within protected areas[33, 34]. In this situation, efforts should focus in forest preservation, and if possible, in increasing the population of these valuable forest tree species.

The potentiality of the plant-soil-microorganism association was explored to optimize both growth and regeneration of the endemic species of *Dalbergia*[35]. These studies illustrated that all 8 studied species formed symbiosis structures with nitrogen-fixing bacteria. Since then, little information was available related to the importance of soil microorganisms on the growth stimulation of *Dalbergia* seedlings. Recently, the presence of arbuscular and vesicular mycorrhizal structures was reported on the root systems of the two endemic species of *Dalbergia* (*Dalbergia maritima* R. Vig)[24]; *Dalbergia trichocarpa* Baker[36]. Then, the first study was conducted exploring the importance of both arbuscular and vesicular mycorrhizas and nitrogen-fixing bacteria on the growth of *D. trichocarpa*[37]. Three strains of arbuscular and vesicular mycorrhizas were used including two native strains (*Glomus* sp1-ME and *Glomus* sp2-ME; isolated from undisturbed stand of *D. trichocarpa*) and one exotic strain of *Glomus* [(*Glomus intraradices*; provided by the Laboratoire Commun de Microbiologie (IRD/UCAD/ISRA) Dakar-Senegal]. A strain of nitrogen-fixing bacteria [from the strain collection of the Laboratory of Environmental Microbiology (CNRE), Antananarivo-Madagascar] was co-inoculated with a single or a multiple strain of arbuscular and vesicular mycorrhizas. This strain of nitrogen-fixing bacteria was isolated from the root system of *D. trichocarpa* collected in an undisturbed stand of this tree.

The results of these experiments illustrated the great importance of native mycorrhiza strains on the development of *D. trichocarpa* seedlings (Table 3). Compared to the control, the total root and shoot growth of seedlings were stimulated 3.5 or 5.8 times, respectively, after inoculation with the nitrogen-fixing bacteria STM 609 and *Glomus sp*1-ME or *Glomus sp*2-ME. At the same time, total growth of roots and shoots was 2.9 times on plants inoculated by the exotic strain *Glomus intraradices*. Shoot and root dry weights of all inoculated plants were significantly higher than valves in the control treatment (with single or multiple strains of arbuscular and vesicular mycorrhizas). Shoot and root development of seedlings was stimulated more in the multiple strain of arbuscular

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and vesicular mycorrhiza than in control or a single strain of arbuscular and vesicular mycorrhiza treatments. For these treatments, the importance of native strains on the stimulation of seedling development was illustrated. Indeed, the high levels of shoot and root developments were observed on plants inoculated by the two native strains of arbuscular and vesicular mycorrhizas with or without the exotic strain of this group of mycorrhizas (Table 3). Similar results were observed on each plant for the mycorrhizal and nodule developments in the root system. The highest levels of mycorrhizal colonization, mycorrhizal dependency and nodule number were registered on plants inoculated by the multiple strains of arbuscular and vesicular mycorrhizas and the nitrogen-fixing bacteria strain.

Effects of ectomycorrhizal symbionts diversity on seedling development of Intsia bijuga (Colebr.) O. Kuntze

Intsia bijuga is found in its native range of Madagascar, the Seychelles, Indonesia, Malaysia, Thailand, Philippines, Papua New Guinea and Australia. This is in addition to its primary distribution in the western Pacific and Indo-Malaysian regions, from New Guinea and Palau in the west to Fiji, Tonga and Samoa in the Southeast, and to the Mariana Caroline and Marshall Islands in the north and northeast in the Pacific. A spreading tree of up to 40 m tall, *I. bijuga* is undoubtedly one of the most highly valuable trees in these regions, both in terms of its traditional cultural and commercial timber values. In Madagascar, *I. bijuga* occurs frequently in the eastern coastal rainforest, in primary or old secondary forests, and in open forests from 0 to 800 m.a.s.l. Trees of *I. bijuga* are in very high demand and permanently decreasing in abundance because of their overexploitation for house posts, canoe making and due to its indiscriminate modern commercial logging.

Belonging to the family of the Fabaceae, subfamily Caesalpinoideae, *I. bijuga* is not a nodulated tree, and it has been found forming exclusively ectomycorrhizas[24, 38]. There is no evidence to date that this tree species associates with vesicular-arbuscular mycorrhiza fungi[11]. *Intsia bijuga* associates with a few groups of ectomycorrhiza fungi[39] despite the exceptional diversity of the ectomycorrhizas fungi associated with native or endemic trees of Madagascar[22, 40]. In natural stands of the Seychelles, only Tedersoo et al (2007) identified 15 species of ectomycorrhiza fungi associated with *I. bijuga* by using DNA sequencing of mycorrhizal root tips[38].

In Madagascar, mycorrhizal inoculation of *I. bijuga* seedlings was initiated by Rakotoarimanga in 2010 by using single or multiple strains of ectomycorrhizal fungi[36]. Four strains of ectomycorrhizas fungi were used in their studies. Two strains of *Scleroderma* (SC02-ME and SC03-ME) were isolated from two fruiting bodies that were collected under (1) *Uapaca bojeri* within the sclerophyllous forest of the Madagascarian highland, and (2) an *Intsia bijuga* stand in the eastern littoral forest of Madagascar, respectively. One strain of *Pisolithus* (*Pisolithus* sp.

Pis02-ME) was isolated from a sporophore collected under *Pinus* and *Eucalyptus* plantations in the central highland of Madagascar. The last isolated strain was a species of *Boletus* (*Boletus sp* BO01-ME), obtained from a sporophore collected under *I. bijuga* in the eastern rainforest of Madagascar. After 4 months of culturing in pots, the effects of each inoculation treatment on seedling growth and mycorrhizal development were as shown in Table 4. Compared to the control, a significant development of shoot seedling biomass was found on all treatments with *Pisolithus* sp. Pis02-ME on single and multiple treatments. However, no significant root development was found between the control and all treatments. For the mycorrhizal dependency and ectomycorrhizal colonization, each type of inoculation (single or multiple) had variable effects depending on the strain used. Generally, high levels of ectomycorrhizal colonization were observed on treatments with Pis02-ME, except on single inoculation with SC03-ME. For this last treatment, no effect of high levels of ectomycorrhizal colonization was recorded on seedling growth (shoot and root biomass). These results illustrated that ectomycorrhizal symbionts associated to exotic trees were able to stimulate the development of *I. bijuga* seedlings.

Effects of dual mycorrhization (endo and ectomycorrhization) on seedling development of Uapaca bojeri L. (Euphorbiaceae)

Some plant species such as *Uapaca bojeri*[22] may contain the two forms of mycorrhizal symbiosis (endomycorrhizae and ectomycorrhizae), in their root system. The importance of each association depends on the developmental stage of the plant[41]. In general, endomycorrhizal (AM) fungi colonize seedlings initially, and then are replaced by ectomycorrhizas through a process of competition after a few months[42].

A native tree species, *Uapaca bojeri*, of the sclerophyllous forest in Madagascar, is highly dependent on both types of mycorrhiza (Table 5). A high occupancy of AM fungi appeared first on young seedlings (3-month-old roots) followed by ECM colonization (Fig. 1)[22]. Chen et al. (2000)[43] described, after studying *Eucalyptus urophylla* growth, that these fungi interact mainly during the first four months of plant growth. AM species colonized first and had little effect on ECM colonization. The succession of these two types of mycorrhizas did not compromise plant development. This was because the greatest growth response was seen on plants colonized by both types of mycorrhiza[41, 44, 45].

Indeed, positive effects of the dual inoculation were shown for seedling growth and root mycorrhizal colonization of *Uapaca bojeri* (Table 5) in comparisson to the non-inoculated control treatment under greenhouse conditions. This co-occurrence of AM with ECM in the same root system might determine the success of plant species to colonize a wide range of habitats and allow plant establishment (i.e. forest restoration) on degraded areas[46].

Nurse plant phenomenon and its importance on late successional plant regeneration and on forest restoration

Following perturbation, it is well known that some plant species (e.g., pioneer or perennial plants) can associate with beneficial soil microorganisms which could have positive effects on late successional plant species[47, 48, 49].

Within two disturbed forest ecosystems of *Uapaca bojeri* (an endemic tree species with high socio-economical value), located at Arivonimamo (Region of Itasy) and Ambatofinandrahana (*Region of Amoron'I Mani*a) in the Central part of Madagascar, another kind of facilitation through shared mycorrhizal fungi was observed. It was first found that the degraded areas, previously occupied by *Uapaca bojeri*, were colonized by shrub species which in most cases were associated with mycorrhizal fungi (Table 6). Some of these shrub species were associated with endo- and ectomycorrhizal fungi like *U. bojeri*, as it was described by Baohanta (2011)[50]. This characteristic might help to explain their ability to establish on poor soils. This is because of the improvement of water and mineral acquisition and plant protection throughout the mycorrhizal symbiosis[11, 51, 52].

Pioneer species, which often reflect the stage of degradation of forest soils, are among the most studied "nurse plants". Many studies have been conducted to determine their impact on soil biological and chemical functioning, and on plant succession[49, 53, 54]. In arid ecosystems, seedling establishment and survival have been greater underneath the canopies of shrubs than in the open interspaces[55]. The ability of such species to persist or to re-establish on disturbed sites might also allow the survival of mycorrhizal fungi propagules in the soil, even though woody mycorrhizal host plants are absent. In turn, the presence of established mycorrhizal fungi in the soils may facilitate the establishment or the re-establishment of mycorrhizal tree seedlings following disturbance[56, 57, 58, 59], potentially contributing to plant succession. As a result, nurse plants might be able to i) resist various environmental stresses, ii) create microclimates or "fertile microclimates" that could facilitate the establishment of other species, iii) be less competitive compared to the target species[60].

Shared mycorrhizal symbionts between two plant species within the same environment and belonging to the same genus, family or different families is an important positive interaction[61, 62, 63]. This kind of association was observed between the two shrubs species, *Leptolaena bojeriana* or *Sarcolaena oblongifolia*, and *Uapaca bojeri* (the native tree species) within the two study sites. Indeed, some ectomycorrhizal species were associated with both shrub species and with *Uapaca bojeri*. This was after the comparison of RFLP-type of ectomycorrhizas collected from harvested *Sarcolaena oblongifolia* roots with those associated with *Uapaca bojeri* by using restriction fragment length polymorphism (RFLP) (Fig. 2).

In a glasshouse study, *Uapaca bojeri* seedlings were grown near established *Leptolaena bojeriana* seedlings (dual cultivation) on soils collected either under exotic species (disturbed soil) or distant from any ectomycorrhizal host (bare soil). Results showed that the presence of the pioneer shrub species enhanced seedling development and root mycorrhizal colonization of the native species *Uapaca bojeri* in all soil samples, in comparison to the control without the shrubs species (Table 7). Increased mycorrhizal colonization of *Uapaca bojeri* seedlings near *Leptolaena bojeriana*, and the consequent increase in seedling nutrient uptake and growth potential, are the possible implications of inter-specific sharing of mycorrhizal fungi[64]. Indeed, sharing of mycorrhizal fungi may allow *U. bojeri* and *L. bojeriana* to form links into a common mycelial network, without initial constrains to establish mycorrhizal colonization[65]. This would also give seedlings a more rapid access to a potentially extensive, established mycelial network[58, 63]. It is also possible that nutrients may be transferred among plants *via* mycorrhizal linkages, fostering seedling development[11].

Facilitation phenomenon for native tree species establishment: are exotic plant species involved?

Most of the forest plantations in the world are carried out with exotic species[66] because of the lack of ecology and sylviculture knowledge of the native species. During 2000 to 2005, plantations in the world showed an expansion of approximately 2.8 million hectares per annum[67] due to the increasing demand for paper pulp, timber and fuelwood[68, 69, 70]. Other objectives of these plantations were to reduce of the pressures on the natural forest ecosystems, and the need for sequestering carbon to meet obligations under the Kyoto Protocol. However, the invasion of exotic plant species constitutes a threat for conservation and restoration of the natural ecosystems[71, 72]. The beginning of the 90s was marked by a new trend, which regarded the forest plantations as a catalyst for regeneration of the native species[73, 74, 75].

In Madagascar, classified among the first ten countries of hot spot of biodiversity with a rate of very high endemisms[25], little importance was granted to exotic plant species. It was considered that these species had a strong capacity of adaptation to hard ecological conditions in comparison to the insular, fragile Malagasy flora[76, 77]. For a few years, this threat became a reality. Binggeli (2003) reported a list of 38 invading exotic species (*Opuntia* spp., *Psidium cattleianum*, *Grevillea banksii*) endangering the Malagasy flora[78]. During a few decades, Madagascar did not have a clear plantation policy[79], despite the advantages and roles of the plantations on the environment (e.g., protection against erosion, production of firewood and paper pulp)[80]. As a result, plantations account for only 2% of the forest cover in the island[81], and the majority of plantation forests are planted with *Pinus* and *Eucalyptus*.

Light in the understory is an important factor to forest regeneration[82]. Tree planting may facilitate the process of forest succession by providing a nurse effect to colonizing native species. Facilitation, the positive effect of plants on the establishment or growth of others, has long been recognized as an important driving force for secondary succession[83]. It was defined by van Andel (2006) as an interaction between individuals of different species, where one of species changes the environment in such a way that is beneficial to the other[84].

In the southern center of Madagascar (commune of Androy, located 400 km south of Antananarivo; 21°22'S, 47°18'E; 1100–1200 m.a.s.l.) the pine plantations (*Pinus patula*) are located near the forest corridor which connects the national parks of Ranomafana and Andringitra. In this context of vicinity of the natural forest and plantations, native species were regenerated in the plantations which underwent various disturbances (wood extraction, cyclones, fires, culture).

We assessed the diversity of naturally regenerated native species (trees, shrubs, herbs and lianas) in the disturbed, exotic tree plantations (*Pinus patula*). Transects were used with this purpose (40 transects, 205 plots of 10m x 10m). The following hypothesis was formulated: gaps in the plantation facilitate native species regeneration. Use of correspondence analysis (CA) allowed identification of three vegetation groups, which corresponded to various stages of succession (Fig. 3): (i) herbaceous vegetation, (ii) mixed herbaceous-woody vegetation and (iii) woody vegetation (forest regrowth). Understory species richness (S), Shannon diversity index (H'), and woody density (D) were studied within 10 plots randomly selected per vegetation group.

One hundred and twenty five (125) species divided into 46 families were inventoried, including 34 endemic species. The most common plant families found under the plantation were Asteraceae (19 species), Poaceae (14 species) and Rubiaceae (14 species). By growth form, there were 58 tree (46%), 55 herb (44%) and 12 liana (10%) species. Mean values for stem density (D) and basal area were 6843 individuals per hectare and 6.29 m² ha^{-1}, respectively, in the woody vegetation (Table 8).

Our results further provide information on the dynamic nature of vegetation. The first stages of succession are characterized by herbaceous vegetation which is replaced by mixed formations and finally by woody formations (forest regrowth). Floristic richness (S) and Shannon Wiener index (H') increased during succession: values were lower in the herbaceous vegetation (S<10 and 1.24<H'<1.46) than in the forest regrowth (27<S<33 and 3.95<H'<4.01).

Although monocultures are deemed to be "biological deserts" by some researchers[85], our results suggest that exotic plantations help to restore native species by stabilizing soil and creating favorable site conditions for plant recolonization. In our study site, the composition of the soil seed bank, and the availability of recent seed sources (forest corridor) in the vicinity of the plantation are important.

Pinus species are dependent on symbiosis to develop optimally under natural conditions[86]. Particularly, *Pinus patula* has the ability to symbiotically

fix nitrogen with the help of certain species of actinomycetes. This relationship causes an increase in soil nitrogen content with time, and facilitates the regeneration of Malagasy native species. Likewise, exotic tree plantations potentially may greatly improve physical and biological site conditions catalyzing subsequent succession processes towards a natural forest[73].

Understanding the process of understory succession might contribute to conserve native biodiversity in Madagascar. The reproduction of the natural regeneration observed in the pine plantation can be used as a model to restore the degraded ecosystems of the region.

Conclusion

Results presented in this chapter show that mycorrhizal symbioses have a real potential to improve the performance of seedlings, especially of endemic trees, and could be used in afforestation programs or in ecological restoration processes of degraded areas in many forest ecosystems in Madagascar. Soil mycorrhizal communities can be managed by (i) using isolated strains in the framework of controlled mycorrhizal inoculation, or (ii) exploring the capacity of pioneer shrub species to stimulate the potentiality of residual mycorrhizal propagules that might facilitate the establishment of others tree seedlings. This second technology would allow to design multispecific reforestations or a two-phase reforestation strategy, mimicking the natural succession process, as soon as most shrub species are able to facilitate the early growth and survival of young forest tree seedlings. However, the development of these technologies is suggested from studies conducted under nursery and /or greenhouse conditions. Further experiments have to be carried out to test the positive effect of each technology both on a longer period of plantation and in an ecological restoration process under field conditions.

Management of native mycorrhizal strains proved to be more interesting than using introduced fungal strains to improve growth of Malagasy endemic tree seedlings. Moreover, use of sun-tolerant shrubs (which can have a positive effect on soil mycorrhizal communities) can be of great importance to the plantation program of endemic trees or to the forest ecosystem regeneration.

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Figure legends

Fig. 1 - Sequence of mycorrhizal colonization on *U. bojeri* seedlings (■: AM colonization; ♦: total ectomycorrhizal colonization)

Fig. 2 - Relative frequency of identified RFLP types based on ITS region sequences on roots of *Uapaca bojeri* and *Sarcolaena oblongifolia*. Relative frequency was calculated as the number of occurrences of each RFLP type divided by the total number of occurrences of all RFLP types.

Fig. 3 - Correspondence analysis for all plots (based on presence/absence of species in the pine plantation, 125 species/205 plots).
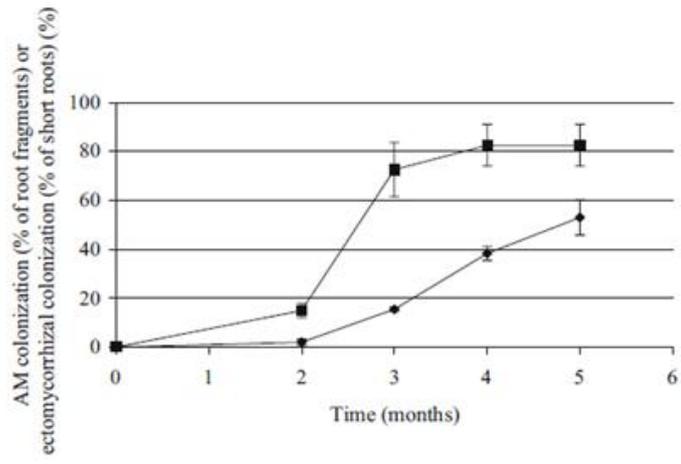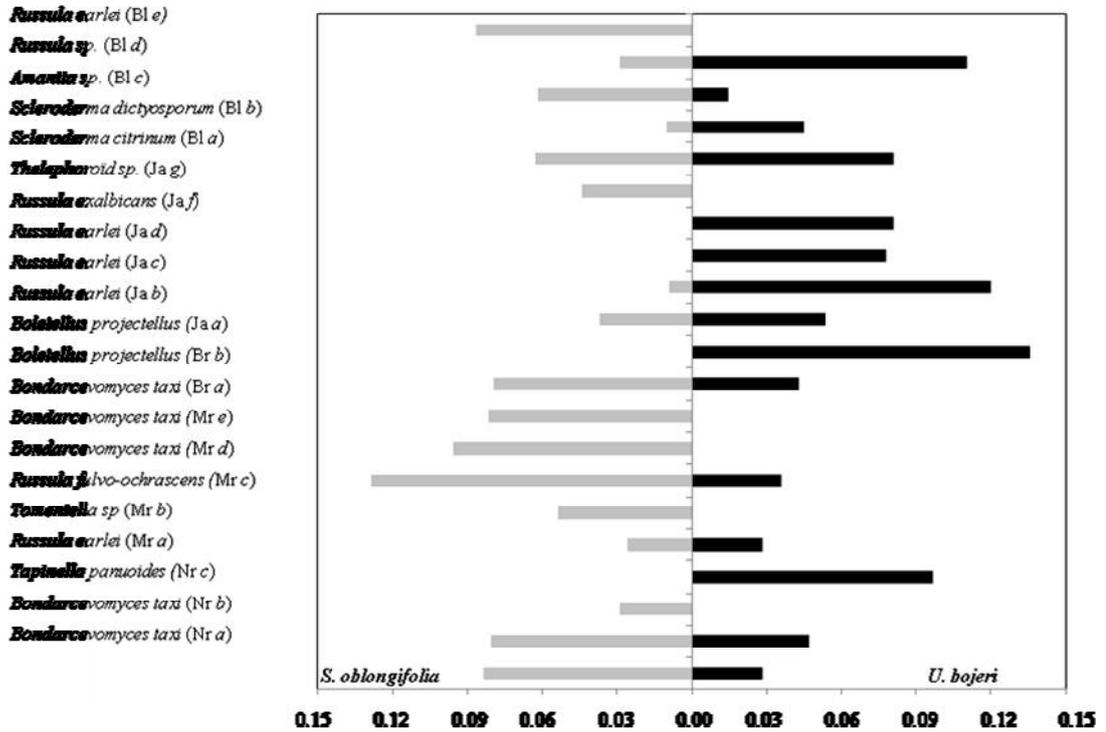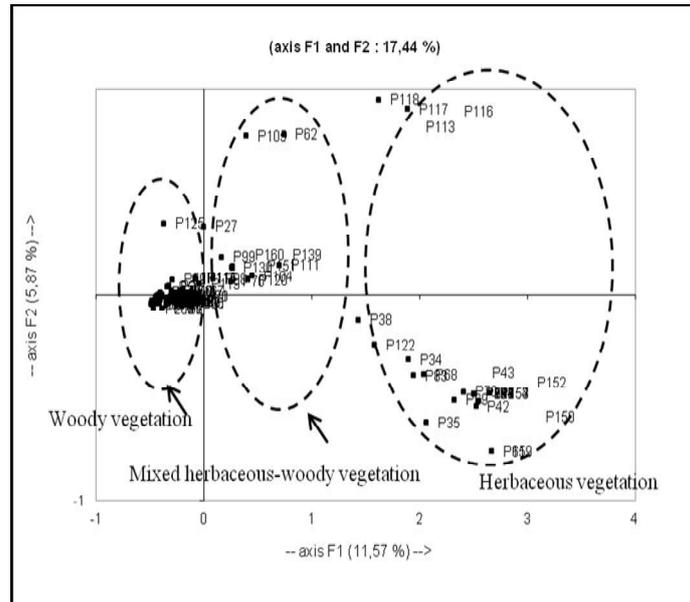
Fig. 1

Fig. 2

Fig. 3

Table 1 - Mycorrhizal status of dominant shrub and tree species from Analalava, Ianjomara and Arivonimamo forests in the eastern and central parts of Madagascar.

Genus/species[1]	Family	Sites[2]	Mycorrhizal status[3]
Amyrea sp. **(?)**	Euphorbiaceae	Ana.	AM
Anthostema madagascariense Baill. **(E)**	Euphorbiaceae	Ana.	AM
Breonia havilandiana Homolle **(?)**	Rubiaceae	Ana.	AM
Canarium madagascariense Engl. **(E)**	Burseraceae	Ana.	AM
Casearia nigrescens Tul. **(E)**	Salicaceae	Ana.	AM
Cynometra capuronii Du Puy et R. Rabev. **(E)**	Fabaceae	Ana.	AM
Clitoria lasciva Bojer ex Benth. **(E)**	Fabaceae	Ana.	AM
Colubrina sp. **(?)**	Rhamnaceae	Ana.	AM
Conchopetalum madagascariense Radlk. **(E)**	Sapindaceae	Ana.	AM
Croton lepidotus Aug. DC. **(E)**	Euphorbiaceae	Ana.	AM+
Cryptocarya acuminata Schinz **(?)**	Lauraceae	Ana.	AM
Dicoryphe sp. **(?)**	Hamamelidaceae	Ana.	AM
Dillenia triquetra (Rottb.) Gilg **(?)**	Dilleniaceae	Ana.	AM
Diospyros bernieri Hiern **(?)**	Ebenaceae	Ana.	AM+
Diospyros sp. **(?)**	Ebenaceae	Ana.	AM+
Dracaena reflexa Lam. **(n)**	Asparagaceae	Ana.	AM+
Dypsis sp. **(?)**	Arecaceae	Ana.	AM
Ellipanthus madagascariensis (G. Schellenb.) Capuron ex Keraudren **(E)**	Connaraceae	Ana.	AM
Erythroxylum sp. **(?)**	Erythroxylaceae	Ana.	AM+
Eugenia louvelii H. Perrier **(?)**	Myrtaceae	Ana.	AM+
Fernelia sp. **(?)**	Rubiaceae	Ana.	AM
Ficus cocculifolia Baker **(n)**	Moraceae	Ana.	AM+
Ficus lutea Vahl. **(n)**	Moraceae	Ana.	AM+
Gaertnera macrostipula Baker **(?)**	Rubiaceae	Ana.	AM
Harungana madagascariensis Lam. ex Poir. **(?)**	Hypericaceae	Ana.	AM+
Homalium involucratum (DC.) O. Hoffm. **(E)**	Salicaceae	Ana.	AM
Landolphia nitens Lassia **(E)**	Apocynaceae	Ana.	(AM)
Leptolaena multiflora Thouars **(E)**	Sarcolaenaceae	Ana.	AM&ECM
Macaranga cuspidata Boivin ex Baill **(?)**	Euphorbiaceae	Ana.	AM+
Macphersonia madagascariensis Blume **(E)**	Sapindaceae	Ana.	AM
Malleastrum minutifoliolatum J.-F. Leroy **(E)**	Meliaceae	Ana.	AM
Mascarenhasia arborescens A. DC. **(n)**	Apocynaceae	Ana.	NM
Memecylon xiphophyllum R. D. Stone **(?)**	Memecylaceae	Ana.	AM
Nesogordonia macrophylla Arènes **(E)**	Malvaceae	Ana.	AM
Paropsia madagascariensis (Mast.) H. Perrier **(E)**	Passifloraceae	Ana.	AM

Psiadia sp. **(?)**	Asteraceae	Ana.	AM+
Psidium cattleianum Sabine **(n)**	Myrtaceae	Ana.	(AM)
Psorospermum lanceolatum (Choisy) Hochr. **(E)**	Hypericaceae	Ana.	AM
Ravenala madagascariensis Sonn. **(E)**	Strelitzaceae	Ana.	(AM)
Ravenea julietiae Beentje **(E)**	Arecaceae	Ana.	AM
Rhodocolea racemosa (Lam.) H. Perrier **(E)**	Bignoniaceae	Ana.	AM
Rhopalocarpus thouarsianus Baill. **(E)**	Sphaerosepalaceae	Ana.	AM
Saldinia proboscidea Hochr. **(E)**	Rubiaceae	Ana.	AM
Suregada boiviniana Baill. **(?)**	Euphorbiaceae	Ana.	AM
Symphonia tanalensis Jum. & H. Perrier **(E)**	Clusiaceae	Ana.	AM
Syzygium emirnense (Baker) Labat & G. E. Schatz **(?)**	Myrtaceae	Ana.	AM
Tabernaemontana coffeoides Bojer ex A. DC. **(n)**	Apocynaceae	Ana.	AM
Tambourissa purpurea (Tul.) A. DC. **(E)**	Monimiaceae	Ana.	AM
Tina fulvinervis Radlk. **(E)**	Sapindaceae	Ana.	AM
Uapaca louvelii Denis **(E)**	Euphorbiaceae	Ana.	AM&ECM
Vepris sp. **(?)**	Rutaceae	Ana.	AM
Zanthoxylum tsihanimposa H. Perrier **(E)**	Rutaceae	Ana.	AM
Aphloia theiformis (Vahl) Benn. **(n)**	Aphloiaceae	Ian.	AM+
Aristida similis Steud. **(?)**	Poaceae	Ian.	AM+
Burasaia madagascariensis DC. **(E)**	Menispermaceae	Ian.	AM
Cinnamumum camphoratum Blume **(n)**	Lauraceae	Ian.	AM+
Cinnamomum zeylanicum Blume **(n)**	Lauraceae	Ian.	AM+
Clidemia hirta (L.) D. Don **(n)**	Melastomataceae	Ian.	(AM)
Colubrina decipiens (Baill.) Capuron **(n)**	Rhamnaceae	Ian.	AM
Commelina sp. **(?)**	Commelicaceae	Ian.	AM+
Dactyloctenium sp **(?)**	Poaceae	Ian.	AM
Dalbergia madagascariensis Vatke **(E)**	Fabaceae	Ian.	AM+
Dombeya dolichophylla Arènes **(?)**	Malvaceae	Ian.	AM
Dracaena reflexa Lam.**(n)**	Asparagaceae	Ian.	(AM)
Dichapetalum leucosia (Spreng.) Engl. **(E)**	Dichapetalaceae	Ian.	AM
Dypsis sp. **(?)**	Arecaceae	Ian.	(AM)
Dypsis nodifera Mart. **(E)**	Arecaceae	Ian.	(AM)
Agelaea pentagyna (Lam.) Baill. **(?)**	Connaracea	Ian.	AM
Gaertnera macrostipula Baker **(?)**	Rubiaceae	Ian.	AM
Gaertnera obovata Baker **(?)**	Rubiaceae	Ian.	AM
Grevillea banksii R. Br. **(n)**	Proteaceae	Ian.	(AM)
Harungana madagascariensis Lam. Ex Poir.	Hypericaceae	Ian.	AM+
Hugonia sp **(?)**	Linaceae	Ian.	AM
Landolphia myrtifolia (Poir.) Markgr. **(E)**	Apocynaceae	Ian.	AM
Landolphia sp. **(?)**	Apocynaceae	Ian.	NM

Species	Family	Site	Status
Landolphia gummifera (Poir.) K. Schum. **(E)**	Apocynaceae	Ian.	(AM)
Macaranga cuspidata Boivin ex Baill **(?)**	Euphorbiaceae	Ian.	AM+
Macarisia lanceolata Baill. **(?)**	Rhizophoraceae	Ian.	AM
Machaerina flexuosa (Boeckeler) J. Kern **(?)**	Cyperaceae	Ian.	AM
Macphersonia madagascariensis Blume **(E)**	Sapindaceae	Ian.	AM
Merremia tridentata (L.) Hallier f. **(n)**	Convolvulaceae	Ian.	AM
Noronhia emarginata (Lam.) Thouars **(E)**	Oleaceae	Ian.	AM+
Osmunda regalis L. **(?)**	Osmondaceae.	Ian.	AM
Ouratea sp. **(?)**	Ochnaceae	Ian.	AM
Panicum luridum Hack. **(?)**	Poaceae	Ian.	AM
Phyllanthus amarus Schumach. & Thonn. **(n)**	Phyllanthaceae	Ian.	(AM)
Poupartia chapelieri (Guillaumin) H. Perrier **(E)**	Anacardiaceae	Ian.	AM+
Psidium cattleianum Sabine **(n)**	Myrtaceae	Ian.	(AM)
Psorospermum fanerana Baker **(E)**	Clusiaceae	Ian.	AM
Ravenala madagascariensis Sonn. **(E)**	Strelitzaceae	Ian.	(AM)
Rubus sp. **(?)**	Rosaceae	Ian.	AM+
Sauvagesia erecta L. **(n)**	Ochnaceae	Ian.	AM
Scolopia maoulidae S. Hul, Labat & O. Pascal **(?)**	Salicaceae	Ian.	AM
Streblus dimepate (Bureau) C.C. Berg **(?)**	Moraceae	Ian.	AM
Symphonia fasciculata (Noronha ex Thouars) Vesque **(E)**	Clusiaceae	Ian.	AM
Tacca leontopetaloides (L.) Kuntze **(?)**	Discoreaceae	Ian.	AM
Trema orientalis (L.) Blume **(?)**	Cannabaceae	Ian.	AM+
Tristemma virusanum Juss. **(n)**	Melastomataceae	Ian.	AM
Trophis montana (Leandri) C.C. Berg **(?)**	Moraceae	Ian.	AM
Uapaca ferruginea Baill. **(E)**	Euphorbiaceae	Ian.	AM &ECM
Urena lobata L. **(n)**	Malvaceae	Ian.	AM
Voacanga thouarsii Roem. & Schult. **(n)**	Apocynaceae	Ian.	NM
Uapaca bojeri L. **(E)**	Euphorbiaceae	Ariv	AM&ECM
Leptolaena bojeriana **(E)**	Sarcolaenaceae	Ariv	AM&ECM
Trema sp **(n)**	Ulmaceae	Ariv	AM
Aphloia theaeformis (Vahl.) Benn. **(n)**	Flacourtiaceae	Ariv	AM+
Rhus taratana (Baker.) H. Perrier **(n)**	Anacardiaceae	Ariv	AM+
Helychrysum rusillonii Hochr. **(?)**	Asteraceae	Ariv	AM+
Psiadia altissima (D.C.) Drake. **(?)**	Asteraceae	Ariv	AM+
Rubus apetalus Poir. **(n)**	Rosaceae	Ariv	AM

[1]Plant species: following the genus, species, and authority names, available data on endemicity are indicated: **(E)**: endemic, **(n)**: nonendemic, **(?)**: not fully established.
www.mobot.org/phillipson/catalogue/catalogue.htm
[2]Collection sites: **Ana** : Analalava, **Ian.** Ianjomara, **Ariv**: Arivonimamo
[3]Mycorrhizal status: **AM**: arbuscular mycorrhiza, **(AM)**: lightly infected, **AM+**: heavily infected, **AM&ECM**: co-existence of arbuscular mycorrhizas and ectomycorrhizas, **NM**: nonmycorrhizal

Table 2 - Shoot and root growth, mycorrhizal dependency and mycorrhizal development and of *A. za* seedlings after 6 months inoculation with *G. intraradices* or native mycorrhizal strains in pot cultures.

	Treatments				
	C*	GI	GL	SC	EN
Shoot biomass (g dry weight plant^{-1})	0.19a**	0.71c	0.88d	0.37b	0.46b
Root biomass (g dry weight plant^{-1})	0.32a	0.95c	1.11d	0.59b	0.65b
Mycorrhizal dependency (%)	0a	72.6d	77.7d	48.4b	58.2c
AM colonization (%)	0a	66.4d	73.19e	37.59b	50.2c

*C: Control; GI: *Glomus intraradices*; GL: *Glomus* sp.; SC: *Scutellospora* sp.; EN: *Entrophospora* sp.
** Data in the same row followed by the same letter are not significantly different (p>0.05) after one-way analysis of variance.

Table 3 - Shoot and root growth, mycorrhizal and nodule development and mycorrhizal dependency of *D. trichocarpa* seedlings inoculated with the nitrogen-fixing bacteria STM 609 and a single or a multiple strain of arbuscular and vesicular mycorrhizas in sterilized soil after 4 months culturing.

Treatments	Number of nodule plant^{-1} (STM 609)	Mycorrhizal colonization (%)	Mycorrhizal dependency (%)	Shoot biomass (g dry weight plant^{-1})	Root biomass (g dry weight plant^{-1})
T*	0 a**	0 a	0 a	0.127 a	0.070 a
A	61 c	47.59 c	29.2 d	0.419 c	0.286 c
B	43 b	34 b	23.2 b	0.359 b	0.229 b
C	85 e	68.19 e	59.5 f	0.722 e	0.422 e
A+B	46 b	36.79 b	26.1 c	0.388 b	0.234 b
A+C	91 f	73.40 f	74.6 g	0.873 f	0.486 f
B+C	66 d	54.20 d	39.6 e	0.523 d	0.393 d
A+B+C	112 g	88.80 g	84 h	0.967 g	0.685 g

*T: Control; A: *Glomus sp1-ME;* B: *Glomus intraradices*; C: *Glomus sp1-ME*

**Data in the same column followed by the same letter are not significantly different (p>0.05) following one-way analysis of variance.

Table 4 - Shoot and root growth, mycorrhizal dependency and mycorrhizal development of *I. bijuga* seedlings after 4 months of culturing and inoculation by a single or multiple ectomycorrhizal strains in pot culture

Treatments	C*	SC02	SC03	Pis02	BO01	SC02+SC03	SC02+SC03+Pis02	SC02+SC03+Pis02+BO01
Shoot biomass (g dry weight plant^{-1})	2.85a	3.12a	3.02a	4.39b	3.01a	3.07a	4.50b	4.13b
Root biomass (g dry weight plant^{-1})	0.82a	0.91a	0.77a	1.18b	0.83a	0.82a	0.94a	0.85a
Mycorrhizal dependency (%)	-	8.02a	1.48a	33.2b	0.91a	5.67a	31.67b	4.21a
Ectomycorrhizal colonization (%)	0.00a	67e	20.6cd	24.6d	7.50b	9.20b	19.09c	18c

*C: Control; SC02: *Scleroderma sp* SC 02-ME ; SC03: *Scleroderma sp* SC 01-ME ; Pis02: *Pisolithus sp* Pis 02-ME ; BO01: *Boletus sp* BO01-ME
** Data in the same row followed by the same letter are not significantly different after a one-way analysis of variance (p>0,05).

Table 5. Shoot growth, mycorrhizal development, and relative mycorrhizal dependency of *U. bojeri* seedlings 5 months after *G. intraradices* and/or *Scleroderma sp* SC 02-ME inoculation in sterilized soil.

Treatments	Shoot biomass (mg plant^{-1})	Ectomycorrhizal colonization (%)	Arbuscular colonization (%)	RMD* (%)
Control	91.1 a	0a	0a	-
Scleroderma sp SC 02-ME	181.2 b	8.7b	0a	47.6a
G. intraradices	160.1b	0a	77.5b	42.7a
Scleroderma sp SC 02-ME + G. intraradices	360.3c	11.5b	82.5b	70.7b

*RMD: Relative mycorrhizal dependency

**Data in the same column followed by the same letter are not significantly different (p<0,05) after a one-way analysis of variance

Table 6 - Mycorrhizal status of pioneer shrub species within the degraded area of two study sites.

Plant species [1]	Family	Mycorrhizal status[2]
Leptolaena pauciflora Baker. (**E**)	Sarcolaenaceae	ECM & MVA
Leptolaena bojeriana (Baill.) Cavaco. (**E**)	Sarcolaenaceae	ECM & MVA
Sarcolaena oblongifolia Cavaco. (**E**)	Sarcolaenaceae	ECM
Trema sp. (**n**)	Ulmaceae	MVA
Vaccinium emirnense Hook. (**n**)	Ericaceae	Endo
Aphloia theaeformis (Vahl.) Benn. (**n**)	Flacourtiaceae	MVA
Rhus taratana (Baker.) H. Perrier (**n**)	Anacardiaceae	MVA
Helychrysum rusillonii Hochr. (**?**)	Asteraceae	MVA
Psiadia altissima (D.C.) Drake. (**?**)	Asteraceae	MVA
Rubus apetalus Poir. (**n**)	Rosaceae	MVA
Erica sp. (**n**)	Ericaceae	Endo

[1]Plant species: following the genus, species and authority names; available data on endemicity are indicated: (**E):** endemic at the genus level; (**n):** nonendemic; (**?):** not fully established (http://www.mobot.org/phillipson/catalogue/catalogue.htm).

[2]Mycorrhizal status: AM arbuscular mycorrhiza; ECM, ectomycorrhiza; AM&ECM, co-existence of arbuscular mycorrhiza and ectomycorrhiza; Endo, endomycorrhizal.

Table 7 - Effect of *L. bojeriana* / *U. bojeri* succession and dual-cultivation of *L. bojeriana* / *U. bojeri* seedlings on growth and ectomycorrhizal colonization of *U. bojeri*

Treatments	*U. bojeri* Shoot biomass (Dry weight in g)	*U. bojeri* ECM Colonization [4] (%)
Bulk soil		
Control [1]	0.1205 [a] ±0.03[5]	29.33 [a] ±9.61
L. bojeriana [2]	0.2769 [b] ±0.02	30.33 [a] ±4.16
L. bojeriana WA [3]	0.3085 [b] ±0.05	65.33 [b] ±2.52
***Pinus patula* soil**		
Control	0.0855 [a] ±0.02	16.33 [a] ±4.16
L. bojeriana	0.2327 [b] ±0.02	65.33 [b] ±5.69
L. bojeriana WA	0.3331 [b] ±0.11	79.33 [c] ±7.02
***Eucalyptus* sp. soil**		
Control	0.0832 [a] ±0.02	36.00[a] ±3.61
L. bojeriana	0.2331 [b] ±0.07	42.00[a] ±10.39
L. bojeriana WA	0.2501 [b] ±0.07	90.33[b] ±5.51

Data in the same column within each soil type followed by the same letter are not significantly different (p>0.05) according to the Newman-Keuls test

[1]*U. bojeri* without pre- or dual cultivation with *L. bojeriana*.

[2]*U. bojeri* with *L. bojeriana* seedlings without the aerial parts

[3]*U. bojeri* after dual-cultivation with *L. bojeriana* seedlings with aerial parts

[4]Root Ectomycorrhizal colonization (%)

[5]Standard error of the mean.

Table 8 - Mean values for vegetation group identified by COA of the pine plantation (standard errors in parenthesis), n: number of plots[87].

Floristic parameters	Vegetation group		
	Herbaceous vegetation n = 10	Mixed herbaceous-woody vegetation n = 10	Woody vegetation n = 10
Species richness S	5.6 (1.89) a*	29 (6.4) b	32.6 (9) b
Shannon H'	1.46 (0.23) a	3.31 (0.78) b	4.01 (0.67) b
Stem density D (No ha^{-1})	-	-	6843 (2276)
Basal area G (m^2 ha^{-1})	-	-	6.29 (4.62)

*Different letters within the same row indicate significant differences ($p<0.05$) following the Tukey HSD test).