# Diversity and Dynamics of Mycorrhizal Associations in Tropical Rain Forests with different Disturbance Regimes in South Cameroon

Neree Awana Onguene

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# CHAPTER 1

# GENERAL INTRODUCTION

Degradation of habitat and loss of biological diversity in tropical rain forests following shifting cultivation and logging practices have become a world-wide environmental concern, thereby prompting the need for planning for the wise use and conservation of forest lands. Though awareness has been raised about consequences of forest destruction at various scales from the local silting up of streams to changes in global climate (Bruijnzeel & Critchley, 1994; WCFSD, 1999), little attention, however, has been given to the below-ground components, processes, and interactions that play important roles in the regulation of the composition, structure, and functioning of rain forests, notably mycorrhizal associations.

## MYCORRHIZAL ASSOCIATIONS

A mycorrhiza is a morphologically and physiologically distinct organ resulting from an intimate and (usually) mutually beneficial symbiosis between particular soil-inhabiting fungi (called mycorrhizal fungi) and roots of most higher plants. In almost all cases, the mycorrhiza is an obligately mutualistic relation because both the mycorrhizal fungus and mycorrhizal plant are unable to exist independently and complete their life cycle under natural conditions (Smith & Read, 1997). The benefit for the fungus is the receipt of photosynthetically derived carbon compounds (energy) from the plant. The plant benefits by increased uptake of mineral nutrients. The immediate outcomes of mycorrhizal formation and improved nutrient uptake are increased growth rate of host plants because they obtain necessary nutrients from poor soils in an effective way and at relatively low cost in ecosystems where external amendment inputs are scarce. Experimental evidence indicated, as a consequence of this improved nutrient uptake and growth status, increased survival, reduced phenotypic variation of seedlings, and increased drought resistance of young plants. In such situations increased production of growth-stimulating substances has also been reported (Sampangiramaiah, 1990), and fertiliser inputs can be decreased.

Initially, it was assumed that all mycorrhizas function in similar ways by enhancing uptake of the immobile ion phosphorus, implying that additional benefits were consequent upon the improved phosphorus status of the plant (Tinker et al., 1992). However, it has become clear that mycorrhizas can have a suite of different beneficial roles for the plant, depending both on the fungus concerned and on characteristics of the root system of the plant (Newsham et al., 1995). Such further benefits include protection against biotic (pathogens) and abiotic (heavy metals, aluminium) stress, mineral weathering, uptake of organic nitrogen and phosphorus compounds, and soil aggregate stability. Hence, mycorrhizal fungi are key components of both plant and soil development (Bethlenfalvay, 1992).

Two types of mycorrhizal associations have been described based on the sitting of the fungus vis-à-vis the root surface: ectomycorrhizas (sheath-forming) with intercellular colonisation and endomycorrhizas with intracellular colonisation. Ectendomycorrhizas combine characters of both types as they possess an external sheath and harbour both inter- and intracellular colonisation. Endomycorrhizal associations differ from ectomycorrhizal ones in that they do

not form mantles and cannot be seen with the naked eye. Based on differences in anatomical structures of the endomycorrhizal fungus (presence or absence of septa in the hyphae) and host plant taxonomy, five endomycorrhizal associations can be distinguished: arbutoid (Ericales), ericoid (Ericales, but also in a group of liverworts), monotropoid (Monotropaceae, an achlorophyllous family in the Ericales), orchid (Orchidaceae), and arbuscular mycorrhiza (various vascular plants, but also some mosses and liverworts). Internal mycorrhizal structures in orchid and ericoid endomycorrhizas consist of hyphal coils, while arbutoid and monotropoid mycorrhizas can harbour sheaths, Hartig net, and septate hyphae in host cells. In fact, the fungi forming arbutoid and monotropoid mycorrhizas are the same as those forming ectomycorrhizas. Differences between ectomycorrhizas and arbutoid and monotropoid mycorrhizas are apparently dependent on the anatomy (and physiology) of the plant root, and not on the fungal taxa involved. The same observation can be made for some orchid mycorrhizas as several achlorophyllous orchids (*Corallorhiza*) are colonised by fungi that form ectomycorrhizas on neighbouring trees (Taylor & Bruns, 1997). The ericoid mycorrhizal fungus *Hymenoscyphus ericae* was recently reported as forming ectomycorrhizas on spruce, called *Piceirhiza bicolorata* (Vrålstad et al., 2000).

Both ecto- and endomycorrhizal associations are ubiquitous soil inhabitants. In fact, the non-mycorrhizal condition of plants is exceptional. Presence of mycorrhizas is considered to be the primitive condition that made the evolution of land plants possible. The non-mycorrhizal habit is most likely due to a number of independent evolutionary losses.

In natural and agricultural ecosystems, the predominant mycorrhizal association is arbuscular mycorrhiza, also known as vesicular-arbuscular mycorrhiza (VAM). Some disagreement persists about the appropriate name for these associations (Smith, 1995; Walker, 1995). In this thesis I will use the name arbuscular mycorrhiza.

**ARBUSCULAR MYCORRHIZA**

Arbuscular mycorrhizal associations are formed between fungi of zygomycetous affinity, belonging to the order Glomales, and roots of a wide diversity of plant life forms, including herbs, shrubs, lianas, and trees. The arbuscular mycorrhizal association evolved only once, more than 400 million years ago. Worldwide there are around 150 species of arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi consist of spores, auxiliary bodies, and a weft of intricately branched, non-septate runner and absorptive hyphae in soil, and in host plant roots of inter- and intracellular non-septate hyphae that sometimes form hyphal coils or ramify within the cortex to produce intricately branched haustoria called arbuscules, and followed in a number of taxa (suborder Glomineae, as contrasted to the suborder Gigasporineae) also by storage structures named vesicles. This sequence of arbuscular mycorrhizal colonisation development was formerly recognised and broadly classified into two types: *Arum*-type with a considerable intercellular phase of hyphal growth and production of terminal arbuscules on intracellular hyphal branches, and *Paris*-type with extensive intracellular hyphal growth yielding hyphal coils. Differences between both types are apparently dependent both on the anatomy of the plant root and the identity of the fungal taxa involved (Smith & Smith, 1997). While most of the (agricultural) plants that have been intensively used in mycorrhizal research exhibit the

*Arum*-type, under natural conditions the *Paris*-type occurs more frequently. In tropical trees arbuscules are usually rare or absent (St John, 1980) whereas hyphal coils are much more evident (Alexander, 1989a). Considering the small number of tropical rain forest taxa investigated for mycorrhizal colonisation, such claims need to be further investigated.
Arbuscules are considered as the major sites of biotrophic exchange between host plants and mycorrhizal fungi but their role in nutrient exchange is still debated, as no clear specialisation between arbuscules and hyphal coils has been demonstrated (Smith & Smith, 1997).

**ECTOMYCORRHIZA**

Ectomycorrhizal associations are formed between basidiomycetous and ascomycetous fungi and gymnosperm and angiosperm plant species belonging to certain tree, shrub, and liana families of both temperate and tropical regions. A few herbaceous plant species, mainly from arctic and alpine regions also form ectomycorrhizal associations. The ectomycorrhizal symbiosis evolved repeatedly with multiple (more than 10) independent origins both for the plants and fungi involved. Worldwide there might be as many as 10,000 species of ectomycorrhizal fungi.
Ectomycorrhizal fungi consist of three structural components: a sheath or mantle of fungal tissue that encloses the root, a labyrinthine hyphal network between root epidermal and cortical cells called the Hartig net, and the extramatrical mycelium, an outwardly growing, intricately branched system of fungal hyphae, sometimes aggregated in linear organs called rhizomorphs that form essential connections with the soil and with the fruitbodies (Smith & Read, 1997). The Hartig net is the site of nutrient exchange between the symbionts. The mantle, which shows a large variation in structure, colour and thickness, covers root tips of host plants and provides outstanding roles in nutrient and water storage and root protection against biotic and abiotic stress factors. The extramatrical mycelium and rhizomorphs are the sites of nutrient uptake and long-distance transport of nutrients and water. But these characteristic structures of ectomycorrhizal fungi may not always be present or be developed to the same extent in all ectomycorrhizal associations (Ashford & Allaway, 1982; Malajczuk et al., 1987; Smits, 1994; Warcup, 1980). Substantial differences seem to exist in size and structural organisation of ectomycorrhizas from cold and warm climates. Alexander & Högberg (1986) noted that the sheaths of tropical angiospermous ectomycorrhizas usually comprised a larger proportion of the composite organ than that of temperate trees, both due to thicker mantles and thinner plant roots. Tropical ectomycorrhizas also showed more prolific rhizomorph development. Comprehensive reviews of ectomycorrhizal characterisation based on anatomical features of mantle and emanating hyphae are covered in Agerer (1994, 1995).
The combined presence of both arbuscular and ectomycorrhizal fungal structures has been noted in the roots of several woody species, both in temperate and tropical regions (Alexander, 1989b; Allen et al., 1999; Chilvers et al., 1987; Lodge, 1989; Moyersoen & Fitter, 1999; Van der Heijden, 2000). Such plants are called dual mycorrhizal. While dual mycorrhizas are structurally defined, the interesting question is whether dual mycorrhizas are functionally mutualistic symbioses. Increased benefit of dual mycorrhizal colonisation has been often suggested but only convincingly demonstrated for *Salix repens* (Van der Heijden,

2000). For tropical trees, it is still unknown how common and how consistent dual mycorrhiza is, especially in plants hitherto considered as ectomycorrhizal.

**MYCORRHIZAL ASSOCIATIONS IN TROPICAL RAIN FORESTS**

Read (1991) has noted that different biomes are characterised by different mycorrhizal types: in the high arctic and alpine biomes ericoid mycorrhiza prevails, in boreal and temperate forest biomes ectomycorrhiza prevails, although on better soils in the more southern part of the temperate forest biomes arbuscular mycorrhiza might be dominant, whereas in the tropics and subtropics arbuscular mycorrhiza prevails, except on extremely infertile sandy soils (white sands), where ectomycorrhizal trees might again be dominant. This shift has been related to a shift in the main limiting nutrient for plant production, where under cold climates nitrogen mineralisation is hampered and where tropical soils are usually limited by phosphorus availability.

Most soils in the tropics are qualified as nutrient-poor, owing to strong acidity, high clay content, high exchangeable aluminium, and low available phosphorus and micronutrients (Sanchez & Salinas, 1981). The major input of mycorrhizal associations in tropical rain forests consists then in improved access to soil nutrients, especially phosphorus and other slowly diffusible nutrients such as zinc, copper, boron, and molybdenum. Increased nutrient uptake is due to the extension of the nutrient-absorbing organ of host plants by the external mycelium, beyond the reach of the depletion zone by roots. In view of the very low nutrient availability in most tropical soils, it is not surprising that very few woody species of tropical trees are non-mycorrhizal (Alexander, 1989a; Janos, 1980a).

While most forests in the tropics are indeed dominated by arbuscular mycorrhizal trees, a few notable exceptions occur both in Asia, where large patches of dipterocarp forest can be found, and in Africa, where large forest stands of caesalps occur both in the savanna (miombo) and rain forest region. In the neotropics, forests dominated by ectomycorrhizal trees are much rarer. If ectomycorrhizal and arbuscular mycorrhizal undisturbed forests (young secondary forest is almost always characterised by arbuscular mycorrhizal trees) in the tropics coexist, they can usually be partitioned in patches with either ectomycorrhizal or arbuscular mycorrhizal canopy dominants.

The majority of tropical tree species forming ectomycorrhizal associations belong to two families, viz. Dipterocarpaceae (mainly in south-east Asia, but with two genera, *Monotes* and *Marquesia*, in east Africa, and one genus, *Pseudomonotes*, in South America) and Caesalpiniaceae (mainly tribe Amherstieae; almost exclusively occurring in the Guineo-Congolian and Sudano-Zambezian region of Africa, but with two genera, *Intsia* and *Afzelia*, in Asia, and one genus, *Dicymbe*, in South America). In Africa the family Uapacaceae (the genus *Uapaca* occurs in tropical Africa and Madagascar) is an important component as well. Native ectomycorrhizal fungal genera in tropical Africa and Asia belong to a large extent to the same basidiomycete taxa as those that provide the fungal partners of temperate ectomycorrhizal plants. Common ectomycorrhizal taxa in the tropics belong to the Cantharellaceae, Boletaceae, Russulaceae, Amanitaceae, and Sclerodermataceae. On species level hardly any ectomycorrhizal fungal species (except when introduced) is shared between tropical and temperate regions. Native arbuscular mycorrhizal fungal taxa from the tropics, classified as

members of the Glomaceae, Acaulosporaceae, and Gigasporaceae, belong to the same taxa found worldwide. Several species of arbuscular mycorrhizal fungi are reported to have a worldwide distribution. However, as only a limited number of spore characteristics are available for species recognition, such observations of cosmopolitan distribution might as well reflect inadequate knowledge and understanding of the taxonomy of the arbuscular mycorrhizal fungi. In general, little information is available on the diversity, abundance, and distribution of ectomycorrhizal and arbuscular mycorrhizal fungal species in natural forests of the Guineo-Congolian region of Africa and their variation with disturbance regimes and stages.

**ECOLOGICAL DIFFERENCES OF MYCORRHIZAL ASSOCIATIONS**

The causes and consequences of the coexistence of ectomycorrhizal and arbuscular mycorrhizal forest types have not been clarified. Newbery et al. (1988) argued that these co-occurring forests would gradually shift (in the absence of climatic changes and large-scale, human-induced disturbance) towards ectomycorrhizal forests, whereas Janos (1996) hypothesised that the balance between ectomycorrhizal and arbuscular mycorrhizal forests is stable. Coexistence of both mycorrhizal forest types depends on both their competitive abilities on soils with various chemical, physical, and biological characteristics, and on their regeneration potential after disturbance. If disturbance regimes (on various temporal scales from local gap formation to periods of climatic deterioration) are more important than edaphic specialisation in determining the competitive ability of arbuscular mycorrhizal and ectomycorrhizal host trees, coexistence of both forest patches is possible even if ectomycorrhizal trees are more efficient nutrient scavengers.

Large differences in plant and fungus species diversity, nutrient status, and litter inputs have been noted between the two types of mycorrhizal forests. Arbuscular mycorrhizal forest patches are rich in phanerogam taxa (many different families, genera, and species) but poor in mycorrhizal fungal species and may be found preferentially on soils where phosphorus is the main limiting nutrient. On the other hand, ectomycorrhizal forest stands are usually poor in plant taxa (usually only one or two plant families, a limited number of genera, and in some cases such stands are even dominated by a single tree species) and rich in ectomycorrhizal fungal species. No hypothesis has been proposed to explain the inverse relationship of diversity of the phytobiont and diversity of the mycobiont. Ectomycorrhizal forest patches may dominate old forests with closed canopy where leaf litter accumulation is more common. Such patches have been related both to nitrogen and phosphorus limitation of the vegetation. Litter accumulation could lead to distinct nutrient pulses, benefitting plants with a better nutrient storage capacity below-ground (e.g. in the ectomycorrhizal sheath) and promoting conditions in which ectomycorrhizal fungi are likely to be effective competitors for nutrients, especially in organic forms (Connell & Lowman, 1989; Newbery et al., 1997, 1998). Litter accumulation could also give rise to soil surface conditions that subsequently reduce the regeneration of non-ectomycorrhizal host plants.

A number of studies reported that the extramatrical mycelia of mycorrhizal fungi can form networks that physically interconnect individuals from the same or different plant species (within the same mycorrhizal type) and allow transfer of both carbon and nutrients (nitrogen

and phosphorus) between plants via hyphal connections (Eason et al., 1991; Francis & Read, 1984; Newman, 1988; Simard et al., 1997a, b). While demonstration of gross transport has been relatively easy, demonstration and quantification of ecologically significant amounts of net transport has been very much harder. The question has also been raised whether materials transferred between plants might primarily benefit competitively inferior plants (Grime et al., 1986) or tree seedlings integrated in the mycorrhizal network of their mother trees (Yasman, 1995), or whether they primarily enhance the fitness of the mycorrhizal fungus, even though this could still indirectly affect competitive relationships between, and allow coexistence of, plants because of different costs. It is possible that the functioning of mycorrhizal interconnections is different for arbuscular mycorrhizal and ectomycorrhizal systems (Robinson & Fitter, 1999).

## DIVERSITY AND ABUNDANCE OF MYCORRHIZAL ASSOCIATIONS UNDER FOREST DISTURBANCE

Regimes and stages of forest disturbance by man could possibly negatively affect soil physical, chemical, and biological properties. In particular, forest disturbance induced by commercial logging practices may reduce or even eliminate mycorrhizal fungi from forest sites (Alexander et al., 1992; Reeves et al., 1979; Schramm, 1966; Smits, 1994). Such reductions of indigenous mycorrhizal fungal populations could have far-reaching implications for the establishment and survival of naturally regenerating seedlings that are dependent on mycorrhizas. There is also some evidence that forest clearance and burning of the woody biomass for growing annual crops, fallowing several years after the cropping period, and permanent plantations in monocropping could affect the quantity and quality of indigenous mycorrhizal populations (Asbjornsen & Montagnini, 1994; Egerton-Warburton & Allen, 2000; Helgason et al., 1998; Johnson, 1993; Thompson, 1987) thereby constraining forest regeneration. However, data on the influence of traditional shifting cultivation, commercial logging activities, and cocoa plantations on the diversity and abundance of native mycorrhizal fungal propagules of soils from tropical rain forests are still lacking. Such information is crucial to assess the impacts of forest land use on the capacity of soils to carry mycorrhizal propagules that are needed to colonise germinating seedlings.

## MYCORRHIZAL INOCULUM POTENTIAL

Assessment of mycorrhizal inoculum potential (MIP) provides a way to investigate the role of mycorrhizal associations in ecosystem processes across different habitats or microsites. The amounts and kinds of infective propagules of mycorrhizal fungi of a site constitute its inoculum potential, i.e. the energy for the growth of an organism at the surface of its host (Garrett, 1956), which is a consequence of the number of active propagules of that organism and their nutritional status. Mycorrhizal inoculum potential can be conceived as the capacity of infective propagules of arbuscular mycorrhizal or ectomycorrhizal fungi present in soils to become established within or on the roots of host plants, to give rise to mycorrhizal colonisations (Infectivity) and to provide benefits (Effectivity) (Brundrett et al., 1996; Janos, 1996; Liu & Luo, 1994). Infective propagules of AM fungi may include spores, dead root fragments, colonised organic matter, and hyphal external networks. Ectomycorrhizal infective

propagules include networks of mycelial strands, old mycorrhizal roots, sclerotia, and spores (Bâ et al., 1991; Brundrett et al., 1996). But the relative importance of these propagules is likely to depend strongly on the relationship between plant, soil, and mycorrhizal fungus. Soils may differ in MIP depending on the amount, type, and effectivity of mycorrhizal fungal propagules. Soil disturbance is likely to affect MIP as well (Alexander et al., 1992; Musoko et al., 1994). Evaluation of MIP will not automatically yield comparable data if different methods are employed.

Several approaches have been used to assess MIP with all showing some limitations (Dalpé, 1992). For example, enumeration of spore populations provides information on a fraction of MIP since other mycorrhizal propagules are not accounted for. The most probable number (MPN) procedure (Porter, 1979) loses information on small scale heterogeneity in inoculum potential by homogenising the distribution of inocula through the soil. The MPN technique enumerates all propagules that have the ability to colonise the test host plant but fails to detect dormant spores and is sensitive to host species selectivity, in addition to many other problems (Liu & Luo, 1994; Van der Heijden, 2000). A method with acceptable biological meaning appears to be the intact soil core which allows to measure total mycorrhizal infectivity due to intact hyphal networks as well as to disturbance-tolerant propagules such as spores and colonised root fragments (Abbott & Robson, 1991; Brundrett, 1991). However, if applied in the field, other factors could hamper comparability, such as different light levels between sites, and if soil cores are taken to the lab, the diameter of the cores might not be large enough to prevent disturbance of the mycorrhizal mycelia. Spatial variability could also be problematic if only a limited number of intact soil cores are sampled, compared to mixed soil samples for spore extraction. Use of different methods to compare MIP among different sites, preferably combined with a test how well the different methods correlate, may circumvent such inherent limitations and allow to assess more accurately impacts of land use practices on infective mycorrhizal propagules.

**MYCORRHIZAL RESPONSIVENESS AND DEPENDENCY**

The quality (in contrast to the sheer quantity) of mycorrhizal inoculum in soils can best be expressed in the responsiveness of host plants to mycorrhizal colonisation. This response depends on the degree to which test plants rely on mycorrhizas to achieve maximum growth at a given set of soil conditions. Mycorrhizal dependency refers to the inability of a host plant to grow without mycorrhiza at its natural level of soil fertility, whereas mycorrhizal responsiveness refers to the difference between mycorrhizal and non-mycorrhizal plants grown under the same environmental conditions. Dependency, an intrinsic property of a plant species, may be correlated with seedling survival. Responsiveness, an emergent property of the interaction between plant, mycorrhizal fungus, and soil characteristics, may be more important for the enhancement of plant primary production. Dependency and responsiveness need not be correlated (Janos, 1993). Data on mycorrhizal responsiveness of seedlings of economically, socially, and ecologically important tree species of tropical rain forests are still lacking. Such information is essential to understand the roles of mycorrhizas in natural forest, to assess effects of land use practices on the regeneration of mycorrhiza-dependent plants, and to evaluate the sustainability of such activities on below-ground processes.

It has been observed that soils with the highest MIP are not always most effective in enhancing plant growth (Asbjornsen & Montagnini, 1994). Such discrepancies could be a consequence of differences in specificity between mycorrhizal fungi or higher carbon costs of more heavily sporulating species (Johnson, 1993). Lack of positive correlation between mycorrhizal colonisation and seedling performance might be expected if the costs for mycorrhiza (in terms of plant carbon) outweigh the benefits (Koide & Elliott, 1989; Koide, 1991) in soils with weedy mycorrhizal species that produce numerous spores. It remains unclear whether mycorrhizal inoculum under weedy vegetation is sufficiently beneficial for regenerating tree species. It has been suggested that prolific spore-forming arbuscular mycorrhizal fungi could exert a larger carbon drain from the plant or effect a larger immobilisation of essential nutrients like nitrogen and phosphorus than the mycorrhizal fungal species of soils from undisturbed forest stands. On the other hand, the concept of "good weeds" (weedy species that contribute to maintenance of soil fertility and apparently facilitate succession), as used by local people, could suggest that at least some of these arbuscular mycorrhizal fungi can sufficiently enhance tree seedling establishment and subsequent growth.

Specificity is not common with mycorrhizal asssociations. The concept of specificity, however, can be used in different meanings. Molina et al. (1992) described six phenomena that have a bearing on the problem of specificity. From the perspective of a host plant or fungus two aspects are paramount, viz. taxonomic specificity (the diversity of mycorrhizal fungi accepted by a host plant or the diversity of host plants acceptable to a mycorrhizal fungus) and ecological specificity (the influence of biotic and abiotic factors on functional mycorrhizas between particular plants and fungi under natural conditions). It is important to note that (taxonomic) specificity phenomena could be quite different between ectomycorrhizal and arbuscular mycorrhizal systems. Ectomycorrhizal plants could still be receptive to arbuscular mycorrhizal fungi, although the opposite is very unlikely. Most, if not all, arbuscular mycorrhizal fungi are able to form functional relationships with a very broad range of plant species, at least under controlled conditions. Ecological specificity, however, could still play an important role, as different arbuscular mycorrhizal fungi selectively colonise certain plants in species-rich vegetation or determine specific plant responses (Bever et al., 1996; Giovannetti & Hepper, 1985; McGonigle & Fitter, 1990; Van der Heijden et al., 1998a; Zhu et al., 2000).

Ectomycorrhizal fungi have, on average, smaller ranges of host trees, although the full spectrum from species with very broad to very narrow host ranges has been observed. Smits (1994) claimed that tropical ectomycorrhizal fungi showed a larger degree of specificity than temperate fungi, but support for this claim has been extremely weak. Data by Thoen & Bâ (1987) could be taken as support for that claim, although their data are also consistent with different habitat preferences for the various fungi. However, if mycorrhizal fungi of the tropical rain forest exhibit a larger degree of specificity, it will undoubtedly have far reaching consequences for rain forest dynamics and for the management and regeneration of ectomycorrhizal timber trees. Yet, knowledge is lacking on the mycorrhizal regeneration requirements of seedlings of currently exploited timber species, especially ectomycorrhizal ones.

**ECOLOGY AND LAND USE OF AFRICAN RAIN FOREST**

Present African rain forests extend as a broad quadrilateral band north and south of the equator from the Atlantic coast eastwards to the "dorsale du Kivu", forming the Guineo-Congolian rain forest refuge. It includes Gabon, both Congo States, Equatorial Guinea, major areas of Central African Republic and southern part of Cameroon, and small satellites in Nigeria, Ghana, Liberia, Guinea, and Côte d'Ivoire. They occur in lowlands with altitude less than 1000 m and on isolated hills. The substratum is underlain by precambrian rocks. The region shows a high degree of endemicity. The greater part of the Guineo-Congolian region was formerly covered with rain forests on well-drained sites. Today, little undisturbed rain forest remains while secondary vegetation at various stages of forest regrowth is extensive. Although some Guineo-Congolian tree species are deciduous the forests themselves are evergreen or semi-evergreen (Richards, 1996; White, 1983).

The Guineo-Congolian rain forest is subjected to different land use practices including large habitations along road sides and small settlements within the forest, permanent agricultural plantations of cash crops like coffee (*Coffea* spp.) and cocoa (*Theobroma cacao*), and of banana (*Musa* spp.), rubber (*Hevea brasiliensis*) and palm oil (*Elaeis guineensis*), traditional shifting cultivation of food crops, selective commercial logging, collection of non-timber forest products, and fishing and hunting. So far, little information is available on the impacts of these land use practices on below-ground components and processes that significantly contribute to the functioning of rain forest ecosystems, in particular on diversity and dynamics of mycorrhizal associations.

**DESCRIPTION OF THE RESEARCH AREA**

The studies described in this thesis were carried out within the Tropenbos Cameroon Programme (TCP) research area. Administratively, the TCP area is found in the South province of Cameroon, sitting astride the Mvila division (subdivision Ebolowa) and the Ocean division (subdivisions Bipindi, Lolodorf, and Akom II). Accessibility to the area is irregular during wet seasons. Landforms, vegetation, and landscape ecology have been compiled in a 1: 100 000 map (Van Gemerden & Hazeu, 1999). Population density is about 12.5 inhabitants per $km^2$ (Lescuyer et al., 1999) and irregularly distributed. Habitation is mainly located along main road axes forming large and small villages. The population consists of a majority of Bantu people and a minority of Bagyeli (Bakola) pygmies. Bagyeli live in small settlements in forests (or in few mixed villages). They survive on gathering of non-timber forest products, fishing, hunting, and product exchange with the Bantu. Four native Bantu ethnic groups are found, the Bulu, Fang, Ngumba, and Bassa. Bulu form the majority and the Bassa the minority. All Bantu groups practice traditional shifting cultivation for subsistence agriculture and cocoa plantation as cash crop. Major food crops include oil-seeds like peanut (*Arachis hypogaea*), starchy plants like cassava (*Manihot esculenta*), plantain (*Musa* spp.), macabo (*Xanthosoma sagittifolium*), numerous condiments and spices, vegetables, food wrapping leaves, and numerous fruits. In addition, Bantu people also collect wild nuts, tree barks, and mushrooms, and practice fishing and hunting. An extensive description of non-timber forest products used by local populations in the TCP area is given by Van Dijk (1999). Large plantations of palm oil (*Elaeis guineensis*) and plantain have recently been introduced by local

elite and common interest cooperative groups (CIG) formed by village inhabitants. The only industrial activity in the area is commercial timber exploitation.
According to Letouzey (1985), the TCP area is subdivided into mid-altitude natural or climax forest rich in Caesalpiniaceae that is mainly ectomycorrhizal, and low altitude secondary forest dominated by *Lophira alata* (Ochnaceae) that is mainly arbuscular mycorrhizal forest. Selective logging and traditional shifting cultivation activities have been carried out for several decades within the TCP reseach area. It is not clear whether the distribution and proportional occurrence of both types of mycorrhizal forests have remained stable after several decades of forest land uses.

**OBJECTIVES OF THE STUDY**

The aim of this thesis is to generate basic knowledge on mycorrhizal fungi and their associations with tree species (mycorrhizal associations) of tropical rain forests at different stages of disturbance, with particular emphasis on the contribution of mycorrhizas to tree establishment and forest recovery after selective logging and traditional shifting cultivation. This information is essential for a better understanding of ecological processes that regulate natural forest regeneration and for the development of appropriate technologies for sustainable forest management. Specific objectives were:

1. To determine the composition and distribution of mycorrhizal associations in rain forests of South Cameroon.
2. To evaluate the influence of man-induced disturbances on mycorrhizal inoculum potential (MIP) of forest soils.
3. To assess the effects of forest soils with different MIP on growth of seedlings of important timber species of tropical rain forest and to determine the responsiveness of these seedlings to inoculum addition.
4. To determine the importance of intact hyphal connections as a source of inoculum for ectomycorrhiza formation and establishment of seedlings in the field.
5. To provide an account of species richness of ectomycorrhizal fungal diversity in rain forests of South Cameroon.

**OUTLINE OF THIS THESIS**

The thesis is divided in four parts. The first part provides background and actual information on mycorrhizal associations in tropical rain forests. CHAPTER 1 introduces the subject of mycorrhiza and mycorrhizal associations, then delineates ecological differences of the major types of mycorrhizal forests. Methodologies for assessing mycorrhizal abundance and functioning are discussed. An overview of tropical rain forests is also provided with a brief presentation of the area investigated and the objectives of the study. In CHAPTER 2, the results of a field survey provide an account of the mycorrhizal status of economically, socially, and ecologically important timber and other tree species, together with the abundance and distribution of mycorrhizal associations in undisturbed forest patches of South Cameroon, comparing them with data from Korup National Park (South-West Province, Cameroon). In CHAPTER 3, a provisional key to ectomycorrhizal fungi is presented to demonstrate species richness in rain forests of South Cameroon.

Mycorrhizal inoculum potential in soils from vegetation types of tropical rain forests has received little attention. The next part of this thesis (CHAPTERS 4-6) is devoted to mycorrhizal inoculum potentials in relation to the coexistence of both types of mycorrhizal forests. CHAPTER 4 presents variations in inoculum potential of arbuscular mycorrhizal fungi following disturbance stages derived from practices of shifting cultivation and logging, while CHAPTER 5 is concerned with changes in inoculum potential of ectomycorrhizal fungi in relation to conservation and management of ectomycorrhizal tree species diversity. In CHAPTER 6, the importance of intact hyphal connections on the ectomycorrhizal formation and survival and establishment of seedlings of indigenous tree species is explored.

The third part of this thesis details the roles of mycorrhizas in growth and mycorrhizal colonisation in seedlings of several important timber species in function of indigenous inoculum potential and addition of soil inoculum. Hence, the growth and nutrient responses of seedlings of native timber species are analysed in soils with inherent inoculum potential and after addition of grass inoculum (CHAPTER 7) and of host tree specific inoculum (CHAPTER 8). Finally, CHAPTER 9 analyses issues related to tropical mycorrhizal associations and raises scientific and practical questions that still need future inquiries. It also provides information for the integration of knowledge on mycorrhizas in planning for ecologically sound forest management.

# CHAPTER 2

## MYCORRHIZAL ASSOCIATIONS IN THE RAIN FOREST OF SOUTH CAMEROON


### ABSTRACT

*Mycorrhizal associations of important tree species were investigated within the research area of the Tropenbos Cameroon Programme, situated on the western portion of the Atlantic Biafrean forest of South Cameroon. Ninety-seven tree species of economic, social, and ecological importance, and 3 lianas were selected in 3 sites that differed in altitude, soil clay content, and soil pH. In each site plots were laid out in undisturbed forest. In each plot, seedlings, saplings, juvenile, and mature trees were identified to species level and counted; girth at breast height measured and basal area calculated; root samples were taken and examined for mycorrhizal type and extent of mycorrhizal colonisation. All 100 species investigated were mycorrhizal. Seventy-four tree species formed exclusively arbuscular mycorrhiza; twenty-three and three* Gnetum *species formed ectomycorrhiza. Five of these ectomycorrhizal plants also harboured arbuscular mycorrhizal structures. Extent of mycorrhizal root colonisation showed large differences for various arbuscular mycorrhizal trees; however, colonisation of more than half of these trees was less than 25%. Colonisation of ectomycorrhizal trees was often higher than 75%. The contribution of ectomycorrhizal trees to basal area varied between 19 and 35%. Ectomycorrhizal trees often occurred in small to large clumps. In sustainable forest management plans, existing ectomycorrhizal forest clumps should be given special conservation value.*


### INTRODUCTION

The rampant deforestation in the tropics due to increasing shifting cultivation and logging practices, urges for the design of appropriate management schemes to safeguard remnants of climax tropical rain forests. Effective strategies and solutions to sustainable forest management require taking into account economic and social interests of all forest dwellers as well as understanding of processes regulating the functioning of tropical rain forests. While the volumes of logs extracted and the kinds of non-timber forest products are well-known, little information exists on factors that sustain growth of trees, determine natural forest regeneration, and maintain floristic biodiversity.

Mycorrhizas (mutualistic associations between specialised basidio-, asco-, and zygomycetous fungi and roots of most higher plants) constitute the most efficient nutrient uptake facilitators, particularly in nutrient-deficient soils of tropical regions. As a consequence, presence of mycorrhizas results in increased plant fitness and forest tree productivity (Smith & Read, 1997).

In the humid tropics, two major types of mycorrhizal associations of trees have been reported, viz. ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM). In general, AM dominates secondary forests and a large number of primary forests (Janos, 1980, 1996). ECM occurs either isolated in a mosaic of AM species or in clumps in undisturbed forest where they dominate the canopy (Alexander, 1989a; Newbery et al., 1988, 1997). In southeast Asia, the major ECM taxa of rain forests belong to Dipterocarpaceae, whereas in tropical Africa the

major taxa belong to Caesalpiniaceae and Uapacaceae (Alexander, 1989a; Fassi & Moser, 1991). Few ECM species are found in the humid neotropics (Alexander & Högberg, 1986; Béreau et al., 1997). In tropical areas, ECM tree species are also encountered in introduced pines and eucalypts (Le Tacon et al., 1989).

Within the Biafrean forest of tropical west Africa, caesalp tree species dominate and these species are strongly aggregated (Letouzey, 1968). Clumping is very conspicuous for ECM caesalps, and species of *Uapaca* (Uapacaceae) usually occur in the same clumps. Newbery et al. (1997, 1998), following an earlier suggestion by Connell & Lowman (1989), hypothesised that the ECM habit and clumping were causally related because these ECM caesalps might be superior competitors for nutrients. However, little is known about the distribution of these clumps. In the rain forest of Korup National Park in southwest Cameroon, 22 caesalp tree species form small clumps (Newbery et al., 1988). Basal area of these ECM trees ranged from 3% to 28% in various transects. In individual 0.32 ha plots where three caesalp species (*Microberlinia bisulcata*, *Tetraberlinia bifoliolata*, and *T. moreliana*) dominated, basal area even ranged between 45% and 68% (Newbery et al., 1988, 1997).

The present study was undertaken to examine the mycorrhizal status of commercially important timbers, of tree species with significance for local forest dwellers, and of ecologically important tree species of the tropical rain forest of south Cameroon. The investigation also aimed at determining the distribution and proportion of major types of mycorrhizal associations in undisturbed forest stands within the research area of the Tropenbos Cameroon Programme (TCP), a programme directed towards sustainable management and use of these forests.

## MATERIALS AND METHODS

### *Research area and sites*

The study was carried out within the TCP research area, which is situated in the western portion of the Atlantic Biafrean forest of south Cameroon (Letouzey, 1985), lying within the Congo-Guinea refuge. The TCP area covers about 2000 $km^2$ and is situated between the cities of Lolodorf (3°14'N, 10°44'E) in the North, Adjap-Essawo (3°02'N, 10°52'E) in the East, Akom II (2°48'N, 10°34'E) in the South, and Bipindi (3°04'N, 10°25'E) in the West. The climate is humid tropical with two distinct wet seasons (mid-August - mid-November, mid-March - mid-May) and two dry seasons. The rainfall decreases in an easterly direction, with an annual mean of 2836 mm in Kribi to 2096 mm in Lolodorf and 1719 mm in Ebolowa. Average monthly temperatures vary between 22.9°C and 27.5°C (Olivry, 1986).

The landscape gradually shifts from lowlands in the southwest to rolling mountains in the central northern portion to abrupt hills in the northeast. Elevation ranges from 50 m a.s.l. near Bipindi to 1057 m a.s.l. in the Bingalanda mountain, near Nyangong. The substratum consists of Precambrian metamorphic rocks and old volcanic intrusions (Franqueville, 1973). In the southwestern lowlands (50 - 350 m a.s.l.), surface soils are sandy clay loam and moderately acid; between 350 m and 500 m a.s.l., surface soils are highly clayey and strongly acid; above 500 m a.s.l., soils are very highly clayey and very strongly acid (Van Gemerden & Hazeu, 1999).

Portions of the area have been recently logged by the Houthandel Gebroeders Wijma and Zonen B.V. (GWZ) timber company in two concessions. Elsewhere in the forest, Bantu populations subsist on shifting cultivation with short fallows of *Chromolaena odorata* (Asteraceae) and long bush fallows, and on cocoa cash crops, while Bagyeli people live in small forest settlements on gathering and hunting. Within the TCP research area, three experimental sites were chosen in Ebimimbang, Ebom, and Nyangong. Location, rainfall data, and soil physico-chemical characteristics are presented in Table 2-1.

**Table 2-1:** Location, elevation, rainfall, soil types, and characteristics of experimental sites

| Locality | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| Location | 3°03'N,10°28'E | 3°05'N,10°41'E | 2°58'N,10°45'E |
| Elevation (m a.s.l.) | 100 | 440 | 550 |
| Rainfall (mm) * | 1707 | 2019 | 1780 |
| Soil types | Ultisols | Ultisols, Oxisols | Oxisols |
| Clay (%) ** | 10-40 | 40-60 | 60-80 |
| pH (water) | 6.1 | 4.7 | 4.3 |
| Carbon (%) | 1.69 | 2.26 | 2.21 |
| Nitrogen (%) | 0.15 | 0.18 | 0.19 |
| Available P in $H_2O$ (µg/ml soil) | 0.01 | 0.005 | 0.002 |

Notes:
* Annual means of rainfall collected from 1995 to 1998
** Data derived from Van Gemerden & Hazeu (1999)

*Selection of plant species*

For the mycorrhizal screening, 97 tree species were selected, belonging to several categories of trees. They include 40 actually commercialised species and 27 potentially exploitable timber species. Actually commercialised timbers were selected on the basis of the percentage of volumes extracted, as recorded at the Kribi Port during the fiscal year 1994-1995 (Anonymus, 1995). Potentially exploitable timbers include lesser-known tree species with commercial potential in the future according to national standards. One timber tree is completely protected under Cameroonian law. Sixteen trees are socially important species that provide non-timber forest products such as fruits, leaves, bark, and medicines to local forest populations; species with cultural values are also included in this category (Van Dijk, 1999). Some of these might also be potentially exploitable as timber trees. Thirteen early-successional trees that could build up mycorrhizal inoculum during forest regeneration are included as ecologically important trees. Three *Gnetum* species (Gnetaceae) were added to the list owing to their particular growth form (liana) and economic position as a food crop.

Identification of tree species was done with the help of local assistants trained earlier by logging companies to recognise native timber species. Bark, leaves, flowers, and fruits were used and these characters were compared with photographs in identification manuals

(Letouzey, 1983; Letouzey & Mouranche, 1952; Thirakul, 1983). For some species herbarium material was taken to the national herbarium in Yaoundé, Cameroon, for additional checks and others were identified at the herbarium of Wageningen University, the Netherlands. Nomenclature of trees follows Aubréville (1970), Letouzey (1985), and Vivien & Faure (1985); De Wildeman (1936) for Uapacaceae and Stevels (1990) for Gnetaceae.

*Plot setting and plant enumeration*

Permanent sampling plots were selected in undisturbed forest stands in each site. Selection criteria of these stands included absence of recent man-made or natural disturbance and presence of different size classes of selected tree species. In each site, 2-ha (200 m x 100 m) plots were laid out in the undisturbed forest. There were two plots per site, 5 km distant from one another. Seedlings, saplings, juvenile, and mature trees were identified to species level, counted and girth at breast height (GBH) determined. Basal area was calculated from GBH.

Seedlings were plants of a small size with 3-5 true leaves and < 1 m height. Saplings were trees of intermediate size between seedlings and juvenile reproducing trees with GBH between 10-49 cm. Juveniles were young reproducing trees different from mature trees with GBH between 50-99 cm. Mature trees had a GBH greater than 99 cm.

*Root sampling and assessment of mycorrhizal colonisation*

Fine roots of saplings, juvenile, and mature trees were collected after tracing larger roots from the stem collar of target trees. For seedlings, the whole plant was uprooted with the surrounding soil. Collection of root samples was complicated by the abundance of intermingling roots of different species near the soil surface and by the difficulty to find the tree collar where primary roots are inserted. Triplicate root samples of each selected tree species were taken per age class. Root sampling was conducted during the periods of July-August 1996, January and July 1997.

ECM roots were easily recognisable as such, by the presence of obvious ECM root surface features (swollen root tips) using a 10x hand lens (Sight Savers and Bausch and Lomb Inc., Rochester, NY). The reliability of scoring of ECM roots was confirmed in the lab by observing the Hartig net in transverse sections of fine roots. ECM roots were placed in a Petri dish containing tap water, gently rinsed to remove soil and adhering organic particles, and then observed under a dissecting microscope at 6.4-40x magnification. Fractional colonisation (100% times the number of ECM root tips divided by the total number of root tips) was estimated from a random subsample (containing at least 100 root tips).

Non-ECM root samples and portions of ECM root samples were first rinsed three times with tap water to remove the alcohol. After rinsing they were cleared with 10% KOH for 24 hours or over the weekend (Phillips & Hayman, 1970). Heavily pigmented root samples were bleached after immersion for 60 minutes in alkaline $H_2O_2$ solution at room temperature. Alkaline $H_2O_2$ was prepared by adding 3 ml of $NH_4OH$ and 30 ml of 10% $H_2O_2$ to 567 ml of tap water (Kormanik & McGraw, 1982). Roots were thoroughly rinsed in water to remove the $H_2O_2$ (Rajapakse & Miller, 1994). The roots were thereafter acidified with 1% HCl for 3 minutes before staining in a solution of acid fuchsin for 2 to 3 days. The acid fuchsin stain was prepared from 875 ml of lactic acid, 63 ml of glycerin, 63 ml of tap water, and 0.15 g of

acid fuchsin (Kormanik et al., 1980). Stained roots were destained for 2 to 3 days in a lactic acid solution devoid of acid fuchsin before observation under a dissecting microscope at 6.4-40x magnification. Five to six one-cm long stained roots were randomly selected, placed on a glass slide, and gently squashed under a cover glass to observe the anatomy of AM fungi under a compound microscope at 100-400x magnification and to estimate fractional colonisation. Root samples of a host tree were considered AM if the following internal and external structures were observed from samples: vesicles, arbuscules, hyphal coils, intra- and intercellular hyphae without septa, auxiliary bodies on soil hyphae, distributive and absorptive hyphae, with or without external spores, attached to the roots.
Estimates of ECM and AM colonisation were categorised in five classes of mycorrhizal root colonisation (MCRI): Class 1, 1-5%; Class 2, 6-25%; Class 3, 26-50%; Class 4, 51-75%; and Class 5, 76-100% (Kormanik & McGraw, 1982).

**RESULTS**

*Mycorrhizal status of selected plant species and extent of mycorrhizal colonisation*

All 100 taxa, belonging to 77 genera and 29 families, were mycorrhizal (Table 2-2). Seventy-four tree species formed only AM. Twenty-three tree and 3 *Gnetum* species formed ECM. Dual mycorrhizal associations were observed in root samples of *Afzelia*, *Anthonotha*, *Uapaca*, and *Gnetum*. AM structures were consistently observed in roots of *Afzelia*. In the other dual mycorrhizal species, only few AM structures occurred on root portions devoid of ECM features.

**Table 2-2:** Mycorrhizal status and extent of mycorrhizal colonisation of selected tree species of economic, social, and ecological importance, including *Gnetum* species

**Category:**

Ta timbers actually exploited by logging companies
Tb potentially exploitable timbers in the future or used by local populations for firewood or other domestical needs
Tp totally protected tree species by Cameroon Forest Law
N tree species providing non-timber forests products such as fibre, fruits, nuts, or bark to local populations
E early successional tree species

**AM** Arbuscular mycorrhiza
**ECM** Ectomycorrhiza
**MRCI** Mycorrhizal root colonisation class; referring to the following classes of mycorrhizal colonisation of fine roots: U = undefined; 1= 1-5 %; 2 = 6-25 %; 3 = 26-50 %; 4 = 51-75 %; 5 = 76-100 %; (+) = few mycorrhizal internal structures
@ presence of nodules only at the seedling and sapling growth stages
@@ presence of nodules at all growth stages

| Species | Family | Category | AM | ECM | MRCI |
|---|---|---|---|---|---|
| Antrocaryon klaineanum | Anacardi. | N | + | | 1 |
| Trychoscypha acuminata | Anacardi. | N | + | | 1 |
| Enantia chlorantha | Annon. | N | + | | 1 |

| | | | | | |
|---|---|---|---|---|---|
| Hexalobus crispiflorus | Annon. | N | + | | U |
| Alstonia boonei | Apocyn. | N | + | | 1 |
| Picralima nitida | Apocyn. | N | + | | U |
| Canarium schweinfurthii | Burser. | Ta | + | | 4 |
| Afzelia bipindensis | Caesalpi. | Ta | + | + | 4 |
| Afzelia pachyloba | Caesalpi. | Ta | + | + | 4 |
| Amphimas ferrugineus | Caesalpi. | Tb | + | | 3 |
| Amphimas pterocarpoides | Caesalpi. | Tb | + | | 2 |
| Anthonotha fragrans | Caesalpi. | Tb | (+) | + | 3 |
| Anthonotha macrophylla | Caesalpi. | Tb | | + | 4 |
| Berlinia bracteosa | Caesalpi. | Ta | | + | 5 |
| Berlinia confusa | Caesalpi. | Ta | | + | 5 |
| Brachystegia cynometroides | Caesalpi. | Ta | | + | 5 |
| Brachystegia eurycoma | Caesalpi. | Tb | | + | 5 |
| Brachystegia zenkeri | Caesalpi. | Tb | | + | 5 |
| Cynometra hankei | Caesalpi. | Tb | + | | 3 |
| Cynometra sanagaensis | Caesalpi. | Tb | + | | 3 |
| Daniella ogea | Caesalpi. | Ta | + | | 1 |
| Detarium macrocarpum | Caesalpi. | Ta | + | | 1 |
| Dialium spp | Caesalpi. | Tb | + | | 1 |
| Didelotia africana | Caesalpi. | Tb | | + | 5 |
| Didelotia letouzeyi | Caesalpi. | Ta | | + | 5 |
| Distemonanthus benthamianus | Caesalpi. | Ta | + | | 4 |
| Erythrophloeum ivorense | Caesalpi. | Ta | +@ | | 4 |
| Gilbertiodendron brachystegioides | Caesalpi. | Ta | | + | 5 |
| Gilbertiodendrom dewevrei | Caesalpi. | Ta | | + | 5 |
| Gossweilerodendron balsamiferum | Caesalpi. | Ta | + | | 2 |
| Guibourtia tessmannii | Caesalpi. | Ta | + | | 3 |
| Julbernardia seretii | Caesalpi. | Tb | | + | 5 |
| Monopetalanthus le-testui | Caesalpi. | Tb | | + | 5 |
| Monopetalanthus microphyllus | Caesalpi. | Tb | | + | 5 |
| Oxystigma buchholzii | Caesalpi. | Tb | + | | 3 |
| Oxystigma mannii | Caesalpi. | Tb | + | | 3 |
| Pachyelasma tessmannii | Caesalpi. | Tb | + | | 3 |
| Paraberlinia bifoliolata | Caesalpi. | Tb | | + | 5 |
| Plagiosiphon longitubus | Caesalpi. | Tb | + | | 3 |
| Plagiosiphon multijugus | Caesalpi. | Tb | + | | 3 |
| Scorodophloeus zenkeri | Caesalpi. | N | + | | 1 |
| Tetraberlinia bifoliolata | Caesalpi. | Ta | | + | 5 |
| Touabouate brevipaniculata | Caesalpi. | Tb | | + | 5 |
| Garcinia kola | Clusi. | N | + | | 1 |
| Garcinia lucida | Clusi. | N | + | | 1 |
| Terminalia superba | Combret. | Ta | + | | 4 |
| Diospyros spp | Eben. | Tp | + | | U |
| Alchornea cordifolia | Euphorbi. | E | + | | 5 |
| Drypetes gossweileri | Euphorbi. | N | + | | 1 |
| Ricinodendron heudelotii | Euphorbi. | Ta | + | | 3 |
| Pterocarpus mildbraedii | Fab. | Tb | +@@ | | 5 |
| Pterocarpus soyauxii | Fab. | Ta | +@@ | | 5 |
| Gnetum africanum | Gnet. | N | | + | 5 |
| Gnetum buchholzianum | Gnet. | N | | + | 5 |

| | | | | | |
|---|---|---|---|---|---|
| Gnetum sp. | Gnet. | N | (+) | + | 5 |
| Sacoglottis gabonensis | Humiri. | E | + | | 1 |
| Irvingia gabonensis | Irvingi. | N | + | | 2 |
| Anthocleista schweinfurthii | Logani. | E | + | | 5 |
| Entandrophragma angolense | Meli. | Ta | + | | 1 |
| Entandrophragma candollei | Meli. | Ta | + | | 1 |
| Entandrophragma cylindricum | Meli. | Ta | + | | 1 |
| Entandrophragma utile | Meli. | Ta | + | | 2 |
| Guarea cedrata | Meli. | Ta | + | | 2 |
| Khaya ivorensis | Meli. | Ta | + | | 2 |
| Lovoa trichilioides | Meli. | Ta | + | | 3 |
| Cylicodiscus gabunensis | Mimos. | N | + | | 2 |
| Pentaclethra macrophylla | Mimos. | N | + | | 1 |
| Piptadeniastrum africanum | Mimos. | Ta | + | | 4 |
| Musanga cecropioides | Mor. | E | + | | 4 |
| Milicia excelsa | Mor. | Ta | + | | 1 |
| Pycnanthus angolensis | Myristic. | Ta | + | | 1 |
| Staudtia kamerunensis | Myristic. | Ta | + | | 1 |
| Lophira alata | Ochn. | Ta | + | | 4 |
| Coula edulis | Olac. | N | + | | 2 |
| Ongokea gore | Olac. | Tb | + | | 1 |
| Strombosia grandifolia | Olac. | E | + | | 2 |
| Panda oleosa | Pand. | Tb | + | | 1 |
| Poga oleosa | Rhizophor. | N | + | | 1 |
| Mitragina ciliata | Rubi. | Ta | + | | 1 |
| Nauclea diederrichii | Rubi. | Ta | + | | 2 |
| Pausynistalia johimbe | Rubi. | N | + | | 3 |
| Fagara heitzii | Rut. | Ta | + | | 2 |
| Allophylus schweinfurthii | Sapind. | E | + | | 2 |
| Blighia welwitschii | Sapind. | E | + | | 2 |
| Deinbollia pycnophylla | Sapind. | E | + | | 2 |
| Eriocoelum macrocarpum | Sapind. | E | + | | 2 |
| Aningeria robusta | Sapot. | Ta | + | | 3 |
| Autranella congolensis | Sapot. | Tb | + | | 2 |
| Baillonella toxisperma | Sapot. | Ta | + | | 2 |
| Chrysophyllum sp. | Sapot. | Tb | + | | 2 |
| Gambeya africana | Sapot. | Ta | + | | 3 |
| Omphalocarpum procerum | Sapot. | Ta | + | | 2 |
| Eribroma oblonga | Sterculi. | Ta | + | | 2 |
| Pterigota macrocarpa | Sterculi. | Tb. | + | | 3 |
| Uapaca acuminata | Uapac. | E | (+) | + | 4 |
| Uapaca guineensis | Uapac. | N | | + | 4 |
| Uapaca staudtii | Uapac. | E | | + | 4 |
| Uapaca vanhouttei | Uapac. | E | | + | 4 |
| Celtis sp. | Ulm. | Tb | + | | 4 |
| Trema orientalis | Ulm. | E | + | | 5 |

Internal AM structures were not observed in root samples of the following tree species: *Diospyros* spp, *Hexalobus crispiflorus*, and *Picralima nitida*. Their mycorrhizal status could not be clearly delineated due to very dense pigmentation. The roots of these taxa were violaceous black and only attached non-septate hyphae and the absence of swollen root tips

allowed us to infer their AM status. *Plagiosiphon* and *Cynometra* possessed swollen root tips that on superficial examination might be interpreted as ECM tips.

Twenty-three tree species had 1-5% roots colonised with AM structures and forty-two had less than 25% AM colonisation. Only *Pterocarpus soyauxii*, *P. mildbraedii*, *Alchornea cordifolia*, *Anthocleista schweinfurthii*, and *Trema orientalis* had always more than 75 % roots colonised with AM. Extent of AM colonisation was equally variable between early- and late-successional tree species. In more than half of the samples investigated internal hyphae only could be observed. Arbuscules and hyphal coils were about equally common. Vesicles were about twice as common as arbuscules. Intraradical hyphae were most often straight with ramifications along the root cortex, sometimes tortuous and thicker. Various kinds of vesicles were observed, viz. oval, irregularly lobed, and rectangular. Both smooth and echinulate auxiliary cells were observed.

ECM colonisation was much higher, and was often more than 75%. Root samples of ECM tree species showed either abundant rhizomorphs or smooth mantles with different colours. ECM of *Uapaca* species were mostly smooth. Root samples of *Gnetum* species harboured either sulphur-yellow, white, brown, or dark brick ECM, covered with abundant rhizomorphs. Roots were not completely covered by a mantle, and numerous root hairs were also observed. In dual mycorrhizal species colonisation was predominantly by ECM.

*Stand composition and occurrence of ECM associations*

In the Ebimimbang site, average density of the investigated tree species was 452 stems per hectare. Basal area was 73 $m^2.ha^{-1}$. In terms of stem numbers *Diospyros* spp. were dominant (45%), but in terms of basal area ECM caesalps dominated these stands. ECM trees contributed 22% of stems and 35% of basal area, whereas *Diospyros* spp contributed only 8% to basal area. In the Ebimimbang area, 6 clumps dominated by ECM caesalps were noted within a 25-km² area, in five North-South, 5-km long transects, distant of 1 km. All of them were situated on flat terrain. In the Ebom site, average density of the investigated tree species was higher, viz. 583 stems per hectare, but basal area lower, viz. 52 $m^2.ha^{-1}$. *Staudtia kamerunensis* was numerically dominant (34% of stem numbers, with 5% contribution to basal area), and *Pycnanthus angolensis* contributed most to basal area (28%, with 10% of stem numbers). These two tree species belonging to the Myristicaceae contributed 33% of total basal area. ECM trees contributed 7% of stems and 19% of basal area. Around Ebom, ECM tree species occurred as individual plants or in small groups scattered within the mass of AM tree species. Two monodominant clumps of *Gilbertiodendron dewevrei* were observed, one at the bottom of a non-flooded valley and the other along the river Melangue (North of Ebom). In the Nyangong site, average density of the investigated tree species was 368 stems per hectare and basal area 64 $m^2.ha^{-1}$. *Uapaca* species were dominant in terms of stem numbers (11%) and basal area (26%). ECM trees contributed 26% of stems and 34% of basal area. Five clumps dominated by *Uapaca* species and two dominated by caesalps were observed. They occurred mostly on hills. Individual *Uapaca* trees occurred near riverside forests.

## DISCUSSION

*Mycorrhizal status of selected plant species and extent of mycorrhizal colonisation*

In our study we did not observe non-mycorrhizal trees, confirming the ubiquity of mycorrhizal associations in the tropical rainforest. Out of 56 species investigated in Korup, Cameroon, 55 turned out to be mycorrhizal and only one species, viz. *Warneckea memecyloides* (Melastomataceae) was non-mycorrhizal (Newbery et al., 1988). In French Guyana Béreau et al. (1997) noted that all 75 tree species investigated were mycorrhizal.

A low amount of AM colonisation was observed in root samples of tree species of several families. Amount of colonisation was often comparable within several members of the same family, e.g. low in Anacardiaceae, Meliaceae, Myristicaceae, and Sapindaceae, and high in Ulmaceae. Colonisation was also high in the nitrogen-fixing legumes *Erythrophloeum* and *Pterocarpus*. It has been noted by Janos (1996) that the correlation between early seral status and independence of mycorrhizas is loose, and it is therefore not surprising that in our study area early- and late-successional trees did not show differences in their amount of colonisation. Characteristics of plant roots usually highly correlate with mycorrhizal formation and abundance (Baylis, 1975). Coarse roots with no or few root hairs are often highly mycotrophic while highly branched, fine, long roots with numerous root hairs have often been observed to derive little nutritional benefit from mycorrhizas in controlled experiments (Manjunath & Habte, 1991a). In our study, roots of most tree species were rather coarse with few root hairs. However, they did not show abundant AM colonisation.

The presence of arbuscules in roots is generally used to designate plants with functional AM. In this investigation arbuscules were rarely observed in most root samples. Hyphal coils were about as common as arbuscules. In most samples only intracellular hyphae were present. Our observations of the lack or scarcity of arbuscules are consistent with those in the tropical wet forests of the neotropics by Janos (1984) and Béreau et al. (1997). Both the amount of arbuscules and coils and the amount of internal colonisation might depend on differences in root morphology. Smith & Smith (1997) reviewed structural diversity of AM and recognised two types, viz. *Arum*-type and *Paris*-type. In the *Arum*-type, extensive intercellular hyphae and arbuscules develop, while in the *Paris*-type these structures are absent and hyphal coils occur commonly.

Three families (Caesalpiniaceae, Uapacaceae, Gnetaceae), 13 genera, and 26 species formed ECM. Thoen (1993) recorded the ECM habit in 18 genera and 50 species of African trees. Most prominent in his list are Caesalpiniaceae (13 genera with 37 species in tribe Amherstieae and 1 genus with 6 species (*Afzelia*) in tribe Detarieae). *Paraberlinia* and *Touabouate* (Amherstieae) have not been reported before as ECM trees. ECM caesalps are not only common in the wet tropical forest but also in the savanna (miombo) of East Africa (Högberg, 1989). The large number of ECM caesalps in Korup (10 genera with 22 species) and the TCP area (11 genera with 19 species) confirm the importance of the Biafrean forest for that group. Most of the ECM caesalps belong to the ekop group. The name ekop has been used by various timber prospectors for a variety of timber species. Letouzey and Mouranche (1952) recognised 10 taxa of ekop with a further 10 unnamed ekops. Of the ten ekop taxa listed explicitly, nine belong to the ECM tribe Amherstieae, whereas the identity of the tenth species was doubtful.

Among the 5 dual mycorrhizal plant species, AM structures were present to a different extent. AM structures were consistently found in roots of *Afzelia* species, suggesting that it is truly dual mycorrhizal (Alexander, 1989b; Moyersoen & Fitter, 1999). In *Uapaca guineensis* and *Gnetum* species, AM occurred incidentally in root portions without ECM, suggesting that in conditions of lower ECM inoculum potential roots might be susceptible to colonisation by AM fungi (Smith et al., 1998). In Africa, reports of AM in roots of these latter genera exist for *Gnetum africanum* (Fassi & Moser, 1991), *Uapaca guineensis* (Thoen & Bâ, 1989), and *U. kirkiana* (Högberg, 1982). In roots of *Anthonotha fragrans* we once encountered arbuscules and AM external hyphae with echinulate auxiliary cells of a *Gigaspora* species. Alexander (1989b) also reported presence of AM fungi in the ECM trees *Gilbertiodendron* and *Pellegriniodendron*, and Moyersoen & Fitter (1999) reported the presence of AM fungi in *Anthonotha*, *Berlinia*, *Didelotia*, *Gilbertiodendron*, *Monopetalanthus*, and *Tetraberlinia*.
The frequent occurrence of vesicles indicates that a large part of the AM fungi belong to the Glomineae, and diversity in vesicle shape indicates the presence of both *Glomus* (vesicles ellipsoid) and *Acaulospora* (vesicles more irregularly shaped or rectangular). Predominance of Glomineae has been reported for tropical sites (Gianinazzi-Pearson & Diem, 1982; Sieverding, 1991). Gigasporineae are much rarer, but smooth auxiliary cells of *Scutellospora* and echinulate auxiliary cells of *Gigaspora* have both been observed.
A large number (more than 125) of ECM fungal species have been observed (CHAPTER 3). Important ECM fungal genera are *Amanita*, *Russula*, *Lactarius*, and various genera of the Cantharellaceae and Boletales. Less important genera include *Scleroderma*, *Cortinarius*, and *Inocybe*. Field observations indicated a low degree of host specificity, as most of these species have been noted under various Caesalpiniaceae and Uapacaceae. *Scleroderma sinnamariense* with its bright yellow mycorrhizas and rhizomorphs has only been encountered with *Gnetum* (Fassi, 1957) and might be considered as host specific.

*Stand composition and occurrence of ECM associations*
Not only the large number of ECM trees (species richness), but also their contribution to stem numbers and basal area (ranging from 19 to 35% in the various sites) confirm that the TCP-area, just as Korup, is a major area for these trees. Basal area of ECM trees was even higher in Bityili, a site about 15 km SE of Nyangong, reaching 73%. The lower contribution of ECM trees to basal area in Ebom can be explained by the fact that these stands are somewhat younger than the stands in Ebimimbang and Nyangong. This can be concluded from the higher number of stems and lower basal area in Ebom and the high representation of members of the Myristicaceae, which are generally characteristic for old secondary forests (Thirakul, 1983; Letouzey, 1985). *Microberlinia bisulcata*, the species showing clumping behaviour to the strongest degree, is absent from the TCP area, although it occurs both in Korup and the Douala-Edea forest (Newbery & Gartlan, 1996). Both Korup and the area around Kribi have been mentioned by Rietkerk et al. (1995) as glacial (pleistocene) refugia. The similar species composition between Korup and the TCP area indicates, however, that neither a very pronounced dry period (as evidenced in Korup) nor the presence of sandy soils that are very prone to leaching are necessary for ECM caesalps to become canopy dominants.

The occurrence of mixtures of ECM and AM trees in the same stands has raised the question whether such forests are or are not in equilibrium (Connell & Lowman, 1989). However, the question of an equilibrium between both functional mycorrhizal types might not be resolved because of the variability of the climate in the last 30,000 years (Maley & Brenac, 1998). Their data from western Cameroon indicate that caesalps generally have a low frequency, even after a recovery from a climatic deterioration (a relatively arid climate) that occurred between 2800 and 2000 years BP. However, their data also indicate that both ECM and AM caesalps reacted in a similar way to climatic changes, which would be consistent with observations that both functional types are equally efficient in extracting phosphorus from these highly leached poor soils (Moyersoen et al., 1998a).

Even if both functional types occupy the same niche, their potential to recolonise these sites after major deforestation could be dissimilar. Plant succession after deforestation will be determined by the availability of AM and ECM inoculum. AM fungi are more capable of surviving land clearing than ECM fungi both because spore viability and longevity is higher (having larger spores with substantial energy reserves) and because host tree specificity is lower. If spore numbers of AM fungi are high, AM inoculum might rapidly build up. If spore numbers are low, succession starts with non-mycorrhizal and facultatively mycorrhizal plants while replacement by obligately mycorrhizal trees will take a longer period of time (Janos, 1996). The more limited ability of the ECM symbiosis to establish after large-scale disturbance (Janos, 1996) might explain their clumped distribution better than their supposed competitive superiority. Management and conservation practices in rain forests should take these clumps into account, as ECM tree recruitment after large-scale deforestation might be a limiting factor.

# CHAPTER 3

# SPECIES DIVERSITY OF ECTOMYCORRHIZAL FUNGI OF RAIN FORESTS OF SOUTH CAMEROON WITH A PROVISIONAL KEY TO THE SPECIES OBSERVED

## INTRODUCTION

Little information is available on species richness of ectomycorrhizal fungi of tropical rain forests in western Africa. For three years, mushroom excursions were carried out in four sites within the TCP area, viz. Ebimimbang, Ebom, Nyangong, and Bityili. In each site, mushroom excursions were carried out in permanent plots in primary forests inside and outside clumps of ectomycorrhizal trees. Excursions took place at the two wet seasons of south Cameroon (April-June and September-November). Collected mushrooms were described in fresh condition and subsequently dried. Exsiccates were investigated in the Netherlands. Most of the material is preserved at the National Herbarium of the Netherlands, Leiden branch. Duplicates of a number of species are kept in Kribi.

Around 125 species of putative ectomycorrhizal fungi have been observed. This number is a minimum estimate and is likely to increase upon completion of the study of the exsiccate collections. The herbarium material will also be made available to monographers of African fungi. Part of the fungal collections could not be identified with the available literature and these taxa are indicated as species nova.

There is no straightforward criterion to determine whether a fungal species is ectomycorrhizal and a number of taxa included are putatively non-mycorrhizal, such as *Amanita aureofloccosa* (found in a cocoa plantation without ectomycorrhizal trees), both species of *Phlebopus* (found without ectomycorrhizal trees close by), and both species of *Paxillus* (found on very decayed wood in ectomycorrhizal clumps).

Fruitbodies of ectomycorrhizal fungi were found mostly in clumps of ectomycorrhizal caesalps or at the stem base of *Uapaca*; a few collections of one gasteromycete species were made near *Gnetum*. As the ectomycorrhizal clumps contained a number of species that grew intermingled, it was not possible to assess with what tree the ectomycorrhizal fungi were most likely associated. For that reason no assessment could be made on host tree specificity on the basis of these observations (but see CHAPTERS 5 and 6). Only *Gnetum* species harboured a host-specific fungus, viz. *Scleroderma sinnamariense*, characterised by its conspicuous bright yellow ectomycorrhizas and rhizomorphs.

Species richness varied with sites. Species richness was highest in Bityli and lowest in Ebom. Species richness matched the contribution of ectomycorrhizal trees in the forest plots (CHAPTER 2). However, all four sites shared a substantial number of species, suggesting that the TCP area is homogeneous in terms of ectomycorrhizal fungal species composition.

In this chapter preliminary keys to the ectomycorrhizal fungi of the TCP area are presented. Keys are arranged per genus, except for the Boletales and Cantharellaceae where the key includes several genera.

## KEY TO CAMEROONIAN SPECIES OF AMANITA

1.a. Spores amyloid; pileus margin usually not grooved-striate → 2
1.b. Spores inamyloid; pileus margin grooved-striate → 18

2.a. Stipe with saccate volva → 3
2.b. Stipe with friable volva → 8

3.a. Pileus margin striate-grooved to 0.3R. Pileus to 50 mm, distinctly viscid, at margin covered by a few remains of porphyry-grey veil, dark brownish grey in centre, pale grey towards margin. Lamellae white. Stipe to 75 x 7 mm, white, with very conspicuous, powdery porphyry-grey veil. Ring not observed. Volva coarsely saccate, conspicuously thick-set, white. Spores 7.0-9.0 x 6.5-8.0 μm. **Amanita sp. 1**
3.b. Pileus margin not distinctly striate-grooved → 4

4.a. Pileus pure white to very pale yellowish, sericeous-smooth, slightly greasy, without velar covering, to 40 mm. Lamellae white. Stipe to 85 x 6 mm, with clavate to marginate bulb (12 mm), white, with a saccate volva; ring pendent, white, not striate. Smell as raw potatoes or more as artificial honey. Spores (6.0-)6.5-7.5(-8.0) x (5.5-)6.0-7.0(-7.5) μm. Clamp-connections absent. **Amanita subviscosa**
4.b. Pileus with various shades of brown → 5

5.a. Pileus with appressed, membranaceous velar patches, to 85 mm, innately fibrillose-striate, reddish brown. Lamellae white. Stipe to 100 x 10 mm, equal, whitish. Ring not observed. Volva thick-set, to 40 mm high, white. Smell absent. Spores 6.0-7.5 x 4.0-5.0 μm. Velipellis with hyphae, without globose cells. **Amanita sp. 2**
5.b. Pileus without velar patches → 6

6.a. Spores (sub)globose, 7.5-9.5 x 7.5-9.0 μm. Basidia 2-spored. Smell strong, as artificial honey. Pileus 45 mm, innately fibrillose, giving the pileus a streaky appearence, dark brown in centre, elsewhere cigar-brown to more greyish brown. Lamellae white. Stipe 85 x 5 mm, slightly swollen-clavate (7 mm) but not bulbous, white. Volva papery, white, thin, rather appressed, saccate. Traces of ring present in upper part of stipe. **Amanita sp. 3**
6.b. Spores ellipsoid, smooth, amyloid. Basidia 4-spored. Smell absent → 7

7.a. Pileus cinnamon at centre, at margin pink. Volva pink. Spores 8.5-10.0(-10.5) x 5.0-6.0 μm. Pileus 40 mm, smooth. Lamellae whitish, with white edge. Stipe 65 x 7 mm, clavate (10 mm) but not bulbous. Ring not observed. **Amanita sp. 4**
7.b. Pileus (very) dark brown to reddish brown in centre, at margin yellowish brown. Volva white, somewhat thick-set, to 35 mm high. Spores 6.5-8.0(-8.5) x 3.5-4.5(-5.0) μm. Pileus to 65 mm, minutely fibrillose to indistinctly squamulose all over. Lamellae whitish. Stipe to 90 x 7 mm, clavate (12 mm) but not bulbous, white to pale greyish buff. Ring indistinct, as some appressed patches on stipe, also with some remains at pileus margin. **Amanita fulvosquamulosa**

8.a. Spores slightly punctate, 5.5-7.0 x 5.0-6.0 μm. Pileus 40 mm, in central part with appressed, dark grey-brown patches, underneath sericeous to indistinctly fibrillose, whitish to pale buff. Lamellae whitish, with white-flocculose edge. Stipe 52 x 6 mm, conspicuously bulbous (14 mm), white; volva indistinct, as appressed grey girdles on outer side of bulb. Ring pendent, white. Smell slightly disagreeable. Pileipellis with subglobose cells, 35-50 μm diameter, with hyaline to brownish pigment. **Amanita sp. 5**

8.b. Spores smooth → 9

9.a. Pileus pure white to very pale yellowish, completely covered by a greyish veil consisting of appressed clusters of globose cells, 45 mm, convex, sericeous-white. Lamellae white, with white edge. Stipe 98 x 8 mm, at base 12 mm, bulbous, whitish, slightly scurfy below, no clear remains of volva visible. Ring appressed, hanging, white. Smell faint, as rubber. Spores 5.0-6.0 x 3.5-4.5 µm. Veil consisting of globose cells, to 60 µm diameter. **Amanita sp. 6**

9.b. Pileus coloured → 10

10.a. Lamellae turning green with age. Pileus to 55 mm, with grey pyramidal warts on brown background, somewhat paler towards margin, somewhat darker in centre. Lamellae white, with flocculose edge; greenish on ageing. Stipe to 70 x 8 mm, with white bulb (15 mm), without distinct velar remains, apically with a membranaceous, non-striate, white ring. Context white in pileus, greening on ageing. Smell reminding of artificial honey. Spores 9.0-10.5 x (6.5-)7.0-8.0 µm. Veil on pileipellis consisting of (broadly) ellipsoid or more subglobose hyaline cells. Clamp-connections absent. **Amanita virella**

10.b. Lamellae not turning green with age → 11

11.a. Context reddening on exposure. Pileus 50-100 m, reddish brown or orange brown, covered with appressed, woolly-pulverulent, whitish to grey-brown then reddish brown velar patches. Lamellae white, with white-flocculose edge. Stipe 40-80 x 5-10 mm, bulbous, at apex with a white-striate, membranaceous ring, elsewhere smooth, whitish, discolouring to pinkish-brownish. Volva reduced, rather squamulose. Smell indistinct. Spores 7.0-8.0 x 6.0-7.0 µm. Basidia 4-spored. Cheilocystidia cylindrico-clavate, thin-walled, colourless. Clamp-connections absent.
**Amanita rubescens**

11.b. Context not reddening on exposure → 12

12.a. Pileus and stipe bright yellow-orange, floccose-pulverulent throughout. Pileus to 80 mm. Lamellae white. Stipe to 140 x 10-15 mm, equal to subbulbous. Ring pendent, subpulverulent, orange-yellow. Smell like flowers of *Iris*. Taste slightly bitter. Spores 7.0-8.5 x 6.5-8.5 µm. Clamp-connections absent. Not ectomycorrhizal. **Amanita aureofloccosa**

12.b. Pileus and stipe without bright yellow colours → 13

13.a. Pileus irregularly diffracted like *Leucoagaricus* sp., 45 mm, dark reddish brown. Lamellae slightly adnate, whitish. Stipe 70 x 5 mm, with a very conspicuous bulb (13 mm), with a similar covering as pileipellis but less densely so, bulb white. Ring not observed; velar remains not observed (except for stipe covering). Spores 7.0-8.5 x 4.0-5.0 µm. **Amanita sp. 7**

13.b. Pileus not irregularly diffracted → 14

14.a. Pileus and stipe squamulose to squamose → 15

14.b. Pileus and stipe with mealy covering of veil → 16

15.a. Spores 7.5-9.5(-10.0) x 6.5-8.5 µm, subglobose. Pileus and stipe grey, brown to black, covered with pyramidal pulverulent, grey-brown warts. Pileus to 40 mm. Lamellae white, sometimes with pale grey-brown edge. Stipe to 70 x 8 mm, with a conspicuous onion-like bulb (to 15 mm), dark grey-fibrillose at base, more hairy upwards. Ring hanging, ephemerous. Smell indistinct. Remnants of volva on pileipellis consisting of subglobose to ellipsoid cells with (dark) brown intracellular pigment. Clamp-connections abundant in hymenophoral trama. **Amanita lanosa**

15.b. Spores 7.0-8.0 x 3.5-5.0 µm, ellipsoid. Pileus 50 mm, applanate to convex, without umbo, in centre with (dark) grey-brown peluchy scales, outwards appressed peluchy-tomentose, buff to

brownish grey. Lamellae white, with white-flocculose edge. Stipe 115 x 7 mm, swollen near base (16 mm), but attenuated below indistinct bulb, grey-brown-squamulose over greater length, especially near base, without distinct volval remains. Ring hanging, striate, white or with a grey lower edge. Smell indistinct, sometimes chemical in base of stipe. Pileipellis with chains of broadly ellipsoid to subglobose cells, to 30-60(-70) x 30-50 μm. Hyphae of pileipellis without clamp-connections. **Amanita sp. 8**

16.a. Stipe prominently bulbous. Pileus covering woolly-peluchy; covering of pileipellis with subglobose cells with brown intracellular pigment, to 50 μm. Pileus to 45 mm, background sericeous, pale grey, velar patches pyramidal in centre, appressed squamulose near margin, grey-brown to black. Lamellae white. Stipe to 80 x 8 mm, prominently bulbous (22 mm), grey-punctate-squamulose in lower part. Ring not observed. Clamp-connections absent. Spores 6.0-8.0(-8.5) x 4.0-5.0 μm. **Amanita sp. 9**

16.b. Stipe to 60 x 5 mm, equal to slightly clavate → 17

17.a. Stipe with velar covering. Pileus to 30 mm, brown-grey. Lamellae white. Stipe to 60 x 3 mm, clavate (at base 6 mm), but not bulbous, with similar covering as pileus (especially in lower part). Ring not observed. Smell slightly earth-like. Spores 7.0-8.0 x (4.0-)4.5-5.5 μm. Remnants of volva consisting of up to 75 μm, globose colourless cells. Clamp-connections absent. **Amanita sp. 10**

17.b. Stipe smooth, without velar covering. Pileus 30 mm, applanate, covered all over with low woolly erect pyramidal warts that crack to show underlying context, slightly glistening, in centre almost blackish brown, outwards more grey-brown. Lamellae pale buff. Stipe 55 x 5 mm, equal, whitish to pale buff, without velar remains. Ring not observed. Smell absent. Spores 6.0-7.5 x 4.0-5.0 μm. Pileipellis with hyaline globose cells, to 40 μm broad. Clamp-connections absent.

**Amanita sp. 11**

18.a. Pileus pure white, sericeous-smooth, striate to 0.5R, covered with appressed white velar patches, to 45 mm. Lamellae white, with white edge. Stipe to 65 x 5 mm, somewhat swollen-clavate below (6 mm) but not bulbous, white, with a relatively thick, saccate volva up to 20 mm high. Ring not observed. Smell indistinct. **Amanita strophiolata**

18.b. Pileus coloured→ 19

19.a. Veil present, pulverulent → 20

19.b. Veil absent, or if present, appressed membranaceous → 22

20.a. Pileus covered with golden-yellow-pulverulent veil, disappearing on handling, showing underlying somewhat greasy, pale yellow pileipellis, to 60 mm, striate to 0.5 R. Lamellae white, with yellow-flocculose edge. Stipe 100 x 6 mm, bulbous (11 mm), pale yellow, with similar covering as pileus. Ring not observed. Volva appressed to bulb, consisting of golden-yellow pulverulent covering, sometimes inconspicuous. Spores 6.5-8.5 x (4.5-)5.0-6.0 μm. Veil of pileipelis consisting of (sub)globose cells, to 50 μm diameter, mixed with scattered hyphae. Clamp-connections absent. **Amanita bingensis**

20.b. Pileus grey-brown to black → 21

21.a. Pileus with conspicuous erect, dark brown to black peluchy scales in central part. Pileus to 50 mm, margin striate to 0.5 R, dark grey-brown to black in centre, pallescent outwards, half-way brown to grey-brown and at margin (pale) grey, buff or whitish. Lamellae pale greyish or buff, with (dark) grey-brown edge. Stipe to 80 x 7 mm, clavate to distinctly bulbous (20 mm), with white ring with black outer lining, lower half with black squamules or more pulverulent, with appressed greyish

volva with black upper lining. Spores 7.0-9.0 x (6.5-)7.0-9.0 µm. Velar covering consisting of (sub)globose cells with brown intracellular pigment. Clamp-connections absent. **Amanita sp. 12**

21.b. Pileus minutely squamulose, at margin more granulose-pulverulent, 13-25 mm, dark brown, in outer half showing white context and therefore seemingly paler, conspicuously striate. Lamellae white, with white edge. Stipe to 30 x 2 mm, bulbillose, with very minute appressed volva, white, minutely hairy in upper part. Spores 7.5-9.0(-9.5) x 4.5-5.5 µm. Veil with globose elements with slightly roughened walls and brownish intracellular pigment, connecting hyphae with roughened walls (pigment minutely incrusting). Clamp-connections absent. **Amanita sp. 13**

22.a. Veil present, appressed, dark grey to brown grey, not consisting of globose cells, on porphyry-grey to violaceous grey background, to 15 mm, striate to 0.5 R. Lamellae white, with floccose, white or porphyry-grey edge. Stipe to 25 x 2 mm, minutely bulbous, grey-brown, at base with narrowly appressed orange-brown, papery volva and one concolorous velar zone just above volva; ring absent. Spores 9.0-13.0 x (5.5-)6.0-8.0 µm. Clamp-connections absent. **Amanita calopus**

22.b. Veil absent on pileus → 23

23.a. Outer side of volva with yellow-brown polygonal scales on ochraceous background. Pileus to 30 mm, brownish grey, somewhat darker in centre, striate. Lamellae white. Stipe 40 x 4 mm, with evanescent, slightly striate ring. Spores 9.0-10.0 x 8.0-9.5 µm **Amanita strobilaceovolvata**

23.b Outer side of volva smooth → 24

24.a. Stipe with a very prominent bulb, to 130 x 18 mm, bulb 33 mm, white. Pileus to 90 mm, striate to 0.3R, buff in centre, at margin white. Lamellae white, with white edge. Ring not observed. Volva saccate, thick-set, to 60 mm high, white, with appressed greyish buff patches. Smell absent. Spores 9.0-11.0x 6.5-8.0 µm. Clamp-connections present. **Amanita sp. 14**

24.b. Stipe equal to clavate → 25

25.a. Lamellae white, yellowing on ageing or drying. Pileus yellow-brown, unicolorous or slightly darker in centre, to 70 mm, conspicuously striate to 0.6R. Stipe to 150 x 8 mm, with appressed to slightly loosened, appressed-woolly, yellow to ochraceous velar patches on whitish background, with small appressed white volva, with very ephemeral ring. Spores 8.0-11.0(-11.5) x 6.0-8.0(-8.5) µm. Clamp-connections not observed. **Amanita sp. 15**

25.b. Lamellae not yellowing on ageing or drying → 26

26.a. Volva large, to 30 mm high, thick-set, saccate, white. Pileus dark brown in centre, outwards yellow-brown to ochraceous yellow, to 60 mm, slightly greasy, striate Lamellae white, with white-flocculose edge. Stipe to 95 x 10 mm, smooth, white, with evanescent ring. Spores not observed. Cheilocystidia (sub)globose, balloon-shaped to broadly clavate. Clamp-connections abundant in hymenium. **Amanita sp. 16**

26.b. Volva rather small, appressed to slightly loosened → 27

27.a. Pileus with black centre, elsewhere grey-brown, seemingly tricolorous, up to 60 mm, outer part conspicuously striate. Lamellae white, with white-floccose edge. Stipe to 80 x 8 mm, pale grey, with an ephemerous grey ring, volval remains not visible. Spores 9.0-12.0 x 6.0-7.5 µm. **Amanita annulatovaginata**

27.b. Pileus, yellow in centre, outwards greenish yellow, striate to 0.6 R, to 30 mm. Lamellae white, with white edge. Stipe 66 x 4 mm, slightly swollen below (5 mm), white, with very small membranaceous white volva. Smell absent. Spores (8.0-)8.5-10.5(-11.0) x 5.5-7.0 µm. **Amanita luteoflava**

KEY TO CAMEROONIAN SPECIES OF BOLETALES

1.a. Hymenophore lamellate to transvenose-lamellate → 2
1.b. Hymenophore poroid → 5

2.a. Hymenium with conspicuous, thick-walled (wall up to 2.5 μm thick, pale yellow), fusiform cystidia, 100-120 x 12-16 μm, with pointed apex. Pileus brown in centre, yellowish brown outwards, tomentose. Hymenophore yellow, on bruising somewhat blue-green. Stipe concolorous with pileus. Context whitish in pileus, not changing on exposure. Spores 6.0-8.0 x 4.5-5.5 μm, ellipsoid, pale yellowish. Clamp-connections absent. **Tubosaeta alveolata**
2.b. Hymenium without conspicuous, thick-walled cystidia → 3

3.a. Spores 3.5-4.5 x 3.0-3.5 μm, pale greyish yellow to yellowish brown, smooth. Clamp-connections present → 4
3.b. Spores 9.5-11.0 x 4.0-5.0 μm, yellowish brown, smooth or very minutely longitudinally striate. Clamp-connections absent. Pileus with erect, dark brown squamules in centre, outwards more subsquamulose-subtomentose on yellowish background. Stipe equal, solid, yellow-brown, at base with yellow tomentum. Lamellae thin, subdistant, intervenose, decurrent, yellow-brown, with fimbriate, concolorous edge.Context somewhat yellowish, especially on drying, not not changing colour on exposure. **Phylloporus depressus**

4.a. Pileus to 50 mm, infundibuliform, minutely subtomentose, yellowish brown. Stipe to 40 x 4 mm, brown. Lamellae thin, crowded, decurrent, brown, on damage with blue-green sheen. Context pale ochraceous buff. **Paxillus sp. 1**
4.b. Pileus to 70 mm, applanate, smooth and almost subshiny, blackish brown in centre, outwards reddish brown. Stipe 38 x 13 mm, attentuate, excentric to almost lateral, very dark brown. Lamellae thin, crowded, decurrent, orange-ochraceous, on damage not changing. Context whitish in pileus, blackish at base of stipe. **Paxillus brunneotomentosus**

5.a. Spores ornamented with very conspicuous wings or longitudinal ridges or spores verruculose, usually dark brown, sometimes brownish yellow → 6
5.b. Spores smooth under light microscope (oil immersion, 1000x), yellow-brown, olivaceous brown, pale pinkish brown, ochraceous cream or almost hyaline → 10

6.a. Spores brownish yellow, with longitudinal ridges, 11.0-14.0 x 5.0-7.0 μm (including ridges). Pileus to 40 mm, strongly cracked, sienna on the patches, showing underlying yellow context. Stipe 60 x 6 mm, yellow, with very conspicuous pale yellow rhizomorphs. Hymenophore slightly olivaceous yellow, bluing on bruising. Context yellow, slightly bluing on exposure. **Boletellus pustulatus**
6.b. Spores dark brown, with conspicuous wings or spores verruculose. Stipe without rhizomorphs → 7

7.a. Spores with wings or crests → 8
7.b. Spores verruculose → 9

8.a. Pileus with dark brown wrinkles on ochraceous background. Spores 12.0-15.0 x 9.0-10.0 μm, with up to 1.0 μm high, longitudinal fine wings. Stipe with very conspicuous black dots over whole length, apex yellow, at base ochraceous, in middle part (dark) brown. Tubes broadly adnate, dirty olivaceous brown. Context yellowish, becoming dark blue (black) on exposure, after 10 minutes slowly discolouring to yellowish. Smell absent. **Strobilomyces luteolus**

8.b. Pileus with blackish scales on pale background. Spores 13.0-16.0 x 11.5-13.0 μm with conspicuous, to 2.0 μm high longitudinal wings. Tubes uneven, partly labyrinthoid, decurrent, olive-grey. Stipe with ring, coarsely squamulose below with elongate reticulum above. Context whitish in pileus, slightly greying on exposure, soon blackening in stipe. Smell absent.
**Strobilomyces lepidellus**

9.a. Spores coarsely reticulately verrucose, 8.0-10.0 x 6.0-7.0 μm. Pileus with coarse appressed to slightly recurvate, squamulose black scales, with age more squamulose-tomentose on paler brownish-greyish background, with margin very strongly appendiculate. Stipe coarsely woolly-tomentose to woolly-squamulose in lower half, black, at apex with small reticulum. Pores white, then grey, darkening to blackish on handling, fairly large, round; tubes shortly decurrent, blackening on exposure. Context white in pileus, reddening and then rapidly blackening on exposure, black in stipe. Smell absent. Clamp-connections absent. **Strobilomyces velutipes**

9.b. Spores with fine, isolated verruculae, 7.0-9.0 x 5.0-6.0 μm. Pileus with erect, later appressed, more or less pyramidal blackish scales, on paler brownish-greyish background, with margin strongly appendiculate. Stipe coarsely squamulose over greater part, at apex with coarse reticulum. Pores white, grey, even, darkening to blackish on handling. Context white in pileus, reddening on exposure, soon dark grey to black. Clamp-connections absent. **Strobilomyces echinatus**

10.a. Not associated with ectomycorrhizal trees. Pileus very large, more than 10 cm diameter. Spores smaller than 10 μm, ellipsoid → 11

10.b. Associated with ectomycorrhizal trees (*Uapaca*, Caesalpiniaceae); Pileus less than 10 cm diameter. Spores variable → 12

11.a. Pileus 50-170 mm, ochraceous, but darkening on handling, silky-smooth, cuticle easily peeling off. Stipe smooth, slightly olivaceous-tinged yellow, with yellow tomentum and bright yellow mycelial cords, dark greenish brown on handling. Pores minute, bright yellow to greenish yellow when young. Context yellow, immediately and strongly bluing in pileus, only slightly so in stipe. Smell absent. Spores 5.0-7.0 x 3.5-5.0 μm. Cheilocystidia abundant. **Phlebopus braunii**

11.b. Pileus to 145 mm, yellow-brown to reddish brown in centre, in outer part more orange-ochraceous, dark red-brown on handling, subtomentose, appressedly scaly with small brown scales, minutely cracking. Stipe to 90 x 35 mm, equal, tapering towards base, without net, very minutely punctate, at apex orange-yellow, downwards soon brown to blackish brown, without mycelial cords. Pores yellow-brown with olivaceous tinge, on bruising dark blue-green. Context white in pileus, blue on exposure in pileus; in stipe yellowish, becoming greenish brown, at base blackish with some irregular white fragments inside. Smell indistinct. Taste with slightly bitterish-astringent aftertaste. Microscopic data unknown. **Phlebopus sp. 1**

12.a. Spores ellipsoid, Q < 1.8, less than 8.0 μ long → 13

12.b. Spores cylindrical-boletiform, l/b > 2.0 → 16

13.a. Hymenophore irregular, consisting of anastomosing pores. Pileus to 55 mm, applanate, tomentose to minutely squamulose, brownish orange, red-brown to red, very slightly cracking and showing reddish context. Stipe to 65 x 6 mm, equal or apically enlarged, red, at apex sometimes more yellow, at base whitish to yellowish. Pores yellow, then mixed with red; tubes ochraceous, not discolouring. Context white to pale yellowish, under pileus red, not discolouring on exposure. Smell absent, to slightly fruity. Spores 6.0-8.0 x 4.0-6.0 μm, $Q_{av}$ < 1.5. Clamp-connections absent.
**Gyrodon sp. 1**

13.b. Hymenophore regular. Spores almost hyaline, $Q_{av}$ > 1.6 → 14

14.a. Stipe hollow. Pileus golden-brown or orange-brown at margin, reddish brown near centre, minutely squamulose, without umbo, rather thin-fleshed. Stipe ± concolorous with pileus, slightly darkening. Pores and tubes (pale) yellowish buff, not discolouring. Context white, unchanging. Smell absent. Spores 6.5-8.0 x 4.0-5.0 μm. Clamp-connections absent. **Gyroporus microsporus**

14.b. Stipe not becoming hollow → 15

15.a. Pileus dark red, subtomentose, slightly cracking, applanate, to 50 mm. Stipe concolorous with pileus, smooth. Pores yellowish; tubes broadly adnate, yellow-brown, not staining on damage. Context whitish to pale yellowish, not bluing on exposure. Smell slightly sweetish. Spores 6.5-7.0(-7.5) x 4.0-4.5 μm. **Rubinoboletus luteopurpureus**

15.b. Pileus when young reddish brown to (dark) brown in centre, at margin yellow-orange, discolouring with age to orange in centre and yellow at margin, coarsely tomentose to minutely squamulose, plano-convex to applanate, to 65 mm. Stipe to 55 x 10 mm, equal, solid, smooth or slightly longitudinally striate, yellow, with yellowish mycelial strands. Pores round to subangular, yellow; tubes white, then buff. Context white, not discolouring. Smell indistinct to slightly fruity. Spores 7.0-8.5(-9.0) x 4.0-5.0(-5.5) μm. **Rubinoboletus luteoporus**

16.a. Hymenophore with very conspicuous, thick-walled, fusiform cystidia → 17

16.b. Hymenophore without setules → 18

17.a. Spores 8.5-12.5 x 4.0-5.5 μm. Pileus blood-red to carmine red, subtomentose, not cracking. Stipe reddish in upper half, more orange-yellow in lower half, reddish punctate near apex, at base with yellowish strands. Pores and tubes pale yellow, not discolouring on bruising. Context almost whitish in pileus, on damaged places with reddish flush, in base of stipe yellow, not bluing on exposure. Smell indistinct to slightly fruity. Cystidia 60-105 x 10-15 μm, fusiform with pointed apex, with up to 2.5 μm thick, yellowish-brownish wall, towards apex reddish-brownish. **Tubosaeta brunneosetosa**

17. Spores 12.0-16.5 x 4.5-6.0 μm. Pileus yellow-brown to brownish red, slightly cracking, showing underlying yellow context, subtomentose under lens. Stipe apex yellow, elsewhere red with a slight violaceous flush, somewhat granulose in upper part. Pores irregular, angular, large, yellow-ochraceous to yellow-brown, bruising to dark blue-green. Context strongly bluing, initially yellow in pileus and stipe apex, red to dark vinaceous elsewhere in stipe. Smell indistinct. Cystidia 70-120 x 9-12 μm, fusiform with pointed apex, thick-walled, with up to 2.5 μm thick yellowish wall, somewhat darker towards apex. **Tubosaeta goossensiae**

18.a. Pileus with conspicuous central papilla, to 40 mm, strongly tomentose, brown to red-brown. Stipe to 55 x 6 mm, swollen below, minutely punctulate, tomentose at base, without net, at apex purplish, downwards reddish brown, more yellowish towards base, blackening on damaging. Pores round, red. Tubes olivaceous yellow. Context whitish to buff in pileus, reddish brown in stipe, unchanging. Smell indistinct. Spores 8.0-10.0(-10.5) x 4.0-4.5(-5.0) μm. Clamp-connections absent. **Boletus sp. 1**

18.b. Pileus without conspicuous central papilla → 19

19.a. Pileus, stipe, and context with greenish-bluish tinges → 20

19.b. Pileus, stipe, and context without greenish-bluish tinges → 23

20.a. Pores large and wide, irregular-angular. Pileus ivory white to pale yellow, mixed with green-yellow tinges on ageing → 21

20.b. Pores normal to fairly large, round → 22

21.a. Spores 7.0-10.0(-10.5) x 3.0-4.0 µm. Pileus 15-25 mm, ivory-white to pale yellowish, ± smooth to subtomentose, with greenish patches, especially on ageing, finally grey-green. Stipe 32 x 2.5 mm, attenuate, yellowish, finally yellowish green, darkening downwards. Pores large and wide, irregular, ochraceous brown with olivaceous flush, slightly greening on bruising. Tubes yellow-brown with olivaceous flush. Context slightly greenish. Smell distinct, as cooked rice. Clamp-connections absent. **Boletus boletiformis**

21.b. Spores 9.0-12.5 x 4.5-5.5 µm. Pileus under lens subtomentose, cracking, whitish to yellowish, on damage blue-green, sticky when moist. Tubes free, olive brown. Pores white, then with blue-green tinges, irregular to roundish. Stipe to 50 x 5 mm, longitudinally striate to almost smooth, white, with blue-green spots on damage. Context white, blueing on exposure. Smell indistinct. Clamp-connections absent. **Boletus sulcatipes**

22.a. Pileus slightly viscid, smooth. Spores 10.0-12.0 x 3.5-4.5 µm. Pileus dark greenish grey, on age somewhat pallescent to ochraceous grey-green. Pores initially white; tubes pinkish brown. Stipe greenish, slightly viscid. Context white, somewhat greenish on exposure. Spores 10.0-12.0 x 3.5-4.5 µm. Clamp-connections absent. **Pulveroboletus viridis**

22.b. Pileus dry, minutely punctulate. Spores 10.5-12.5 x 5.5-6.0 µm. Pileus 13 mm, glaucous. Pores white; tubes yellow-brown. Stipe 22 x 2 mm, greenish at apex, more brownish yellow at base. Context sightly greenish on exposure. Clamp-connections absent. **Pulveroboletus aberrans**

23.a. Spores (almost) hyaline, 11.0-14.0 x 4.0-5.5 µm. Hymenophoral trama with cystidia with yellow-brown contents (somewhat resembling chrysocystidia), to 15 µm broad. Pileus to 45 mm, subtomentose to minutely squamulose, clay buff. Stipe 60 x 10 mm, slightly tapering downwards, solid, whitish to pale buff in upper part, (dark) brown in lower part, slightly tomentose. Tubes and pores white, yellow to brown on handling. Context (pale) sulphur-yellow. Smell sweetish. Clamp-connections absent. **Boletus macrocystis**

23.b. Spores yellow-brown. Chrysocystidioid cystidia lacking → 24

24.a. Pores yellow. Pileus to 105 mm, subtomentose, not or slightly cracking, (cinnamon-)brown, clay pink to ochraceous-orange in centre, at margin yellowish brown. Stipe to 80 x 13 mm, equal, at apex yellow, towards base slightly reddish cinnamon or brown, smooth or red-brown punctulate throughout. Pores angular, yellow to brownish yellow, on bruising blue-green. Tubes yellow. Context whitish to yellowish buff, yellow just above tubes, pinkish brown in stipe, not or hardly bluing on exposure, finally whitish. Smell absent. Spores 9.0-14.0 x 4.0-5.5(-6.0) µm. Pileipellis a cutis with pigment (very) minutely incrusting. Clamp-connections absent. **Boletus subspinulosus**

24.b. Pores white, pinkish or greyish → 25

25.a. Stipe to 80 x 6 mm, equal, with faint longitudinal striation or reticulation at apex, elsewhere smooth, yellowish brown, with age concolorous with pileus. Spores 12.0-15.0 x 3.5-4.0(-4.5) µm (Q > 3.0), pale pinkish brown, smooth. Pileus reddish brown to sepia vinaceous, to 40 mm, convex, almost smooth to subtomentose, minutely cracking and showing underlying white context. Pores white, later greyish-brownish. Context white, somewhat greying on exposure. Smell agreeable. **Tylopilus violaceus**

25.b. Stipe to 60 x 6 mm, equal, minutely punctulate, especially in upper part, grey-brown. Spores 10.0-12.0 x 5.0-5.5 µm (Q < 2.5), yellow-brown, smooth. Pileus to 40 mm, applanate, with margin slightly extending over pores, coarsely tomentose, minutely cracking, buff, later greyish brown. Pores white, then greyish vinaceous. Context white, becoming brown in stipe. Smell when fresh as rubber, on drying mixed with a sweet component. **Leccinum excedens**

KEY TO CAMEROONIAN SPECIES OF CANTHARELLACEAE

1.a. Spores verruculose, ochraceous, 7.0-9.0 x 4.0-5.5 µm. Hymenium almost smooth to irregularly venose, decurrent, buff, then brown, finally porphyry-brown. Pileus funnel-shaped, almost smooth, flesh-coloured yellowish when moist, whitish on drying. Stipe yellowish brown. Clamp-connections present. **Gomphus brunneus**

1.b. Spores smooth, hyaline → 2

2.a. Hymenium almost smooth. Clamp-connections absent → 3

2.b. Hymenium distinctly merulioid-venose to almost lamellate with very narrow lamellae → 4

3.a. Pileus to 50 mm, infundibuliform, coarsely tomentose, with slightly lacerate margin, dark grey-brown to fuscus black. Stipe to 40 x 6 mm, concolorous with pileus. Hymenophore (almost) smooth to slightly wrinkled, violaceous grey to violaceous black. Smell sweetish. Spores (7.5-)8.0-15.0(-15.5) x (5.5-)6.0-11.0(-11.5) µm. **Craterellus cornucopioides**

3.b. Pileus to 15 mm, fibrillose, (dark) grey. Hyphae of stipitepellis short, with frquent septa. Stipe central, concolorous with pileus. Hymenophore smooth, grey. Spores 8.5-10.0 x 6.0-7.0 µm. **Craterellus crispus**

4.a. Pileus buff and darkening with age or damage or pileus dark grey-brown with violaceous sheen. Clamp-connections present → 5

4.b. Pileus yellow, orange to red → 6

5.a. Context blackening. Pileus to 65 mm, infundibuliform with involute margin, tomentose, when young buff to ochraceous, darkening with age and damage to dark brown or blackish. Stipe to 55 x 10 mm, equal, fibrillose, concolorous with pileus, blackening. Hymenophore consisting of narrow, reticulate wrinkles, decurrent, grey, finally blackish. Context white, finally black on exposure. Smell slightly sweetish. Spores 6.0-7.0(-7.5) x 4.0-5.0 µm. **Cantharellus congolensis**

5.b. Context orange-ferrugineous, not blackening on exposure. Pileus to 33 mm, with involute margin, coarsely fibrillose to subsquamulose, very dark grey-brown with violaceous sheen. Stipe to 37 x 8 mm, equal, saffron-yellow, especially on handling, at apex with violaceous sheen. Hymenophore reticulately wrinkled, orange-yellow. Spores 6.0-7.0 x 4.0-5.0 µm. **"Cantharellus" dichrous**

6.a. Hymenium reticulate with (sub)distant veins; if more lamellate, then with (sub)distant and broad lamellae → 7

6.b. Hymenium lamellate with very crowded and narrow, furcate or ramose, decurrent, buff to orange-yellow lamellae. Pileus to 60 mm, pale orange-yellow to brownish buff, minutely to distinctly squamulose, especially in centre. Stipe to 30 x 10 mm, tapering, buff to pale orange-yellow, somewhat darkening on handling. Context thin, yellow-orange. Smell sweetish. Spores 5.0-7.0 x 4.0-5.0(-5.5) µm. Clamp-connections absent. Pileipellis hyphae in centre thick-walled. **Cantharellus densifolius**

7.a. Clamp-connections present → 8

7.b. Clamp-connections absent → 9

8.a. Hymenophore venose, veins narrow, distant, often reticulate, pinkish orange to pale yellowish. Fruitbodies to 30 mm, orange-red to orange-ochraceous, smooth to subtomentose. Stipe to 25 x 2 mm, equal, concolorous with pileus. Smell not distinct. Spores 5.5-7.5 x 4.0-5.5 µm. **Cantharellus miniatescens**

8.b. Hymenophore merulioid, consisting of narrow, reticulate, whitish to pale yellow or yellowish buff wrinkles, with age more distinctly crwamy-yellow. Pileus to 110 mm, irregularly shaped or

infundibuliform, with margin involute, rather smooth to somewhat subtomentose, orange, orange-brown to reddish brown, slightly pallescent on ageing. Stipe to 70 x 6 mm, equal, smooth, whitish when young, then pale yellow. Context whitish, slightly yellowing on exposure. Smell indistinct. Spores 6.0-7.0 x 4.0-5.0 μm. **Cantharellus sp. 1**

9.a. Pileus distinctly scaly. Pileipellis with short, thick-walled elements → 10
9.b. Pileus smooth to subtomentose, not scaly. Hyphae of pileipellis thin-walled → 11

10.a. Hymenium rather lamellate, transvenose, yellow. Pileus with red-brown scales. Pileus to 70 mm, yellow-orange. Stipe to 50 x 10 mm, yellowish, distinctly squamulose-punctate in upper part. Context whitish. Smell sweetish. Spores 6.0-8.5 x 4.5-6.0 μm. **Cantharellus rufopunctatus**
10.b. Hymenium narrow, merulioid, conspicuously venose-wrinkled, pale yellow. Pileus to 40 mm, whitish, in centre with small erect orange red scales, at margin more appressed squamulose, pale orange. Stipe to 23 x 4 mm, equal, yellowish, ± smooth. Context white. Smell indistinct. Spores 6.0-7.5 x 4.0-5.0 μm. **Cantharellus luteopunctatus**

11.a. Pileus to 22 mm, slightly infundibuliform, yellow to orange, slightly pallescent on drying, smooth to subtomentose. Stipe to 40 x 3 mm, equal, concolorous with pileus. Hymenophore lamellate-venose, pale cream to yellow. Context thin. Smell indistinct or sweetish. Spores 5.5-8.0 x 4.0-5.0 μm. **Cantharellus sp. 2**
11.b. Pileus larger, 40-70 mm → 12

12.a. Pileus to 50 mm, infundibuliform, with involute margin, slightly hygrophanous, tomentose to minutely squamulose (especially on drying), buff, greyish brown, brownish yellow to orange buff. Stipe to 60 x 6 mm, equal, smooth, whitish to pale yellow buff, more distinctly so on handling, towards base more orange, at apex minutely scurfy. Hymenium rather truly lamellate, not reticulate or intervenose, but sometimes furcate, yellow to orange. Context white, orange-yellow in stipe base. Spores (6.5-)7.0-9.0 x 5.0-6.5 μm. **Cantharellus isabellinus**
12.b. Pileus to 70 mm, infundibuliform, hygrophanous, smooth, (deep) yellow. Stipe to 50 x 7 mm, equal, concolorous with pileus. Hymenium rather truly lamellate, transvenose, (pale) yellow. Context whitish. Smell sweetish. Spores 7.5-8.5 x 6.0-6.5 μm. **Cantharellus pseudocibarius**

## KEY TO CAMEROONIAN SPECIES OF CLAVULINA

1. Carpophores to 11 mm high, branched, when young creamy white, with age grey-brown to vinaceous buff, at base yellowish. Spores 5.5-8.0 x (4.5-)5.0-6.5 μm, smooth, hyaline. Basidia 2-spored. Clamp-connections present. **Clavulina vanderystii**

## KEY TO CAMEROONIAN SPECIES OF COLTRICIA

1. Pileus to 15 mm (young specimens!), slightly zonate. Pores narrow, 4-5(-6) / mm. Spores 5.0-6.5 x 4.0-5.0 μm, smooth, very pale brown, with slightly thickened, congophilous wall. Hyphae without clamp-connections. Pileipellis a trichoderm of erect, brown hyphae, at apex often furcate. **Coltricia spathulata**

## KEY TO CAMEROONIAN SPECIES OF CORTINARIUS

1.a. Pileus not hygrophanous, dry to slightly tacky, yellow-brown with appressed brown scales (appressedly imbricate). Stipe smooth to slightly fibrillose, bulbous, violaceous blue, bulb white, upper part lined with yellow buff veil. Lamellae violaceous blue, not staining on damage. Context pale violaceous blue throughout, but white in bulb. **Cortinarius sp. 1**

1.b. Pileus hygrophanous, dry → 2

2.a.. Pileus not translucent striate, violaceous when moist, arachnoid-tomentose, becoming mixed with greyish-brownish tinges on drying, to 15 mm. Lamellae violaceous, then brown, with flocculose, violaceous edge. Stipe to 30 x 2.5 mm, slightly swollen at base (3.5 mm), silvery grey, at apex with a violaceous sheen, slightly fibrillose. Spores (5.5-)6.0-7.0(-7.5) x (5.0-)5.5-6.0 μm, verruculose. Cheilocystidia 23-45(-47) x 4-10 μm, cylindrical-sublageniform, often with 1 or 2 septa, thin-walled, hyaline. **Cortinarius sp. 2**

2.b. Pileus translucent striate to 0.3R, brown when moist, smooth, 13 mm, conical. Lamellae reddish brown with flocculose white edge. Stipe 46 x 1.5 mm, equal, concolorous with pileus, covered with appressed, aeriferous white velar patches. Spores 8.0-9.0 x 5.0-5.5 μm, verruculose. Cheilocystidia not reviving. **Cortinarius sp. 3**

## KEY TO CAMEROONIAN SPECIES OF INOCYBE

1.a. Pileus strongly squarrose on woolly background covering, brown, with margin extending beyond lamellae, to 55 mm. Lamellae with conspicuously serrate, dark brown edge, broadly adnate, pale ochraceous brown. Stipe to 65 x 7 mm, equal, densely squarrose throughout with dark brown scales on yellow background. Context white, rather firm. Smell indistinct. Spores (6.0-)6.5-8.0 x 5.5-7.0 μm, smooth. Cheilocystidia as pseudocystidia, consisting of chains of up to 15-20 clamped cells, in all up to 1500 μm long, most elements thick-walled and brown, elements usually less than 70 μm long and 15 μm thick. Clamp-connections present. **Inocybe bipindensis**

1.b. Pileus and stipe not squarrose throughout; lamellae without dark brown, serrate edge → 2

2.a. Pleurocystidia absent. Cheilocystidia present, thin-walled. Spores smooth → 3

2.b. Pleurocystidia present, usually thick-walled. Cheilocystidia present, thick-walled, mixed with thin-walled paracystidia. Spores nodulose → 4

3.a. Pileus in centre recurvately squamulose, towards margin appressedly squamulose, not radially rimulose, to 8 mm. Lamellae narrowly adnate, clay-brown, with white-flocculose edge. Stipe to 13 x 1 mm, whitish, on damage brownish, not pruinose. Smell absent. Spores (7.0-)8.0-9.0 x 5.0-6.0 μm. Cheilocystidia (22-)23-31(-32) x 11-14(-16) μm, clavate to very broadly utriform. **Inocybe sp. 1**

3.b. Pileus smooth in centre, radially rimose outwards. Spores 7.0-8.0 x 5.0-5.5 μm. Cheilocystidia 23-40(-41) x 10-16 μm, (broadly) utriform. Further data unknown. **Inocybe sp. 2**

4.a. Stipe with white marginate bulb; stipe pruinose throughout, but not well visible in lower half→ 5

4.b. Stipe equal to slightly swollen, not pruinose in upper part → 6

5.a. Spores 7.5-9.0 x (6.0-)6.5-8.0 μm, conspicuously nodulose with 9-13 nodules. Pileus 17 mm, applanate, with low umbo, in centre very dark brown, in outer half yellow-brown, tomentose in centre, outwards recurvately squamulose, at margin indistinctly radially rimulose, without velipellis. Lamellae thin, crowded, narrowly adnate to almost free, greyish brown to olivaceous

brown, with minutely fimbriate whitish edge. Stipe 36 x 3 mm (bulb 5 mm), brownish in greater part, blackish brown at base. Smell absent. Pleurocystidia (34-)38-60 x 14-21 μm, ventricose-clavate to broadly fusiform, ± sessile, very thick-walled, with up to 3.0-4.0 μm thick, almost hyaline to slightly yellowish wall, at apex not crystalliferous. Stipe with caulocystidia throughout. **Inocybe sp. 3**

5.b. Spores 6.0-6.5 x 5.5-6.0 μm, angular to minimally subnodulose, but nodules impossible to count. Pileus 24 mm, convex, slghtly umbonate, reddish brown, radially rimose, withour velar remains, slightly tacky. Stipe 34 x 3 mm, bulbous (-4 mm), but not distinctly so, bulb white, elsewhere concolorous with pileus or slightly paler, pruinose over greater length. Lamellae thin, crowded, narrowly adnate, somewhat greyish buff. Context whitish in pileus, reddish brown in stipe, white in bulb. Smell somewhat as artificial honey. Pleurocystidia 35-45 x 14-16(-17) μm, cylindrico-clavate to slenderly clavate, ± sessile, thick-walled, with up to 2.0(-3.0) μm thick, almost colourless wall, with apex only slightly or not crystalliferous. Stipe with caulocystidia and cauloparacystidia throughout. **Inocybe sp. 4**

6.a. Pileus tomentose-smooth → 7

6.b. Pileus recurvately squarrose in centre, outwards tomentose, at margin not distinctly rimulose, reddish brown with slightly darker scales in centre, to 20 mm. Lamellae thin, subdistant, narrowly adnate, brown, with white flocculose edge. Stipe 32 x 2 mm, equal, concolorous with pileus, irregularly fibrillose, near apex more subsquamulose, not pruinose. Smell slightly dust-like. Spores 10.0-11.5(-13.5) x 8.5-10.0 μm, (strongly) nodulose, with 11-14 nodules that are easy to count, with apical nodule sometimes prolonged. Pleurocystidia (62-)63-79(-87) x 12-18 μm, lageniform or sublageniform-subcylindrical, thin-walled, without apical crystals, often with brownish yellow contents. Cheilocystidia similar to pleurocystidia. Caulocystidia absent. **Inocybe sp. 5**

7.a. Pileus very conspicuously radially rimose, blackish brown, to 40 mm, applanate, with a prominent umbo. Lamellae thin, crowded, narrowly adnate, pale buff, with almost even, concolorous edge. Stipe 52 x 4 mm, equal, buff in apical part, darkening downwards and in lower half blackish brown throughout, not pruinose. Smell faint, slightly acidulous. Spores (8.5-)9.0-10.0 x 7.0-8.0 μm, subnodulose, with 9-12 nodules, usually rather difficult to count. Pleurocystidia (59-)63-73(-74) x (15-)16-19(-20) μm, usually subfusiform, with up to 3.0 μm thick, almost hyaline to slightly yellowish wall, at apex not crystalliferous, but with some slimy deposits that give apex a swollen appearance. Cheilocystidia similar to pleurocystidia. Caulocystidia not observed. **Inocybe sp. 6**

7.b. Pileus smooth, not rimulose, pale orange-brown. Lamellae brown with concolorous edge. Stipe concolorous, slightly pruinose in upper part. Spores 7.0-8.0(-9.0) x 6.0-7.0 μm, subdistinctly nodulose, with 7-10 nodules that are ± difficult to count. Pleurocystidia (50-)51-60(-61) x 19-22(-23) μm, scarce, usually close to lamella edge, broadly sublageniform or subfusiform, sessile or shortly pedicellate, thick-walled, with up to 2.0 μm thick, yellow(ish) wall, at apex not crystalliferous. Caulocystidia not observed. **Inocybe sp. 7**

## KEY TO CAMEROONIAN SPECIES OF LACTARIUS

1.a. Latex watery-whitish to white, distinctly darkening on exposure to brownish violaceous, sometimes finnaly even blackening → 2

1.b. Latex whitish (sometimes scarce), not discolouring → 3

2.a. Pileus large. Latex whitish, rapidly becoming violaceous, finally strongly blackening **Lactarius denigricans**

2.b. Pileus small, 11 mm, applanate with distinctly involute margin, concentrically zoned, reddish brown in centre, at margin buff, minutely hairy-squamulose under lens. Lamellae thin, crowded, decurrent, whitish, then buff. Stipe 26 x 2 mm, equal, cylindrical, covered with appressed reddish brown squamules all over (as pileus) on buff background. Milk watery-milky white, brownish violaceous on exposure. Smell absent. Spores 7.0-8.5 x 5.5-7.0 μm, ornamentation to 1.5 μm high, forming a rather incomplete to fairly complete reticulum. Pileipellis a hymeniderm, consisting of chains of cells, with terminal cell pyriform, to 12-15 μm broad, with brown inracellular pigment.
**Lactarius acutus**

3.a. Pileus very small, to 10 mm diameter, under lens conspicuously wrinkled, very finely hairy on wrinkles, distinctly striate-crenulate, brown buff in centre, paler outwards. Lamellae thin, slightly distant, decurrent, buff. Stipe 10 x 1 mm, equal, pale yellow buff. Milk slightly watery. Spores 7.0-8.5 x 6.0-7.0 μm, ornamentation consisting of a reticulum that is less than 0.5 μm high.
**Lactarius pumilus**

3.b. Pileus larger, 30-110 mm → 4

4.a. Pileipellis with thick-walled hairs; pileus minutely hairy under lens → 5
4.b. Pileus without thick-walled hairs → 9

5.a. Stipe at (extreme) apex with an appressed white patch (remains of veil), elsewhere smooth, 40 x 10 mm, tapering downwards, ochraceous buff. Pileus 65 mm, infundibuliform, distinctly transversely wrinkled, ochraceous, at margin with some white patches (remains of veil). Lamellae thin, very crowded, decurrent, pale ochraceous yellow. Context whitish. Milk very sparse, white. Smell slightly fruity. Spores 6.5-8.0 x 6.0-6.5 μm, ornamentation consisting of warts, up to 1.0 μm high that form a very incomplete reticulum. Pileipellis with abundant, thick-walled hyaline hairs that are partly furcate. **Lactarius annulatoangustifolius**
5.b. Stipe and pileus without remains of veil → 6

6.a. Lamellae thick, (very) distant. Pileus margin not grooved-sulcate. Pileus very conspicuously reticulately wrinkled, to 110 mm, ochraceous yellow, orange yellow to yellow-brown, pallescent on drying to yellow. Lamellae deeply decurrent, extending as longitudinal veins on stipe, whitish buff to pale yellowish, with age buff to finally brown. Stipe to 60 x 15 mm, cylindrical, somewhat venose at apex because of extending lamellae, concolorous with pileus. Context brittle, white, browning in exsiccate. Latex white, browning with age. Smell indistinct to slightly disagreeable. Taste mild, fungoid. Spores 6.5-8.0 x 5.5-6.5 μm, with ornamentation to 1.0 μm high, consisting of irregular warts and fine connective lines, forming an incomplete reticulum. Pileipellis a hymeniderm, consisting of erect hyphae, up to 15(-18) μm broad with thick (to 3.0 μm) yellow wall. **Lactarius gymnocarpus**
6.b. Lamellae thin, crowded. Pileus margin grooved-sulcate or costate → 7

7.a. Spores 8.0-9.5 x 8.0-9.5 μm, zebroid-winged, with ornamentation up to 2.0 μm high. Pileus 30 mm, infundibuliform, umbonate in centre, wrinkled all over, dark yellowish brown. Stipe to 40 x 4 mm, equal, smooth, dark brown. Lamellae decurrent, café-au-ait. Latex not observed (material old). Smell indistinct. Pileipellis a trichoderm of slightly thick-walled hyphae with brown intracellular pigment. **Lactarius pulchrispermus**
7.b. Spores not zebroid-winged, with amyloid ornamentation less than 1.0 μm high → 8

8.a. Surface of pileus not reticulately wrinkled, 60 mm, smooth to slightly hairy under lens, orange-ochraceous. Lamellae subdecurrent, pale yellowish orange. Stipe 28 x 8 mm, attenuate towards base, concolorous to pileus but much paler. Latex scarce, white. Smell indistinct. Spores 7.0-9.0 x 5.5-7.0 μm, with ornamentation consisting of irregularly located warts (up to 0.4 μm high),

connected with fine lines but not forming a complete reticulum. Pileipellis a weakly developed trichoderm, consisting of erect thick-walled hyphae, up to 6.0 μm broad, mixed with ascending to almost erect thin-walled elements. **Lactarius sesemotani**

8.b. Surface of pileus, especially in centre, conspicuously reticulately wrinkled. Pileus to 90 mm, deeply infundibuliform, orange to brownish yellow. Lamellae very crowded, partly furcate, decurrent, pale yellow, with sparse white latex, not discolouring. Stipe 35 x 12 mm, equal, smooth, concolorous with pileus, at apex whitish. Smell weak to strong, fruit-like. Spores 6.0-8.0 x 6.0-7.5 μm, with ornamentation consisting of very low (< 0.2 μm high), isolated warts that are not or exceptionally interconnected. Pileipellis with thick-walled hairs, up to 250(-300) μm long, often repeatedly branched. **Lactarius medusae**

9.a. Spores winged. Pileus 41 mm, infundibuliform, furrowed-wrinkled at margin, very dark brown with reddish flush, smooth. Lamellae thin, crowded, decurrent, greyish buff, with even, brownish edge. Stipe 31 x 6 mm, attenuate, concolorous with pileus, smooth. Context brownish. Latex not observed. Smell somewhat fungoid. Spores 7.0-9.0 x 7.0-9.0 μm, with ornamentation consisting of up to 2.0 μm high, slightly amyloid spines that form an incomplete reticulum, embedded in an inamyloid net. Pileipellis a trichoderm consisting of long and slender elements with brownish, intracellular pigment. **Lactarius undulatus**

9.b. Spores verruculose, sometimes with some interconnections, not winged → 10

10.a. Pileus to 80 mm, applanate with slightly depressed centre or infundibuliform, with margin slightly involute, slightly grooved at margin, distinctly wrinkled especially in central part, orange sienna to reddish-tinged brown in centre, towards margin pinkish buff. Lamellae thin to slightly thickish, crowded to subdistant, decurrent, pale cream, orange-ochraceous or pale orange, with (watery-)milky-white latex, not discolouring on exposure, or with brownish spots. Stipe to 40 x 9 mm, equal, smooth, concolorous with pileus or slightly paler. Smell indistinct-fungoid to slightly fruity. Spores 6.5-9.0 x 5.5-6.5 μm, with ornamentation consisting of scattered low warts and lines (up to 0.5 μm high, often lower), interconnected to form a rather incomplete, but fairly dense reticulum. Pileipellis consisting of isodiametrical to more fusiform, hyaline elements, without thick-walled hairs. **Lactarius kivuensis**

10.b. Pileus 90 mm, infundibuliform, minutely aeriferous-subtomentose throughout, pale orange-ochraceous. Lamellae thickish, distant, decurrent, very pale orange buff, with white latex, not discolouring on exposure. Stipe to 35 x 17 mm, equal, smooth, concolorous with pileus. Smell sweet, fruity. Spores 6.0-7.5(-8.0) x 6.0-7.5 μm, with ornamentation consisting of low (< 0.5 μm high), partly interconnected warts, not forming a complete reticulum. Pileipellis with abundant dermatocystidia, at apex submoniliform or capitulate. **Lactarius claricolor**

## KEY TO CAMEROONIAN SPECIES OF RUSSULA

1.a. Lamellulae abundant → 2

1.b. Lamellulae absent or scarce (less frequent than lamellae) → 5

2.a. Pileus to 30 mm, brown, diffracting, under lens with globose cells visible → R. diffusa

2.b. Pileus larger, to 80-250 mm→ 3

3.a. Pileus, stipe, and lamellae blackening on age → 4

3.b. Pileus, stipe, and lamellae not blackening on age. Pileus to 80 mm, very pale buff, at margin slightly crenulate-striate. Stipe to 35 x 17 mm, whitish, with blue-green sheen. Lamellae white. Smell slightly fish-like. Spores 7.0-8.0 x 6.5-7.5 μm, ornamentation consisting of up to 1.0 μm

high conical projections that are partly interconnected (but in some spores hardly reticulate). Hymenial cystidia abundant, with oleiferous contents. **Russula albospissa**

4.a. Pileus to 250 mm, with surface cracking areolate, grey-brown, finally blackish. Stipe black. Lamellae thick, very distant, decurrent, buff, with black spots. Context white, reddening, then blackening. Spores 8.0-9.5 x 7.0-8.0 µm, ornamentation consisting of up to 0.5(-1.0) µm high, ± complete reticulum. Hymenial cystidia frequent, moniliform. **Russula areolata**

4.b. Pileus without areolate surface. Pileus grey-brown to blackish, minutely tomentose. Stipe blackish with whitish apex. Lamellae thick, subdistant, cream, staining dark grey. Context white, reddening, finally blackening. Hymenial cystidia very abundant, cylindrical, filled with distinct pigment in clods or crystals. **Russula afronigricans**

5.a. Stipe with ring (or pileus margin with remains of ring) → 6

5.b. No remains of ring at stipe or margin of pileus → 11

6.a. Pileocystidia present. Context very thin → 7

6.b. Pileocystidia absent. Context thickish. Pileus red, to 45 mm, at margin distinctly striate, minutely scurfy-hairy under lens. Stipe to 23 x 6 mm, equal, grey-brown. Lamellae thickish, decurrent, white. Smell absent. Spores 7.0-8.0 x 6.5-7.5 µm, ornamentation consisting of up to 1.5(-2.0) µm high spines, interconnected with low lines. Pileipellis articulate, terminal elements clavate and slightly thick-walled. **Russula annulatobadia**

7.a. Ornamentation of spores with isolated verruculae, 0.5-1.0 µm high. Pileus small, less than 10 mm. On branches and leaves. Pileus greyish pink. Stipe white. Lamellae white. Spores 6.5-8.0 x 6.5-7.5 µm. Pileocystidia abundant. Pileipellis consisting of chains of broadly ellipsoid elements, with fusiform-subulate terminal element. **Russula mimetica**

7.b. Ornamentation of spores consisting of an almost complete reticulum. Pileus larger. Terrestrial → 8

8.a. Pileus ochraceous yellow, brownish yellow to yellow-brown, to 50 mm, distinctly striate and slightly tuberculate at margin. Stipe to 45 x 7 mm, equal, white, with yellow to brown ring. Lamellae thin, (very) crowded, adnate to subdecurrent, white, with white edge. Smell indistinct. Spores 7.5-11.0 x 7.5-11.0 µm, with ornamentation consisting of 1.5-2.5(-3.0) µm high spines that form a ± complete reticulum. Pileipellis with rather inconspicuous chains of ellipsoid to (sub)globose cells, often becoming smaller towards upper cell and at apex partly capitulate, but sometimes upper cell ± swollen. **Russula yaeneroensis**

8.b. Pileus pink, red or violaceous → 9

9.a. Pileus (deep) violaceous, shiny, at margin striate, pelliculate, irregularly breaking up in patches. Stipe with violaceous tinge to violaceous. Ring thick, dark violaceous. Lamellae white, with concolorous or violaceous edge. Spores 7.5-9.5 x 7.0-9.0 µm, (sub)globose, ornamentation consisting of up to 1.0-2.0 µm high spines in an incomplete reticuum of lower and narrow ridges. Pileipellis consisting of cylindrical elements, with terminal element cylindrical, slenderly fusiform or more elliptical-subclavate and somewhat inflated. Pileocystidia abundant. **Russula annulata**

9.b. Pileus pinkish red, red to orange-red → 10

10.a. Terminal cells of pileipellis rather short, ellipsoid to somewhat swollen. Pileus 23-30 mm, at margin striate-furrowed, pelliculate, at margin with remains of ring. Stipe 34-37 x 5 mm, equal, fistulose, white. Lamellae thin, crowded, white (with yellowish sheen), with white edge. Smell absent. Spores 7.5-9.0 x (7.0-)7.5-9.0 µm, ornamentation consisting of an almost complete reticulum, 1.0-2.0 µm high. Pileocystidia frequent. **Russula pseudocarmesina**

10.b. Terminal cells of pileipellis long and slender. Pileus pelliculate, pure red, shiny. Stipe equal, fistulose, white. Ring with reddish edge. Lamellae thin, crowded, white, with white to reddish edge. Spores 7.0-8.0 x 7.0-7.5 μm, ornamentation consisting of up to 1.0-1.5 μm high verruculae in an incomplete reticulum. Pileipellis cells with slightly thickened walls. Pileocystidia scarce, inconspicuous. **Russula intricata**

11.a. Lamellae elastic, not brittle → 12
11.b. Lamellae brittle, not elastic → 13

12.a. Pileus olivaceous greenish to green, unicolorous or in centre darker, olivaceous brown, outwards more yellowish green. Pileus 80 mm, irregularly shaped, not to indistinctly striate but slightly wrinkled at margin, smooth, greasy. Stipe 25 x 15 mm, equal, white. Lamellae (very) crowded, often forked at base, white, with white edge. Smell absent. Spores 6.5-8.0 x 6.0-7.5 μm, ornamentation consisting of fairly dense, up to 0.5-1.0(-1.5) μm high, verruculae that are isolated or only slightly interconnected. Pileocystidia very frequent. **Russula striatoviridis**

12.b. Pileus grey to violaceous grey, in centre more distinctly violaceous or purplish. Pileus 60 mm, deeply infundibuliform, at margin wrinkled to indistinctly striate, slightly innately fibrillose, somewhat greasy-viscid. Stipe 43 x 14 mm, slightly swollen below, white, slightly greasy. Lamellae (very) crowded, white. Smell absent to slightly fruity. Spores 5.5-8.0 x 5.0-7.0 μm, ornamentation consisting of (very) low, up to 0.5(-1.0) μm high warts that are mostly isolated but partly interconnected by low lines, sometimes spores hardly ornamented. Pileipellis a (strongly) gelified cutis with scattered to frequent dermatocystidia. **Russula pseudopurpurea**

13.a. Pileus dull, brown-grey to dark brown or black → 14
13.b. Pileus with bright colours → 23

14.a. Pileus covered with brown to blackish, appressed to slightly erect, hairy scales. Stipe with similar covering → 15
14.b. Pileus and stipe without hairy scales. Cheilocystidia and pleurocystidia similar → 16

15.a. Pleurocystidia thin-walled, 40-70 x 9-14 μm; cheilocystidia thin-walled, 90-120 x 6-8 μm, with brown intracellular pigment. Pileus 28 mm, not striate at margin, dark brown to blackish brown. Stipe 24 x 4 mm, pale buff. Lamellae thin, crowded, buff, with blackish edge. Context (dark) brown. Smell absent. Spores 6.5-7.5 x 6.5-7.0(-7.5) μm, ornamentation consisting of up to 1.0(-1.5) μm high spines that are partly to distinctly interconnected in a low, irregular reticulum. **Russula sp. 1**

15.b. Pleurocystidia with thick, yellow wall, 40-60 x 14-17(-20) μm; cheilocystidia similar, strongly staining in Melzer's reagent. Pileus to 40 mm, not striate at margin, blackish on buff background. Stipe 15-40 x 4-8 mm, with similar covering as on pileus on whitish to buff background. Lamellae thin, crowded, with a few lamellulae, pale café-au-lait with black, coarsely flocculose edge. Context sepia greyish. Smell absent. Spores 6.0-9.0 x 5.5-8.0 μm, ornamentation consisting of low (< 0.5 μm) isolated warts that are partly interconnected in a (very) incomplete reticulum. **Russula sp. 2**

16.a. Cheilocystidia conspicuously thick-walled, with up to 4.0 μ thick wall. Pileus 28 mm, slightly infundibuliform, at margin somewhat plicate-furrowed, covered with continuous patch in centre that ruptures towards margin and shows underlying context, covering dark grey-brown. Stipe 25 x 3 mm, fistulose, whitish to buff, minutely pruinose. Lamellae slightly decurrent, whitish, with grey-brown edge. Smell absent. Spores 8.0-10.5 x 8.0-10.0 μm, ornamentation consisting of dense spines, up to 1.5-2.0 μm high, that are interconnected in a low, incomplete reticulum. Pileipellis

with chains of ellipsoid elements with terminal element elongate, with brown intracellular pigment. **Russula lamprocystidiata**

16.b. Thick-walled cheilocystidia absent → 17

17.a. Pileus minutely tomentose; pileipellis with slightly thick-walled hyphae. Pileus very dark brown, at margin not striate-furrowed. Lamellae conspicuously furcate, lamellulae absent, very pale buff with concolorous edge. Stipe somewhat tomentose, concolorous with pileus. Smell and taste indistinct. Spores 7.5-9.0 x 7.5-8.5 µm, ornamentation consisting of 2.0-2.5 µm high, interconnected spines that form an incomplete network. Pileipellis with somewhat thick-walled hyphae. Pileocystidia not observed. **Russula velutina**

17.b. Pileus smooth, not tomentose → 18

18.a. Pileus surface with globose cells (visible under lens); lamella edge white → 19

18.b. Pileus pelliculate, somewhat plicate-furrowed, 25-40 mm, brown to dark brown. Stipe 20-40 x 3-4 mm, brown-grey, smooth to very minutely punctulate. Lamellae thin, crowded, adnate, white, with brownish buff to dark brown edge. Spores 8.0-9.5 x 7.0-9.0 µm, with ornamentation consisting of up to 1.0-2.0 µm high, obtuse to somewhat acute spines that form a ± complete but somewhat irregular reticulum. Pileipellis articulate, with chains of broadly ellipsoid to subglobose cells, but with terminal element lageniform or fusiform. Dermatocystidia in pileipellis frequent to abundant, often occurring in clusters, fusiform to slenderly spathuliform, with yellowish refringent contents. **Russula declinata**

19.a. Pileus diffracting and showing white context in outer part, to 30 mm, cuticle smooth, chocolate-brown, at margin indistinctly striate. Stipe minutely brown-punctate on whitish background. Lamellae crowded, white; lamellulae present. Smell fungoid. Spores 6.5-7.5(-8.0) x 6.5-7.5 µm, ornamentation consisting of up to 1.0 µm high, slender, isolated spines. Pileipellis consisting of slenderly fusiform to lageniform cells, with brown intracellular pigment. **Russula diffusa var. fissurans**

19.b. Pileus not diffracting, at margin not showing white context; lamellulae absent → 20

20.a. Pileus large, to 115 mm. Stipe to 90 x 25 mm, with brownish fibrils or squamules or brown over greater length → 21

20.b. Pileus small, 42 mm. Stipe 35 x 11 mm, white to brown. Pileus with granular-subtomentose surface, not or slightly striate, grey to grey-brown at margin to grey-brown to blackish brown in centre. Lamellae very crowded, not intervenose, white to very pale buff; no lamellulae observed or with a few lamellulae. Context white. Smell and taste indistinct. Spores 6.0-7.5(-8.0) x 5.5-7.0(-7.5) µm, ornamentation consisting of low (< 0.5 µm) fairly dense, isolated warts without interconnections or slightly interconnected. Hymenial cystidia present, at apex rounded or mucronate-appendiculate. Pileipellis consisting of dense chains of short cells. **Russula liberiensis**

21.a. Spore ornamentation very low, usually less than 0.3-0.5 µm high, consisting of isolated, minute verruculae. Spores 6.0-7.5 x 5.0-6.0 µm. Pileus 100 mm, subtomentose-smmoth, very dark brown to blackish brown. Stipe 85 x 20 mm, grey-brown, at base yellow-brown. Lamellae (very) thin, crowded, pale buff. **Russula sp. 3**

21.b. Spore ornamentation higher, to 1.0-1.5 µm high, slightly to distinctly reticulate → 22

22.a. Spores 7.0-8.0 x 6.0-8.0 µm, ornamentation consisting of to 1.0(-1.5) µm high isolated warts, occasionally interconnected with low lines (< 0.5 µm high), but not forming a reticulum. Pileus to 100 mm, smooth, not striate, dark brown to blackish brown. Stipe to 90 x 20 mm, with brownish

fibrils or squamules on pale background. Lamellae very crowded, intervenose near base, white. Hymenial cystidia present, at apex mucronate or moniliform. **Russula cellulata**

22.b. Spores 5.5-6.5 µm, ornamentation consisting of up to 1.0 µm high, ± complete reticulum. Pileus 115 mm, infundibuliform, smooth, not striate, slightly glistening, hazel-brown with yellow streaks. Stipe 45 x 25 mm, equal, solid, concolorous with pileus but white immediately below lamellae. Lamelle very crowded, with a few lamellulae, whitish. **Russula sp. 4**

23.a. Pileus greyish green to green → 24
23.b. Pileus yellow, red or violaceous → 25

24.a. Spores 7.0-8.0 x 5.5-6.0 µm, ornamentation consisting of isolated obtuse cylindrical projections that are 1.0 µm high, without interconnections. Pileus 33 mm, at margin indistinctly crenulate, smooth, shiny. Stipe 27 x 7 mm, white. Lamellae thick, subdistant, subdecurrent, white, with white edge; lamellulae scarce. Pleurocystidia very abundant, with refringent contents, with mucronate apex. **Russula pseudostriatoviridis**

24.b. Spores 7.0-8.0 x 7.0-8.0 µm, ornamentation consisting of up to 2.0 µm high, rather coarse spines that form a low and incomplete net. Pileus greenish, margin somewhat striate. Lamellae white, with greenish edge. Stipe with olive-green tinge. Pileipellis with articulate hyphae, and terminal element broadly ellpitical to cylindrico-sublageniform. **Russula pausiaca**

25.a. Pileus brownish ochraceous, yellow to orange-yellow → 26
25.b. Pileus red or violaceous → 28

26.a. Pileus strongy striate-plicate, to half-way, infundibuliform, with minute appressed scales that become more dense towards the centre, brownish ochraceous, honey colour to more saffron. Lamellae orange-honey-coloured, more distinctly orange on age or with damage. Stipe concolorous with pileus. Smell strongly fruit-like. Spores 7.0-8.0 x 6.5-7.0(-7.5) µm, ornamentation consisting of moderately dense, isolated, acute spines, to 1.0(-1.5) µm high, spines sometimes minimally interconnected. Pileocystidia not observed. Pileipellis trichodermal, with rather long and slender elements, somewhat shortened towards apex, but terminal element rather variable. **Russula aurantiofloccosa**

26.b. Pileus surface somewhat granular-punctate → 27

27.a. Pileus minutely punctulate, to 50 mm, with margin (weakly) brownish gold to golden yellow, somewhat pallescent on drying. Stipe 55 x 6 mm, equal, concolorous with pileus. Lamellae thin, crowded, adnate, white, with flocculose, golden-yellow edge. Context white. Smell absent. Taste mild. Spores 6.0-8.0 x 5.0-7.0 µm, ornamentation consisting of low, to 0.5 µm, warts, partly inter-connected by lower lines, forming an incomplete reticulum, or isolated. Pileipellis distinctly epithelioid, consisting of chains of subglobose to broadly ellipsoid, slightly thick-walled cells. **Russula testaceoaurantiaca**

27.b. Pileus to 20 mm, pale lemon-yellow, on damage becoming beautifully intensely chrome-yellow, with margin striate. Lamellae chrome-yellow. Stipe chrome-yellow. Spores 7.5-9.5 x 7.5-9.0 µm, ornamentation consisting of an incomplete, irregular reticulum up to 2.0(-2.5) µm high. Pileipellis conspicuously epithelioid with globose elements. Pileocystidia present. **Russula discopus**

28.a. Pileus deep violaceous, at margin striate, pelliculate. Stipe with violaceous tinge. Lamellae white. Spores 7.5-9.0(-9.5) x 7.0-9.0 µm, ornamentation consisting of up to 2.0 µm high spines in an incomplete reticuum of lower and narrow ridges → see R. annulata above

28.b. Pileus red → 29

29.a. Stipe grey-brown. Pileocystidia absent → see R. annulatobadia above
29.b. Stipe white or yellow. Pileocystidia present → 30

30.a. Stipe white, sometimes reddish punctulate → 31
30.b. Stipe yellow → 32

31.a. Stipe reddish punctulate, 27 x 3.5 mm, equal. Pileus 24 mm, at margin slightly striate, red, minutely punctulate. Lamellae thin, crowded, white, with concolorous edge. Smell absent. Spores 7.5-9.0(-9.5) x 7.5-9.0 μm, ornamentation consisting of dense, narrow spines, up to 1.5-2.0 μm high, in an almost complete, fine reticulum. Pileocystidia abundant, conspicuous, with granular contents. Pileipellis with terminal element fusiform-lageniform, with obtuse apex; walls slightly thickened. **Russula kivuensis**
31.b. Stipe white, solid. Pileus minutely punctulate-squamulose, red, conspicuously striate-furrowed. Lamellae white, with white edge. Smell and taste indistinct. Spores 6.5-7.5(-8.00 x 6.0-7.0 μm, ornamentation consisting of fairly dense, small isolated spines, 1.0-1.5 μm high, without interconnections. Pileocystidia not observed. Pileipellis articulate, with terminal cell relatively broad, broadly ellipsoid to subglobose. **Russula echinosperma**

32.a. Pileus pinkish red to red, with age with minute yellowish fibrils (and then centre more orange), to 30 mm, with smooth or strongly grooved margin. Stipe to 25 x 3 mm, white in upper part, pinkish-reddish in ower half, yellow on handling, because of minute yellow, glandular dots. Lamellae thin, crowded, adnate, white with concolorous edge. Smell absent or slightly fruity. Spores 6.5-9.0 x 6.0-8.5 μm, ornamentation consisting of 1.0(-2.0) μm high, conical warts or spines that are partially interconnected in an incomplete reticulum, but some spores more heavily ornamented and almost zebroid. Dermatocystidia frequent. Pileipellis almost a hymeniderm with obovate to subulate elements, mixed with slightly to distinctly thick-walled, very long velar hyphae with yellowish-orange pigment. **Russula sp. 5**
32.b. Pileus dark red to purplish, with age paler and in centre mixed with yellow, minutely granulose under lens, 15-20 mm, with striate margin. Stipe lemon- to chrome-yellow, 15-20 x 3-4 mm. Lamellae whitish to (very) pale yellowish, with white edge. Context yellow in stipe cortex, white elsewhere. Smell indistinct. Spores 7.5-9.0 x 7.0-8.0 μm, ornamentation consisting of dense, 1.0-2.0(-2.5) μm high, narrow, isolated spines that are not interconnected. Pileocystidia abundant, cylindrical, slenderly clavate to spathuliform, with yellowish refringent pigment. Hyphae of pileipellis articulate, with broadly elliptical cells, and terminal element slightly swollen.
**Russula fulvoochrascens**

## KEY TO CAMEROONIAN SPECIES OF SCLERODERMA

1.a. Peridium papery, bright ochraceous yellow, smooth, to 30 mm; rhizomorphs and mycorrhizas bright yellow. Associated with *Gnetum*. Spores (exclusive ornamentation) 6.0-7.5 μm, covered with 1.0-1.5 μm long spines that form an incomplete reticulum. **Scleroderma sinnamariense**
1.b. Peridium leathery, yellowish brown; rhizomorphs and mycorrhizas white; associated with Caesalpiniaceae and *Uapaca*. Carpophore stipitate, to 80 mm high, with a (sub)globose fertile part, to 30 x 35 mm, and stipe to 55 x 10 mm. Peridium with low, pyramidal polygonial warts, yellow-brown to brown. Stipe whitish to pale yellow-brown. Spores (exclusive ornamentation) 7.0-8.5 μm, covered with 1.5-2.0 μm high, isolated, hyaline spines. **Scleroderma roseocarneum**

**CHAPTER 4**

# INOCULUM POTENTIAL OF ARBUSCULAR MYCORRHIZAL FUNGI FOLLOWING SHIFTING CULTIVATION AND SELECTIVE LOGGING PRACTICES


## ABSTRACT

*Impacts of land use practices on mycorrhizal inoculum potential (MIP) in tropical rain forests have been little investigated. This study was conducted to determine changes in arbuscular mycorrhizal fungal abundance and infectivity following shifting cultivation and selective logging in southern Cameroon. Spore numbers and mycorrhizal colonisation of bait plants were investigated in late and early successional forest stands, fields of food crops before and after burning, 3-year old* Chromolaena odorata *fallow, and skid trails and landings at three sites with varying elevation, soil texture, and soil acidity characteristics. Both intact soil cores and disturbed (mixed) samples of surface soil were used to assess mycorrhizal colonisation. As a bait plant the timber tree* Distemonanthus benthamianus *was used. Spore numbers and mycorrhizal colonisation in intact and disturbed soil cores were significantly positively correlated, indicating that mainly spores contributed to MIP. Spore density and mycorrhizal colonisation increased after converting forests to agricultural fields or fallow and gradually declined during secondary succession. Spore numbers and mycorrhizal colonisation were much lower in skid trails and landings compared to primary forest. These patterns were similar in the three sites. Increased MIP after converting forest to agricultural lands suggests that these land use practices might still be sustainable, whereas the decreased MIP in part of the logged over forest indicates negative impacts of forestry practices that persist for a considerable amount of time. Soil management for sustainable forestry could require to boost infectivity in part of the logged-over forest by arbuscular mycorrhizal fungal inoculation of nursery soils.*


## INTRODUCTION

Land use practices in the humid tropics have raised a world wide concern, because degradation of rain forests might be followed by the loss of biological and functional diversity. Tropical soil fertility and productivity is to a large extent determined by biological activity since fertilisers are seldom used by small shifting cultivation farmers. Most tropical plant species rely on mycorrhizas, mutualistic symbioses between plants roots and certain fungi, for survival, growth, and reproduction. Many tropical plant species, both trees and major food crops, are obligately dependent on and highly responsive to mycorrhizal fungi in their natural habitats, especially on phosphorus-poor, lowland humid tropical soils (Janos, 1980a, 1996; Sieverding, 1991). In order to increase understanding of mycorrhizal dynamics and their roles in ecosystem productivity and forest regeneration, knowledge on how soil disturbance due to various land uses affects mycorrhizal propagules and activities is imperative.

Janos (1980b, 1996) hypothesised that disturbance of tropical forests could negatively affect mycorrhizal fungi and that depauperate populations of mycorrhizal fungi could constrain subsequent rehabilitation of such disturbed sites. Under that hypothesis changes in

mycorrhizal populations after soil disturbance could be a convenient tool for assessing and predicting impacts of forest use on ecosystem productivity, and thus could become an indicator for measuring the sustainability of land use practices with regard to below-ground ecosystem functioning. Yet, such information has not been included in management plans designed for sustainable use of tropical rain forests, owing to lack of knowledge of the effects of shifting cultivation and logging practices on the abundance and infectivity of indigenous mycorrhizal fungi.

Various attempts have been made to define (mycorrhizal) inoculum potential. Originally inoculum potential has been defined as the fungal energy for growth per unit area of host surface (Garrett, 1956). As such the concept of inoculum potential is not quantifiable and various methods have been proposed to make the concept operational. Hayman (1983) identified two aspects of mycorrhizal inoculum potential (MIP), viz. propagule number and their infective behaviour, i.e. their capacity to become established in roots of host plants giving rise to mycorrhizal colonisation. Thus, MIP can be assessed by enumeration of mycorrhizal propagules (spores, colonised dead root fragments, colonised organic material and networks of mycorrhizal hyphae) or by measurement of the colonisation of host plant roots using bait plants (Brundrett et al., 1996; Liu & Luo, 1994; Tommerup, 1994). Mycorrhizal colonisation can be assessed in situ or under experimental conditions, using intact soil cores or soils that have been mixed. Different methods have their own advantages and problems, and for that reason are not always indicating similar inoculum potentials. Enumeration of propagules is only possible for spores, but this method has various drawbacks, viz. spore longevity is unknown, their viability variable, their production might be fungal species specific, host plant specific, and seasonally fluctuating, and their extraction efficiency size dependent. Assessing mycorrhizal colonisation might be affected by pretreatment of the soil samples (amount of disturbance), the choice of bait plants (root architecture, mycorrhizal responsiveness, mycorrhizal specificity), seasonality in mycelial activity, and by plant limits to mycorrhizal colonisation (Adelman & Morton, 1986; Brundrett, 1991; Habte & Fox, 1989; Sigüenza et al., 1996; Sutton & Barron, 1972).

The nature and abundance of mycorrhizal propagules in soils vary in response to disturbance. In undisturbed forests and under equitable climatic conditions, hyphal networks are considered to be particularly significant as a source of arbuscular mycorrhizal colonisation (Jasper et al., 1989a, 1989b, 1991) while spores of arbuscular mycorrhizal fungi are considered to be the main sources of colonisation in disturbed soils and in seasonal climates where the hyphal network is regularly disrupted (Abbott & Gazey, 1994; Hayman & Stovold, 1979; Janos, 1996). When comparing different land uses such as shifting cultivation and logging, a diversity of methods to assess MIP is therefore necessary.

The objective of our investigation was to study changes in MIP, assessed by spore number and mycorrhizal colonisation, of indigenous AM fungal communities in soils of the rain forest of south Cameroon, following traditional shifting cultivation and selective logging. For practical purposes we will consider practices that lead to a long-term decline in MIP, compared to late successional (undisturbed) forest, as an indicator for the lack of sustainability of below-ground ecosystem functioning.

## MATERIALS AND METHODS

### *Site description and selection*

The study was carried out within the Tropenbos Cameroon Programme (TCP) research area, which is situated in the western portion of the Atlantic Biafrean forest of south Cameroon, lying within the Congo-Guinea refuge. The TCP area is situated between the cities of Lolodorf (3°14'N, 10°44'E) in the North, Adjap-Essawo (3°02'N, 10°52'E) in the East, Akom II (2°48'N, 10°34'E) in the South, and Bipindi (3°04'N, 10°25'E) in the West. The climate is humid tropical with two distinct wet seasons (August-December: heavy rainy season; March-May: short rainy season) and two dry seasons (December-March: long dry season; May-August: short dry season). Rainfall decreases in an easterly direction, with an annual mean of 2836 mm in Kribi to 2096 mm in Lolodorf and 1719 mm in Ebolowa (Waterloo et al., 1997). Average monthly temperatures vary between 23°C and 28°C. Elevation ranges from 50 m a.s.l. near Bipindi to 1057 m a.s.l. in the Bingalanda mountain near Nyangong. The substratum consists of Precambrian metamorphic rocks and old volcanic intrusions. In the southwestern lowlands (50 - 350 m a.s.l.), surface soils are sandy clay loam and moderately acid; between 350 m and 500 m a.s.l., surface soils are highly clayey and strongly acid; above 500 m a.s.l., soils are very highly clayey and very strongly acid (Van Gemerden & Hazeu, 1999).

Slash-and-burn agriculture is the main subsistence activity with plantain (*Musa spp.*), cocoyam (*Xanthosoma sagittifolium*), groundnut (*Arachis hypogaea*), and cassava (*Manihot esculenta*) as major crops. Cocoa (*Theobroma cacao*) is cultivated for cash revenues. The only industrial activity has recently been selective logging for about ten to fifteen years.

In the research area three experimental sites were selected. Location, rainfall data, and soil physico-chemical characteristics are presented in Table 4-1. In each site, undisturbed late-successional forest stands, stages of disturbance caused by shifting cultivation, and stages of disturbance caused by logging were randomly selected. The successional series created by shifting cultivation included early successional forest stands, fields of food crops, and fallow. In late-successional stands both arbuscular mycorrhizal and ectomycorrhizal trees occur, the latter group often occurring in small to large clumps (CHAPTER 2). Sampling was done outside these ectomycorrhizal clumps. Early successional forest stands differ in being very dense due to the abundance of climbers, young saplings, juveniles, and ground vegetation. Agricultural fields are created by slashing the undergrowth vegetation and felling existing trees at the onset of the dry season, then allowing it to dry. Removal of surface debris is done by dragging large branches to field edges and burning slashed and dried vegetation in stacked piles, not over the whole field plot. Planting at the beginning of the wet seasons is done by minimum tillage with short hand hoes. Weeding by hand hoeing occurs a month after planting. Exploitation of a forest plot for subsistence production of food crops lasts two to three years. Thereafter, abandoned plots are rapidly invaded by the exotic weed *Chromolaena odorata* (Asteraceae), which forms monodominant stands that completely cover the soil surface. After fallowing four to six years, fallow plots may be cleared again for cultivation or abandoned. In the latter case the land reverts to early secondary forest. Selective logging involves felling and extraction of logs from late successional stands with wheeled skidders and crawler tractors, in 2500 ha concessions, for one to three years. Thereafter, skid trails and

landings are abandoned. Landings are slowly invaded by the early successional tree *Musanga cecropioides* (Moraceae). Most late successional stands within the research area have been selectively logged at least twice, thus ages of skid trails used in this study vary with site.

**Table 4-1:** Location, elevation, rainfall, soil types, and characteristics of experimental sites.

| Locality | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| Location | 3°03'N,10°28'E | 3°05'N,10°41'E | 2°58'N,10°45'E |
| Elevation (m a.s.l.) | 100 | 440 | 550 |
| Rainfall (mm) * | 1707 | 2019 | 1780 |
| Soil types | Ultisols | Ultisols, Oxisols | Oxisols |
| Clay (%) ** | 10-40 | 40-60 | 60-80 |
| pH (water) | 6.1 | 4.7 | 4.3 |
| Carbon (%) | 1.69 | 2.26 | 2.21 |
| Nitrogen (%) | 0.15 | 0.18 | 0.19 |
| Available P in $H_2O$ (µg/ml soil) | 0.01 | 0.005 | 0.002 |

Notes:
* Annual means of rainfall from 1995 to 1998
** Data derived from Van Gemerden & Hazeu (1999)

*Soil sampling*

In each site two late and four early successional forest stands, eight shifting cultivation fields (four before burning, four after slashing and burning), four 3-year old fallows of *C. odorata*, and eight skid trails and landings without or with *M. cecropioides* were selected. These stands are considered disturbance stages.

From each disturbance stage, intact soil cores and mixed surface soil samples were taken. Intact soil cores were about 2.5 kg cylindrical monoliths. They were collected *in situ*, by dropping a hammer from a constant height (about 10-15 cm) onto a flat steel plate, placed on top of PVC tubes. Tubes were 10 cm diameter and 25 cm long. They were removed intact from soil by digging around the core. Portions (2.5 kg; dry mass basis) of mixed samples without removing roots or sieving were transferred into black plastic bags. There were 3 replicated cores and composite samples for each disturbance stage per site.

Plots in late successional forest were 2-ha large with ten 0.2-ha subplots and five 0.04-ha quadrats per subplot. In each quadrat, ten 0.5 kg surface soil samples (0-20 cm) were taken in a criss cross manner, and bulked into one sub-composite sample. Five sub-composite samples were further bulked into one composite sample per subplot. Hence, ten composite (mixed) samples were collected from each late-successional forest stand. Intact soil cores were taken at the two end- and middle subplots. In early successional forest stands and in places affected by logging practices (skid trails, landings), about one kg surface soil sub-samples were taken every 10 m along a 100 m distance and bulked into one composite sample. Age of skid trails and landings was 1, 3, 6, 10, and 15 years. Trails and landings of the last three age classes had

been colonised by *M. cecropioides* at the time of sampling. Intact soil cores were taken at 0, 50, and 100 m spots from a reference point corresponding to the edge of undisturbed forest. In three years old fallows of *C. odorata* at distances at least 1 km, ten 0.5 kg surface soil samples were taken, in criss cross, from a 100 m² (10 m x 10 m) quadrat. Three intact soil cores were collected from the middle and near edges of a quadrat. Samples were also taken from one and six years old fallows of *C. odorata*. In shifting cultivation fields, ten 0.5 kg surface soil subsamples were collected after clear-felling and burning, and on bare surface soil not burnt, in a criss cross manner, starting from the different corners of the fields, and bulked into one composite sample. Intact soil cores were collected on bare or burnt soil, from the top, middle and low areas of fields.
All soil samples were collected during the dry season in February 1997.

*Spore extraction and enumeration*
Soil samples were air-dried for 6 days on greenhouse benches. Air-dried soil samples were sieved through a 2-mm mesh size to remove coarse particles and big roots. Spore extraction was carried out by a modified wet-sieving method followed by centrifugation (Brundrett et al., 1996; Pacioni, 1994). Triplicate portions of 25 g sieved soil and fine root sub-samples from each composite sample were soaked in 100 ml of tap water and left to stand for five minutes to allow sedimentation of coarse sand. The sample was suspended by stirring briskly for two minutes and then allowed to settle for 30 seconds. The suspension was decanted over a series of three sieves (mesh width respectively 850 µm, 500 µm, and 250 µm). Suspending and decanting were repeated three times with 25 ml of tap water. Then, equal aliquots were transferred by weighing into 120 ml centrifuge tubes, and centrifuged at 2,000 rpm (about 70 x G) for five minutes. Afterwards, the supernatant was discarded and the pellet was resuspended in 20 ml of a local grade sugar solution (480 g sugar in 1,000 ml sterile water), by gently mixing with a glass rod. The suspension was again centrifuged at 2,000 rpm for two minutes. Spores in the supernatant were then poured over a 38µm sieve, and rapidly washed with abundant water to remove sugar. Spores were transferred with a water flow from a wash bottle onto a filter paper fitted in a funnel placed on top of an Erlenmeyer to allow water filtration under gravity. The filter paper with spores was finally placed in a Petri dish.
Spore abundance (total number of spores in 25 g dry soil) was determined by counting all spores with a normal appearance under a compound microscope at 100x magnification. On the basis of differences in spore morphology (colour under transmitted light, shape, size, and wall ornamentation) various taxa were recognised. Arbuscular mycorrhizal fungi were not determined to species.

*Root colonisation*
A local timber species, movingui, *Distemonanthus benthamianus* (Caesalpiniaceae) was used as the bait plant. Movingui is commercially popular, yielding timber classified as yellow light hardwood. In a preliminary bioassay to assess the rate of mycorrhiza formation with time including three other timber species, *Erythrophloeum ivorense* (Caesalpiniaceae), *Lophira alata* (Ochnaceae), and *Piptadeniastrum africanum* (Mimosaceae), root samples of movingui became colonised more rapidly than those of the other test tree species (data not shown).

Seeds of *Distemonanthus* were manually scarified with a nail scissor, sterilised in 70% alcohol for 1 minute, rinsed three times in sterile water, and grown on filter paper for 2 days at room temperature. One pregerminated seed was placed in a black plastic bag or in a PVC tube. The soil did not receive nutrients. Bags and tubes were watered to field capacity by weighing every 3 days (150 ml water/kg dry clayey soil and 200 ml water/kg sandy soil). All soil cores and bags were kept on greenhouse benches in Kribi, Cameroon (2°57'N; 9°55'E). Plants were grown for 12 weeks. At harvest, 0.5 g portions of fine root samples were taken. The proportion of root length colonised by arbuscular mycorrhizal fungi was estimated by the grid-line intersect method after bleaching (immersion for 60 minutes in alkaline $H_2O_2$ solution at room temperature), clearing (10% KOH for 24 hours), subsequent acidifying (1% HCl for 3 minutes), staining in a solution of acid fuchsin for 2 to 3 days, and destaining.

*Statistical analyses*

Statistical analyses were performed using the SPSS package (SPSS Inc., 1993). Data of spore numbers and percent root colonisation were tested for normality and homogeneity of variances using the Levene test. As variances were not homogeneous, spore numbers were log transformed and percent root colonisation arcsin square root transformed before a two-way analysis of variance (ANOVA) was done with disturbance stages and sites as factors. Average means were separated by Duncan's multiple range test. Correlations (Pearson's correlation coefficient) were calculated between spore numbers, root colonisation in intact cores, and root colonisation in mixed samples. A one-way analysis of variance was done to test whether age of skid trails and landings (irrespective of site) affected spore number or root colonisation. A further two-way analysis of variance was done with age of *Chromolaena* fallow (1, 3, and 6 years) and site as factors.

**RESULTS**

Spore numbers, mycorrhizal colonisation of *Distemonanthus* in intact cores, and in mixed samples were all significantly positively correlated (spore number versus colonisation in intact cores: $r = 0.78$, $n = 18$, $p<0.01$; spore number versus colonisation in mixed samples: $r = 0.73$, $n = 18$, $p<0.01$; colonisation of *Distemonanthus* in intact versus mixed samples: $r = 0.90$, $n = 18$, $p<0.01$). The various methods therefore assess the same propagule type(s).

Analysis of variance indicated that spore numbers were significantly affected by disturbance stage and site, whereas the interaction between site and disturbance stage was not significant (Table 4-2). Spore numbers were lowest in skid trails and landings. Compared to the late successional stand logging practices decreased spore numbers by 10-50%. Spore numbers in early successional forests were twice as high as in late successional stands. In the shifting cultivation cycle both *Chromolaena* fallow and fields after burning showed the highest spore abundance, with fields after slashing but before burning having substantially lower spore numbers. Spore abundance in early and late successional forests were similar across sites but spore abundance in disturbed sites in Nyangong was generally higher than in Ebimimbang (Table 4-3).

**Table 4-2:** Two-way ANOVA of site and disturbance stage effects on spore density of arbuscular mycorrhizal fungi.

| Source of variation | Df | F | P |
|---|---|---|---|
| Site | 2 | 5.42 | 0.007 |
| Disturbance stage | 5 | 109.95 | 0.000 |
| Site x Disturbance stage | 10 | 1.57 | 0.137 |

**Table 4-3:** Spore abundance (number/g soil) in soils of different disturbance stages at three different localities. Disturbance stages include primary forest (LS), secondary forest (ES), skid trails and landings (FP), *Chromolaena* fallow (FA), agricultural fields before burning (FI), and agricultural fields after burning (FB). Values followed by different letters are significantly different at $p < 0.05$.

| Disturbance stage | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| LS | $12.1^{de}$ | $11.8^{de}$ | $11.1^{def}$ |
| ES | $21.3^{c}$ | $20.8^{c}$ | $22.0^{c}$ |
| FP | $5.9^{f}$ | $7.8^{ef}$ | $10.0^{ef}$ |
| FA | $32.2^{b}$ | $44.3^{b}$ | $42.8^{b}$ |
| FI | $10.7^{ef}$ | $17.8^{cd}$ | $14.1^{d}$ |
| FB | $34.9^{b}$ | $37.9^{b}$ | $62.9^{a}$ |

Analysis of variance indicated that root colonisation in intact cores was significantly affected by disturbance stage, site, and the interaction between disturbance stage and site (Table 4-4).

**Table 4-4:** Two-way ANOVA of site and disturbance stage effects on mycorrhizal colonisation of *Distemonanthus* in intact cores.

| Source of variation | Df | F | P |
|---|---|---|---|
| Site | 2 | 10.12 | 0.000 |
| Disturbance stage | 5 | 31.55 | 0.000 |
| Site x Disturbance stage | 10 | 7.45 | 0.000 |

Root colonisation of *Distemonanthus* was lowest in agricultural fields in Ebimimbang and in skid trails and landings in Nyangong. On average logging practices reduced colonisation with 30% compared to late successional forest. Late successional sites showed only a slightly lower colonisation than early successional sites. Fallow and fields after burning yielded the highest colonisation, with slashed but unburned fields having substantially lower colonisation (Table 4-5).

Root colonisation in mixed samples was significantly affected by disturbance stage and by the disturbance stage * site interaction, but not by site (Table 4-6). Although colonisation levels of *Distemonanthus* were lower in mixed samples than in intact cores, the same patterns were noted. However, skid trails and landings had the same colonisation as late successional forest, being 30% lower than early successional stands (Table 4-7).

**Table 4-5:** Root colonisation of *Distemonanthus* (%) in intact soil cores of different disturbance stages at three different localities. Disturbance stages include primary forest (LS), secondary forest (ES), skid trails and landings (FP), *Chromolaena* fallow (FA), agricultural fields before burning (FI), and agricultural fields after burning (FB). Values followed by different letters are significantly different at $p < 0.05$.

| Disturbance stage | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| LS | $26^{def}$ | $26^{def}$ | $38^{bcd}$ |
| ES | $24^{def}$ | $34^{bcd}$ | $44^{bc}$ |
| FP | $26^{de}$ | $22^{ef}$ | $11^{g}$ |
| FA | $61^{a}$ | $69^{a}$ | $60^{a}$ |
| FI | $13^{ef}$ | $44^{bc}$ | $25^{de}$ |
| FB | $29^{cde}$ | $63^{a}$ | $55^{ab}$ |

**Table 4-6:** Two-way ANOVA of site and disturbance stage effects on mycorrhizal colonisation of *Distemonanthus* in mixed cores.

| Source of variation | Df | F | P |
|---|---|---|---|
| Site | 2 | 1.61 | 0.209 |
| Disturbance stage | 5 | 21.25 | 0.000 |
| Site x Disturbance stage | 10 | 7.25 | 0.000 |

**Table 4-7:** Root colonisation of *Distemonanthus* (%) in composite soil samples of different disturbance stages at three different localities. Disturbance stages include primary forest (LS), secondary forest (ES), skid trails and landings (FP), *Chromolaena* fallow (FA), agricultural fields before burning (FI), and agricultural fields after burning (FB). Values followed by different letters are significantly different at $p < 0.05$.

| Disturbance stage | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| LS | $18^{de}$ | $12^{def}$ | $18^{de}$ |
| ES | $19^{de}$ | $23^{cd}$ | $26^{bcd}$ |
| FP | $26^{bcd}$ | $14^{de}$ | $8^{f}$ |
| FA | $33^{abc}$ | $43^{a}$ | $43^{a}$ |
| FI | $13^{de}$ | $22^{cd}$ | $18^{de}$ |
| FB | $15^{de}$ | $36^{ab}$ | $42^{a}$ |

Subsequent analyses showed that spore number in skid trails and landings did not increase after invasion by *Musanga* after 6 to 10 years. However, colonisation of *Distemonanthus* indicated recovery after trails and landings were invaded by *Musanga* (Fig. 4-1). Spore abundance in 3-year old *Chromolaena* fallow was higher than in 1-year and 6-year old fallows. Colonisation of bait plants showed the same pattern (Fig. 4-2).

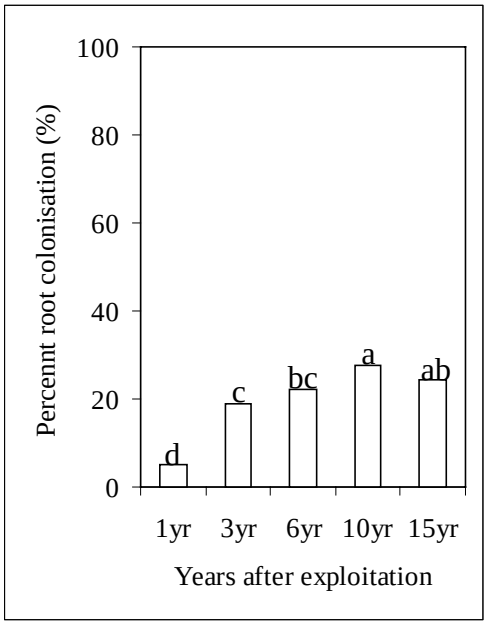
**DISCUSSION**

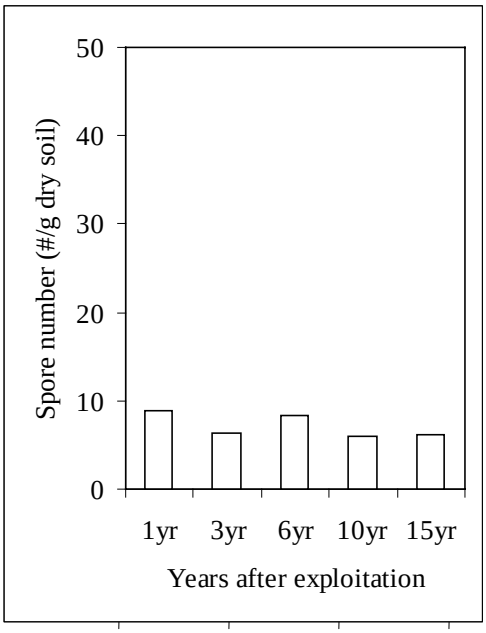
The high correlations between spore numbers and mycorrhizal colonisation of bait plants in both soils demonstrate that mycorrhizal inoculum in the humid zone of south Cameroon is to a

large extent determined by fungal spores. Prevalence of spores as mycorrhizal inoculum could make these sites more resilient against disturbance. The significantly positive correlation between these parameters furthermore indicates that in Cameroonian soils the relative importance of the various propagules does not change very much after disturbance. Whereas Fischer et al. (1994) also established a significant positive correlation between number of viable spores and mycorrhizal colonisation of bait plants, other authors (An et al., 1990; Johnson et al., 1991) did not find a significant correlation, possibly because of differences in the relative importance of the various propagule types in different vegetations. Lack of significant differences in MIP in forest stands across sites is consistent with our observations (CHAPTER 2) that vegetation composition and the relative importance of ectomycorrhiza and arbuscular mycorrhiza are similar even though sites have different soil characteristics.

Average spore densities of 10 spores/g soil in late and 20 spores/g soil in early successional stands are higher than reported by earlier authors. Janos (1992) stated that the data available did not allow an accurate estimate of arbuscular mycorrhizal fungal spore abundance in tropical soils but suggested that the top 10 cm of tropical soils may harbor few spores of these fungi, about 0.5 to 5 spores/g soil. Janos (1996) reiterated that humid tropical soils contain few arbuscular mycorrhizal fungal spores and that therefore many tropical forest soils are not well buffered against large-scale disturbance. However, comparison of spore numbers between sites is problematical, because of the variety of different methods used. Methods that apply wet sieving and decanting without centrifugation would yield high numbers of unviable spores. Fisher et al. (1994) found around 10 empty spores/g soil in Costa Rican secondary rain forest, but less than 0.2 viable spores/g soil were noted in their samples. The mesh size of sieves exerts a large effect on spore extraction efficiency. In a dry tropical forest Johnson & Wedin (1997) noted around 140 spores/g soil, of which 85% belonged to a *Glomus* species with extremely small spores with a diameter of 16-23 μm. Sampling time affects spore number, at least in more seasonal tropical climates. In such climates the onset of the dry season might induce spore formation in arbuscular mycorrhizal fungal species (Sutton & Barron, 1972). Our samples were made during the dry season. Earlier investigations in the semideciduous forests in Cameroon (about 250 km to the northeast of our research site) indicated higher spore numbers in the dry season than in the wet season (Musoko et al., 1994). Sampling depth is a further confounding factor, as in tropical forests most spores occur in the upper 5 cm where the dense root mat is found. Finally, soil drying before spore extraction could affect spore abundance. However, extractive assays of late successional forest soils immediately after sampling yielded similar spore numbers (10-15 spores/g soil).

Spore morphotype richness and diversity were not different between undisturbed forest, early successional forest, and fallow at Ebimimbang and Ebom (Y. Mbarga & N.A. Onguene, unpublished observations). After disturbance a few spore types increased in dominance, consistent with observations by Helgason et al. (1998) in agricultural soils in England.


Percennt root colonisation (%)
100
80
60
40
20
0
d
c
bc
a
ab
1yr
3yr
6yr
10yr
15yr
Years after exploitation



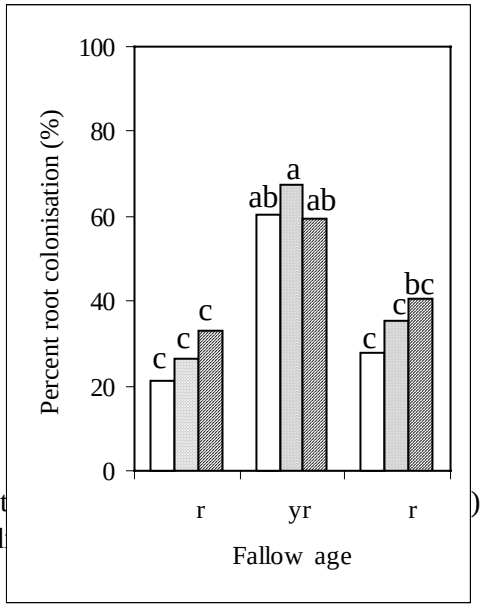




**Fig. 4-1:** Spore abundance (number/g dry soil) and root ) in composite samples in function of age of skid trails and land

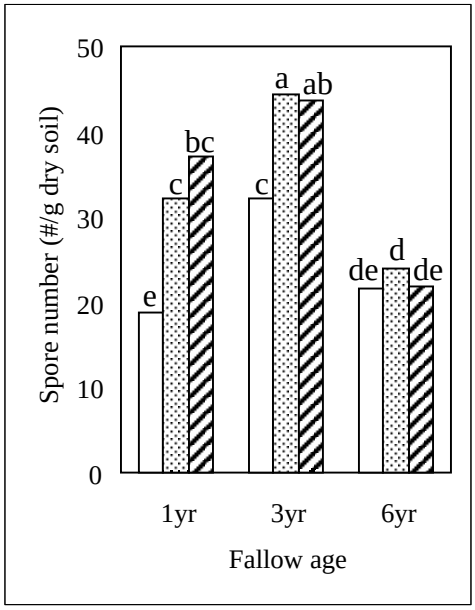



**Fig. 4-2:** Spore abundance (number/g dry soil) and (b) root colonisation of *D. benthamian*us (%) in composite samples in function of age o*f Chromolaena* fallow. (In clear bars = Ebimimbang; Dashed = Ebom; Oblique stripes= Nyangong).

Deforestation could negatively affect spore numbers because viable spores might not persist long enough after disturbance. However, conversion of forest to agricultural land increased spore numbers. These observations are in line with those of Sieverding (1991) who stated that slash-and-burn agriculture has little negative impact on propagules of arbuscular mycorrhizal fungi. High numbers of spores might be caused by higher soil temperatures inducing spore formation after canopy opening. A more important factor might be the short rotation cycles of the plants whereby spores become the dominant propagules as the mycorrhizal network is frequently destroyed by plant senescence and soil disturbance.

Spore abundance and root colonisation of *Distemonanthus* in shifting cultivation fields were generally lower in Ebimimbang than in Nyangong. Aerial photographs have shown that at Ebimimbang shifting cultivation practice has been intensified and fallowing period shortened in the past 20 years more than at the other two sites (Van Gemerden & Hazeu, 1999). The possible connection between agricultural intensification and a decrease in mycorrhizal inoculum deserves further study, as such a decrease could suggest that agricultural practices tend to become less sustainable (Munyanziza et al., 1997). Fallowing maintained the high infectivity of soils as assessed by spore number and root colonisation. The role of the exotic weed *C. odorata* might deserve more attention in this respect as it is possible that vegetative fallowing enhances the availability of nutrients such as phosphorus or potassium through mycorrhizal activity, thus explaining the observation of shifting cultivators that productivity of local peanut (*Arachis hypogaea*) varieties is always increased following vegetative fallowing.

A decline in spore numbers, on the other hand, was found in sites where heavy machinery was used for logging practices. In such sites removal of the mycorrhiza-rich surface soil leads to exposition of inoculum-free subsurface soils. In skid trails and landings spore numbers dropped on average by 30%. Alexander et al. (1992) noted a severe decrease of arbuscular mycorrhizal fungal spores in a Malaysian forest after heavy logging, whereas selective logging somewhat increased spore abundance. Spore numbers in our sites in Cameroon did not show recovery with time, even after skid trails and landings had been invaded by *M. cecropioides*. These data indicate that changes in the soil ecosystem caused by heavy machinery cannot easily be reverted and that the contribution of facultative mycorrhizal trees like *M. cecropioides* to buildup of mycorrhizal inoculum is small. Slow recovery of mycorrhizal inoculum might retard secondary succession. Such retardation of vegetation reestablishment after bulldozer clearing of the topsoil in a Venezuelan savanna was reported by Cuenca & Lovera (1992).

While spore abundance of skid trails and landings did not show signs of recovery over time, root colonisation gradually increased. Establishment of facultative mycorrhizal trees such as *M. cecropioides* might increase recovery rates, but after more than a decade negative effects of intensive logging practices could still be seen. Apparently, the build up of mycorrhizal inoculum in the presence of facultatively mycorrhizal plants might be slow and hence establishment of obligate mycorrhizal trees, if they need a higher inoculum density, might be retarded. This might explain the failure of tree species like *Lophira alata* and the nitrogen-fixing *Pterocarpus soyauxii* (Fabaceae) to establish; although many seedlings of these species were observed on landings, they all died within a few years. Reduction in infectivity in logged-over plots may be due to active removal of topsoil with mycorrhizal propagules by physical action or heavy trafficking and mechanical pressure. In our study area forestry practices led to soil compaction (bulk density in skid trails and landings was about 20% higher than in undisturbed forest). In Ebom about 5% of the forested area was impacted by these forestry practices. However, that site has only recently and sparsely been logged and under normal selective logging practices the area affected by forest roads, skid trails, and landings varies between 10 and 18%. The relative importance of deforestation, soil compaction, and erosion has only seldomly been addressed, but data from Alexander et al. (1992) show that heavy logging decreased MIP with 75%, heavy logging with soil compaction with 90%, and heavy logging with subsequent erosion with 98%. Habte (1989) also noted that erosion drastically reduced mycorrhizal inoculum potential in a tropical oxisol.

The question is relevant whether MIP can be used as an overall indicator of sustainability of below-ground processes. There is no reason to simplistically assume that sites with the highest spore number are managed most sustainably. In fact, there might be different strategies of mycorrhizal fungi in undisturbed systems where the fungi are more K-selected and hyphal networks play a more important role, and in disturbed systems where the fungi are more r-selected and sporulate prolifically. Such a shift in mycorrhizal fungal strategies might explain the increase of spore numbers after conversion of forest to agricultural land. Large spore numbers do not necessarily increase the fitness of the mycorrhizal plant. Asbjornsen & Montagnini (1994) observed that soils with a higher mycorrhizal inoculum potential were not always the most effective in enhancing plant growth. Increased sporulation of arbuscular

mycorrhizal fungi with decreased plant benefits might be a further example of selection for less mutualistic fungi after disturbance (Johnson, 1993).

Janos (1992, 1996) redefined MIP as the capacity of inocula existing at a site to produce mycorrhiza sufficient to affect plant performance (positively or negatively) and assessed MIP as time until a seedling of a mycorrhiza-dependent plant attains a threshold growth rate indicative of having adequate mycorrhizas to affect growth. This definition allows for the possibility that different tree species experience different MIP. Adequacy of MIP should include determination of growth or nutrient uptake of baited plants. These measurements are ongoing to realistically assess the sustainability of the various land use practices (CHAPTERS 7 and 8).

However, our data already provide evidence that logging practices reduce abundance and activities of arbuscular mycorrhizal fungi and that their negative impacts persist for a long time. These negative impacts could not only jeopardise sustainable forest management but could also reduce tree species richness. Sustainable forest management should adopt practices that allow persistence of arbuscular mycorrhizal fungi on sites until new trees become established, by reducing trafficking and multiplication of landings. Managing arbuscular mycorrhizal fungi on a landscape level consists in creating (or maintaining) sufficiently large and connected habitats to sustain viable populations of all species. To achieve this goal, managers should assure a steady supply of readily accessible habitats that produce sufficient mycorrhizal propagules after exploitation before the next harvest cycle. Forest types, successional stages, and unique microhabitats harbouring high levels of arbuscular mycorrhizal fungi need to be identified, mapped, and inventoried. Critical aspects of mycorrhizal ecology with management implications will consist in the initial assessment of mycorrhizal inoculum potential of stands recommended for plantation enrichment with tree species that are poorly naturally regenerating. Mycorrhizal dependency and responsiveness of these trees also need to be determined. If the target enrichment sites include old skid trails and landings, our findings imply that the management strategy may require to boost arbuscular mycorrhizal infectivity by inoculation of nursery soils. Outplanting seedlings that have previously been inoculated in nurseries or shade houses with mycorrhizal inoculum from field-collected soils might be a feasible technique. Species selection of arbuscular mycorrhizal fungi (or the use of native species diversity) would under these conditions be crucial (Herrera et al., 1997).

**CHAPTER 5**

# INOCULUM POTENTIAL OF ECTOMYCORRHIZAL FUNGI IN AN AFRICAN RAIN FOREST IN RELATION TO CONSERVATION AND MANAGEMENT OF ECTOMYCORRHIZAL TREE SPECIES DIVERSITY


## ABSTRACT

*Inoculum potential of ectomycorrhizal fungi was studied in soils from the rain forest area of South Cameroon. Inoculum potential was assessed as fractional ectomycorrhizal colonisation of roots of seedlings of the caesalp species* Tetraberlinia bifoliolata *(characteristic for clumps of ectomycorrhizal trees) and* Afzelia bipindensis *(a species that usually grows solitarily). Both tree species experienced differences in ectomycorrhizal inoculum potential, suggesting ectomycorrhizal specificity.* For T. bifoliolata*, ectomycorrhizal inoculum was absent in soils from skid trails and landings, agricultural fields, and fallow without* Gnetum*. Some ectomycorrhizal inoculum was present in soils from fallow with* Gnetum*. Ectomycorrhizal inoculum was highest in late successional forests within clumps of caesalps. Lack of ectomycorrhizal inoculum in soils from forestry practices indicates that selective logging is no guarantee for the maintenance of ectomycorrhizal trees. For* A. bipindensis *ectomycorrhizal inoculum was highest under conspecific mature trees, somewhat lower in agricultural fields and fallow, and even lower in forests. No ectomycorrhizal inoculum was present in a late successional clump of caesalps. These data indicate that maintenance of late successional ectomycorrhizal clumps depends on ecologically sound forest management and conservation. Maintenance of* Afzelia*, which was consistently dual mycorrhizal, although fractional colonisation by arbuscular mycorrhizal fungi was always low, is apparently less critical. Lack or scarcity of arbuscular mycorrhizal inoculum in ectomycorrhizal clumps indicates that differences in the regeneration niche of ectomycorrhizal and arbuscular mycorrhizal trees contribute to their coexistence.*


## INTRODUCTION

Sustainable functioning of tropical rain forest ecosystems depends on key ecological processes that maintain soil fertility, such as decomposition of organic matter, mineralisation of nutrients, and mycorrhizal activities, which improve access to soil nutrients for almost all terrestrial plants. Such key processes might be altered by deforestation resulting from logging and shifting cultivation practices, if they lead to changes in species composition and the disappearance of species. Decline of species richness of mycorrhizal fungi and a decrease in abundance of mycorrhizal propagules has been linked to changes in above-ground species diversity and altered ecosystem functioning (Janos, 1996; Perry et al., 1989, 1990). Yet, data on the impact of changes in land use on mycorrhizal populations remain scarce.

In the rain forest of tropical western Africa two kinds of mycorrhizal associations occur, viz. arbuscular mycorrhiza and ectomycorrhiza (Alexander, 1989a; CHAPTER 2). Whereas most timber trees form arbuscular mycorrhiza, the ectomycorrhizal habit occurs in a very limited number of plant families, viz. Caesalpiniaceae (a family that is partly arbuscular mycorrhizal) and Uapacaceae. Co-occurrence of both mycorrhizal types has raised the question whether

trees with different kinds of mycorrhiza show niche partitioning. Niche partitioning could occur along various axes. Ectomycorrhizal and arbuscular mycorrhizal trees could show edaphic specialisation. This niche differentiation has been suggested for forests in the Amazonian region, with forests on podzolic, white sands being dominated by ectomycorrhizal trees and forests on brown sands by arbuscular mycorrhizal trees (Singer & Araujo, 1986). Some supportive evidence for this kind of niche differentiation has also been obtained for the rain forest of Korup National Park, Cameroon (Newbery et al., 1988).

However, spatial separation of ectomycorrhizal and arbuscular mycorrhizal trees does not necessarily provide support for a hypothesis on edaphic niche differentiation. Tropical ectomycorrhizal trees often show conspicuous gregarious behaviour in monospecific or at least species-poor stands (Connell & Lowman, 1989) and this habit is well-known from ectomycorrhizal trees from the African rain forest (Letouzey, 1968; Voorhoeve, 1964; Hart, 1995; Newbery et al., 1997; Wieringa, 1999; CHAPTER 2). In such clumps taxa from the Caesalpiniaceae, tribe Amherstieae, constitute the largest contribution to basal area. The occurrence of related tree taxa in these clumps has been earlier noted by tree prospectors in Cameroon and Gabon, where these caesalps are collectively known as ekop and andoung respectively (Letouzey & Mouranche, 1952; De Saint Aubin, 1963). Richards (1996) stated that the gregarious behaviour might be a consequence of the limited dispersal ability of either partner that forms the ectomycorrhizal symbiosis. As both ectomycorrhizal tree and ectomycorrhizal fungus do not possess the capacity to grow and reproduce independently of the other symbiotic partner, mycorrhizal establishment on new sites might be a rare chance event (Janos, 1996). After the ectomycorrhizal symbiosis is initiated, such trees could serve as focal points for establishment of other trees that are compatible with the ectomycorrhizal fungus.

This hypothesis ascribes a large role to initial chance factors, with edaphic differences between ectomycorrhizal and arbuscular mycorrhizas stands being due to the different biochemical capabilities of both fungal groups. A further implication of this hypothesis is that ectomycorrhiwal recovery after large-scale natural (climatic deterioration) and human-induced disturbances (forestry, slash-and-burn agriculture) could be very slow, demanding special management if these ectomycorrhizal stands are to be preserved (Janos, 1996). However, other ectomycorrhizal trees, such as members of the genus *Afzelia* (Caesalpiniaceae, tribe Detarieae), occur patchily in these rain forests in a matrix of arbuscular mycorrhizal trees. Its different behaviour might be related to the fact that *Afzelia* species are consistently dual mycorrhizal (Alexander, 1989b; CHAPTER 2), forming functional relationships with both arbuscular and ectomycorrhizal fungi. Their establishment and initial growth might therefore not be restricted to sites of ectomycorrhizal dominance. It is unclear, however, whether sites with *Afzelia* can equally serve as a focal point for ectomycorrhizal clumping, that being dependent on the extent of host specificity of ectomycorrhizal fungi.

The objectives of this investigation were: (1) to determine the impacts of (selective) logging and shifting cultivation practices on the ectomycorrhizal inoculum potential in south Cameroon; (2) to study changes in inoculum potential of ectomycorrhizal fungi during forest succession; and (3) to relate ectomycorrhizal inoculum potential to growth of seedlings of ectomycorrhizal trees. By comparing an ectomycorrhizal tree species that grows in clumps

and a dual mycorrhizal tree species that grows solitarily, we also intended to assess the potential for sustainably managing the mixed ectomycorrhizal – arbuscular mycorrhizal rain forest. In a separate study, the inoculum potential of arbuscular mycorrhizal fungi as affected by selective logging and shifting cultivation practices has been assessed (CHAPTER 4).

**STUDY SITE**

The study was carried out within the research area of the Tropenbos Cameroon Programme (TCP), a programme directed towards sustainable management and use of tropical forests (Van Gemerden & Hazeu, 1999; Eba'a, 2000). The area is situated in the western portion of the Atlantic Biafrean forest of south Cameroon (Letouzey, 1985). The TCP area covers around 2000 $km^2$. The climate is humid tropical with two distinct wet seasons and two dry seasons. Rainfall decreases in an easterly direction, with around 3000 mm in Kribi to around 1700 mm in Ebolowa. Soil texture ranges from sandy clay loam in the lowlands to very highly clayey in the hilly areas. Along the same gradient pH and phosphorus availability decrease. Intensity of land use and consequently forest vegetation also change from the lowlands in the western parts to the hilly areas and plateaus in the eastern part of the area. In the lowlands only few fragments of undisturbed rain forests remain and a large part of the forest is very degraded; in the hilly areas late secondary and undisturbed forests occur more commonly. Within the TCP area three sites were selected in Ebimimbang (low elevation), Ebom (mid elevation), and Nyangong (high elevation).

Two types of mycorrhizal forest exist: ectomycorrhizal and arbuscular mycorrhizal. In the former stands members of the Caesalpiniaceae and Uapaceae commonly occur in clumps where they tend to dominate the canopy. Surrounding these clumps are old, predominantly arbuscular mycorrhizal stands. These stands sometimes constitute the rotational head of traditional shifting cultivation fields. Late successional and undisturbed forest stands are given out as concessions or "vente de coupe" to logging companies. After exploitation such stands are either colonised by the exotic weed *Chromolaena odorata* (Asteraceae) or the early successional tree *Musanga cecropioides* (Moraceae), after which other early-successional trees re-establish, forming early successional stands. In early successional stands most trees are arbuscular mycorrhizal, but a few isolated ectomycorrhizal trees could be found. Age of undisturbed, late successional and early successional stands could not be accurately determined, due to lack of historical data on land and forest use. However, species composition, stem numbers, and basal area (the latter two parameters being inversely related during succession) can be used to infer their relative age. Data on forest vegetation in the area are provided by Van Gemerden & Hazeu (1999) and data on the mycorrhizal associations of the important tree species in CHAPTER 2.

**METHODS**

*Soil sampling*

In each site, nine 100 $m^2$ (10m x 10m) quadrats were selected in seven vegetation types, viz. ectomycorrhizal forest clumps (EF), late successional forest stands outside the crown projection of ectomycorrhizal clumps (LS), early successional forest stands (ES), agricultural fields of food crops with plantain, cocoyam, groundnut, and cassava as the major crops (FI),

*Chromolaena* fallow (FA), *Chromolaena* fallow with the liana *Gnetum* (GN), and sites of forestry practices (FP), such as skid trails and bare landings.The presence of *Gnetum*, an ectomycorrhizal plant, was considered important as this liana might provide inoculum that facilitates establishment of ectomycorrhizal seedlings. The vegetation types will hereafter be referred to as disturbance stages. Canopy dominance in the clumps varied with site: in Ebimimbang dominants were Ekop species (a collective pilot name for a number of species of tribe Amherstieae), in Ebom *Gilbertiodendron dewevrei*, and in Nyangong *Uapaca* and Ekop species (CHAPTER 2).

In each quadrat soil cores were made in three spots, each 50 m apart. Relatively undisturbed, intact, about 4.2-4.5 kg (wet weight basis) cylindrical soil monoliths were collected by driving a 15 cm diameter x 45 cm long PVC tube into the ground with a hammer dropped from a constant height (10-20 cm) onto a flat steel plate, placed on top of the PVC tubes.

*Mycorrhizal bio-assays*

Two native timber species, both belonging to the Caesalpiniaceae, were used for the bio-assay. *Tetraberlinia bifoliolata* (pilot name Ekop ribi) is an ectomycorrhizal tree (with very occasional arbuscular mycorrhizal structures: Moyersoen & Fitter, 1999) that is valuable as a potential novel timber tree. *Afzelia bipindensis* (pilot name Doussie rouge) is a dual mycorrhizal tree (Newbery et al., 1988; CHAPTER 2) that provides a highly priced timber. Hereafter, the trees will be designated by their generic names only. *Tetraberlinia* occurs usually in clumps together with other Ekop species, while *Afzelia* usually occurs isolated between arbuscular mycorrhizal trees. Both tree species have large pods (10-20 x 5-8 cm) with a small number of large and heavy seeds; average seed size of *Tetraberlinia* is 20-30 x 15-25 x 5-7 mm and of *Afzelia* 30-40 x 20-30 x 10-20 mm; average seed mass of *Tetraberlinia* is 1.5 g (0.8-2.7 g) and of *Afzelia* 11.5 g (6.2-17.4 g). Seedlings of both species possess coarsely branched seedlings with few root hairs.

Seeds were germinated for a week in steam-sterilised sand without pregermination treatment. One 1-week old seedling of each tree was placed in a small hole in the centre of the soil core. Cores were placed on benches and grown under natural light conditions in a greenhouse in Kribi (02°57’N, 09°59’E) in a randomised complete block design, and watered every three days to maintain soils at field capacity. Soil cores did not receive nutrient amendments. Seedlings of *Tetraberlinia* were grown from February 7$^{th}$, 1999 to June 8$^{th}$, 1999; seedlings of *Afzelia* from August 29$^{th}$, 1999 to December 30$^{th}$, 1999.

At harvest, shoots and roots were separated. Shoots were dried at 70° C for 72 hrs and shoot dry weight subsequently determined. Root systems were cleared of soil debris by gently washing under a water flow, immersed in tap water, and observed under a dissecting microscope at 40x magnification. Ectomycorrhizal fractional colonisation was assessed by the gridline intersect method. Afterwards, portions of the root sample of *Afzelia* were stained with acid fuchsin and fractional colonisation by arbuscular mycorrhizal fungi was assessed by the gridline intersect method.

To determine whether ectomycorrhizal and arbuscular fungal propagules share the same niche, a local variety of cowpea (*Vigna unguiculata* (Fabaceae)) was grown in plastic bags in soil cores from ectomycorrhizal clumps. In addition, four soil cores were collected around the

stem base of ectomycorrhizal tree species, at 5 and 10 m distance away from the stem base of *Afzelia bipindensis* and *Brachystegia cynometroides* in Ebom, and *Tetraberlinia bifoliolata* and *Paraberlinia bifoliolata* in Ebimimbang. Cowpea plants were raised for a month in the greenhouse under the same conditions. Afterwards, arbuscular mycorrhizal fractional colonisation was assessed by the gridline intersect method.

*Experimental design and statistical analysis*

For *Tetraberlinia* the experiment was a full factorial with two factors, site (3 levels) and disturbance stage (7 levels). Because of a limited number of seeds of *Afzelia*, a full factorial experiment was not possible. A smaller factorial experiment was executed with soils from 3 sites and 3 disturbance stages (ectomycorrhizal clumps, late successional stands, early successional stands). For the Ebom site, where *Afzelia* is fairly common and widespread, soils from forestry practices, agricultural fields, and fallow were included, but we did not differentiate between fallow with and without *Gnetum*. We also investigated ectomycorrhizal inoculum potential of soils directly under *Afzelia* mother trees (CO).

The SPSS package (SPSS Inc., 1993) was used for statistical analysis. Data were tested first for normality and homogeneity of variances using the Levene test in the one-way analysis of variance (ANOVA). Data on ectomycorrhizal fractional root colonisation by *Tetraberlinia* contained many zeroes and did not meet the requirements of normal distribution and homogeneous variances. Therefore the non-parametric Kruskall-Wallis test was applied. When the analysis was restricted to the three forested disturbance stages (ectomycorrhizal clumps, late successional stands, early successional stands) data of fractional ectomycorrhizal root colonisation, after arcsin square root transformation, did meet the requirements for ANOVA. For *Afzelia*, ectomycorrhizal colonisation in soils from the three forested disturbance stages was very variable, resulting in variances that did not meet the requirement of homogeneity. Again, the non-parametric Kruskall-Wallis test was applied. For the Ebom soils, data did meet the assumptions for ANOVA after arcsin square root transformation. Data on arbuscular mycorrhizal colonisation were also arcsin square root transformed. Shoot dry weight of both species was square root transformed and analysed in the same way. Average means were separated by Duncan's multiple range test. Spearman's rank correlation coefficients between ectomycorrhizal fractional colonisation, arbuscular mycorrhizal fractional colonisation, and shoot dry weight were calculated.

**RESULTS**

*Ectomycorrhizal and arbuscular mycorrhizal colonisation*

Non-parametric analysis of variance indicated that fractional ectomycorrhizal colonisation of *Tetraberlinia* was significantly influenced by disturbance stage ($p < 0.001$), but not by site (p $p > 0.1$). Seedlings grown in soils from sites of forestry practices, agricultural fields and fallow without *Gnetum* remained free of colonisation. Seedlings in soils from fallow with *Gnetum* from all three sites were colonised to some extent. Colonisation was highest in soils from ectomycorrhizal clumps and late successional forests (Fig. 5-1). A two-way analysis of variance for ectomycorrhizal colonisation of the three forest stands (EC, LS, and ES) indicated that both site and disturbance stage were statistically significant, whereas the

interaction was not (Table 5-1). Ectomycorrhizal inoculum increased during succession, with forest clumps showing a significantly higher colonisation than late successional stands, and colonisation being low in early successional stands. Colonisation was highest in soils from Nyangong and lowest in soils from Ebom (Table 5-2).

**Table 5-1:** Two-way analysis of variance of site and disturbance stage on ectomycorrhizal fractional colonisation of seedlings of *Tetraberlinia*.

| Source of variation | Df | F | p |
|---|---|---|---|
| Site | 2 | 52.7 | 0.000 |
| Disturbance stage | 2 | 88.9 | 0.000 |
| Site x Disturbance stage | 4 | 0.4 | 0.836 |

**Table 5-2:** Ectomycorrhizal colonisation (percent root length colonised) of seedlings of *Tetraberlinia* in soils from various forest stands. Forest stands include ectomycorrhizal clumps (FC), late successional forest (LS), and early successional forest (ES). Different letters indicate significant differences according to Duncan's Multiple Range Test at $p < 0.05$.

| Forest type | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| FC | $48^{bc}$ | $32^{cd}$ | $81^{a}$ |
| LS | $28^{d}$ | $12^{e}$ | $52^{b}$ |
| ES | $4^{f}$ | $0^{f}$ | $20^{de}$ |

Non-parametric analysis of variance indicated that ectomycorrhizal colonisation of *Afzelia* in soils from the three forest stands (EC, LS, and ES) was neither significantly influenced by site nor by disturbance stage ($p > 0.1$). No or very little colonisation by ectomycorrhizal fungi was observed in soils from a *Gilbertiodendron* clump in Ebom and in soils from early successional forest from Ebimimbang and Nyangong (Table 5-3). For the Ebom soils only, cores taken under a mature *Afzelia* (CO) resulted in the highest fractional ectomycorrhizal root colonisation. Colonisation was high in sites of agricultural practices (fields, fallow) and declined in soils from later successional stages. In soils from forestry practices and ectomycorrhizal clumps, no ectomycorrhizal colonisation was observed (Fig. 5-2). In the three forested disturbance stages there was no correlation between ectomycorrhizal inoculum potential as assessed by *Tetraberlinia* and *Afzelia* ($r = 0.50$, $n = 9$; $p > 0.1$).

**Table 5-3:** Ectomycorrhizal colonisation (percent root length colonised) of seedlings of *Afzelia* in soils from various forest stands. Forest stands include ectomycorrhizal clumps (FC), late successional forest (LS), and early successional forest (ES).

| Forest type | Ebimimbang | Ebom | Nyangong |
|---|---|---|---|
| FC | 26 | 0 | 13 |
| LS | 22 | 5 | 34 |
| ES | 1 | 11 | 0 |

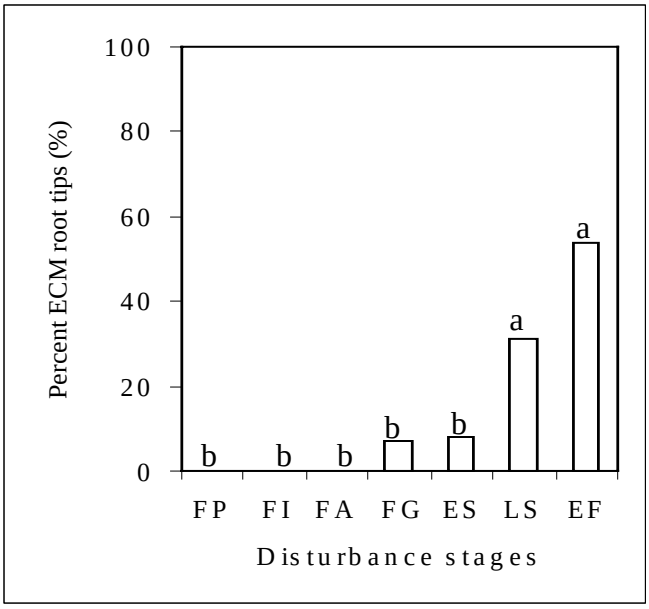



**Fig. 5-1:** Ectomycorrhizal fractional colonisation of seedlings of *Tetraberlinia*, grown in soils from different disturbance stages (average from three sites). Significant differences between disturbance stages (Mann-Whitney U-test; $p < 0.05$) are indicated by different letters. See text for abbreviations of disturbance stages.

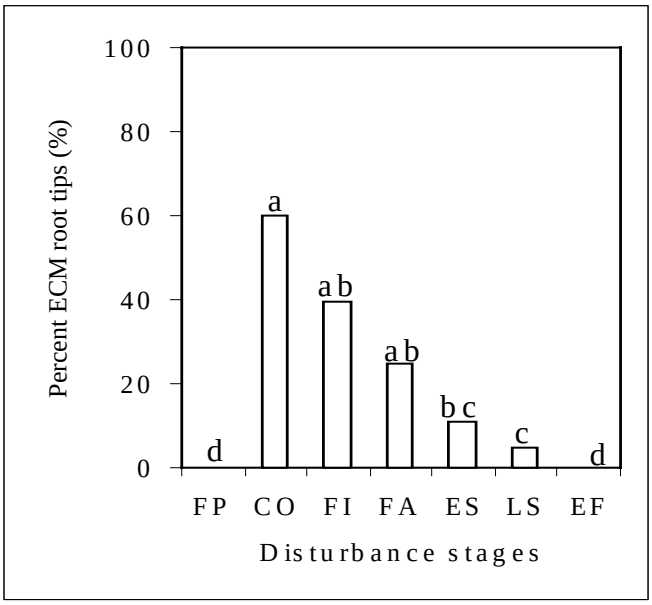



**Fig. 5-2:** Ectomycorrhizal fractional colonisation of seedlings of *Afzelia*, grown in Ebom soils from different disturbance stages. Significant differences between disturbance stages (Duncan's Multiple Range test; $p < 0.05$) are indicated by different letters. See text for abbreviations of disturbance stages.

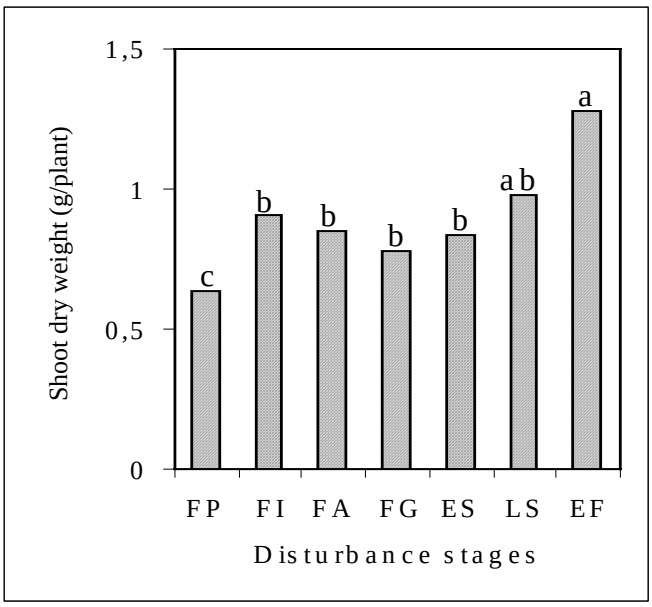



**Fig. 5-3:** Shoot dry weight of seedlings *of Tetraberlinia*, grown in soils from different disturbance stages (average from three sites). Significant differences between disturbance stages (Duncan's Multiple Range test; $p < 0.05$) are indicated by different letters. See text for abbreviations of disturbance stages**.**

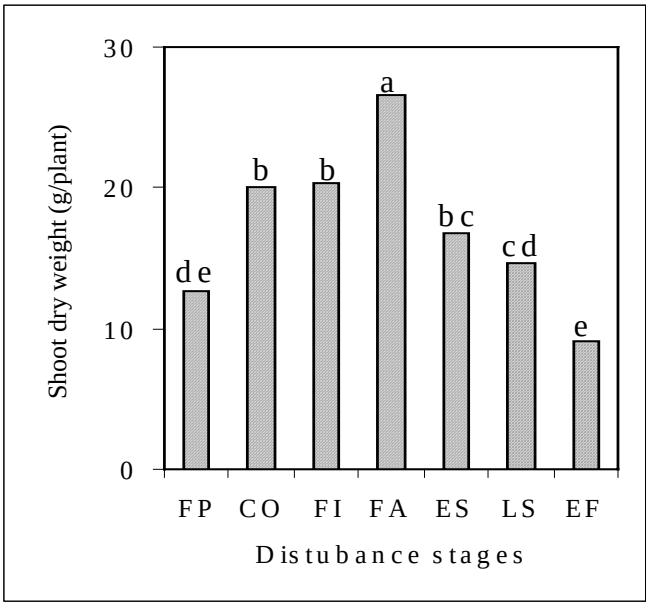



**Fig. 5-4:** Shoot dry weight of seedlings of *Afzelia*, grown in Ebom soils from different disturbance stages. Significant differences between disturbance stages (Duncan's Multiple Range test; $p< 0.05$) are indicated by different letters. See text for abbreviations of disturbance stages.

Fractional arbuscular mycorrhizal colonisation was almost always lower than 5%. Neither site, nor disturbance stage, nor the interaction were significant for the three forest stands (Table 5-4). No colonisation by arbuscular mycorrhizal fungi was observed in soil cores from forestry practices and ectomycorrhizal clumps. Fractional colonisation by ectomycorrhizal and arbuscular mycorrhizal fungi *in Afz*elia roots was neither correlated for the range of disturbance stages of Ebom soils, nor for the three forest stands ($p > 0.1$ in both cases).

**Table 5-4:** Two-way analysis of variance of site and disturbance stage on arbuscular mycorrhizal fractional colonisation of seedlings of *Afzelia*.

| Source of variation | Df | F | p |
|---|---|---|---|
| Site | 2 | 1.87 | 0.183 |
| Disturbance stage | 2 | 0.30 | 0.745 |
| Site x Disturbance stage | 4 | 1.31 | 0.303 |

*Shoot dry weight*

Shoot dry weight o*f Tetraberlinia* seedlings was significantly affected by disturbance stage, site, and the interaction between both factors (Table 5-5). Seedlings growing in soils from ectomycorrhizal clumps and late successional forests outside clumps had largest mass, in soils from forestry practices smallest mass (Fig. 5-3). Seedlings growing in soils from Ebimimbang had largest mass. There was a significant positive correlation between fractional ectomycorrhizal colonisation and seedling dry weight ($r = 0.324$; $n = 63$; $p < 0.01$). Analysis of variance on shoot dry weight restricted to the three forest types yielded similar results: significant effects of site and disturbance stage, whereas the interaction was not significant (data not shown). However, for the three forested disturbance types, fractional ectomycorrhizal colonisation was not correlated with seedling weight ($r = 0.175$, $n = 27$, $p > 0.1$).

**Table 5-5:** Two-way analysis of variance of site and disturbance stage on shoot dry weight of seedlings of *Tetraberlinia*.

| Source of variation | Df | F | p |
|---|---|---|---|
| Site | 2 | 4.36 | 0.019 |
| Disturbance stage | 6 | 4.39 | 0.001 |
| Site x Disturbance stage | 12 | 2.44 | 0.017 |

Shoot dry weight of *Afzelia* grown in soils from the three forest types was significantly affected by site and by the site x disturbance stage interactions, but not by disturbance stage (Table 5-6). However, for the data set from Ebom, shoot dry weight was significantly affected by disturbance stage ($p < 0.001$). Seedlings grown in soils from fallow had the highest biomass, and seedlings grown in soils from forestry practices and from ectomycorrhizal clumps had the smallest biomass (Fig. 5-4). For the Ebom soils, fractional ectomycorrhizal colonisation was significantly positively correlated with seedling dry weight ($r = 0.596$, $n = 21$, $p < 0.01$). However, for the three forested disturbance types, fractional ectomycorrhizal

colonisation was not correlated with seedling weight ($r = 0.163$, $n = 27$, $p > 0.1$). Fractional arbuscular mycorrhizal colonisation was not significantly correlated with seedling dry weight both for the Ebom soils and for the three forested disturbance types ($p > 0.1$).

**Table 5-6:** Two-way analysis of variance of site and disturbance stage on shoot dry weight of seedlings of *Afzelia*.

| Source of variation | Df | F | p |
|---|---|---|---|
| Site | 2 | 13.2 | 0.000 |
| Disturbance stage | 2 | 0.7 | 0.529 |
| Site x Disturbance stage | 4 | 3.3 | 0.036 |

*Inoculum potential of arbuscular mycorrhizal fungi in ectomycorrhizal clumps*

Most soil cores did not produce abundant arbuscular mycorrhizal fungal colonisation on roots of *Vigna unguiculata*. Arbuscular mycorrhizal colonisation was detected in 56% (54 out of 96) soil cores from forest clumps collected in the TCP area. The sparse arbuscular mycorrhizal colonisation varied with sites; it was very low to low in clumps in Ebimimbang and Ebom, and completely absent in Nyangong. No arbuscular mycorrhizal colonisation was observed in soil cores taken 5 m and 10 m away from the stem base of *Afzelia*, *Brachystegia*, and *Paraberlinia*, but arbuscular mycorrhizal colonisation varied from 2.5 to 22.5% around *Tetraberlinia*.

**DISCUSSION**

Various types of propagules, such as spores, sclerotia, hyphal fragments or rhizomorphs, and dying roots, can contribute to the mycorrhizal inoculum potential. It is not clear what the relative importance of these various sources is in our soil cores. Baiting techniques to assess ectomycorrhizal inoculum might yield different results if plants are baited in the field within reach of live mature ectomycorrhizal plants or are baited in the greenhouse. Studies where seedlings were baited in intact vegetation in the field supported the view that the species colonising naturally regenerating seedlings in natural vegetation were similar to that of the ectomycorrhizas of that surrounding vegetation (Jonsson et al., 1999), whereas in the absence of surrounding vegetation of ectomycorrhizal plants a different suite of ectomycorrhizal fungi will be encountered (Taylor & Bruns, 1999). The concept of Late-Stage and Early-Stage ectomycorrhizal fungi (Last et al., 1987) could also be applicable to ectomycorrhizal succession after deforestation in the tropics. Whereas individual tree deaths in late-successional forests would allow colonisation of seedlings via the mycelial network of established fungi, larger-scale deforestation would select for early-stage mycorrhizal fungi that show more efficient spore dispersal. As a consequence baiting in the lab would probably underestimate mycorrhizal inoculum in late-successional forests, but not in more disturbed sites.

Inoculum potential of ectomycorrhizal fungi was very strongly reduced after selective logging. In fact, in soils from forestry practices both seedlings of *Tetraberlinia* and *Afzelia* remained devoid of ectomycorrhizas. Although selective logging at present concentrates on arbuscular mycorrhizal trees (most ectomycorrhizal trees, except *Afzelia* species, are not yet

actually logged for timber purposes, but are considered potential timber species), lack of ectomycorrhizal inoculum on skid trails and landings within the forest suggests that dispersal and survival of ectomycorrhizal propagules is limited. Lack of ectomycorrhizal inoculum after severe disturbances is consistent with suggestions by Boerner et al. (1996), Brundrett et al. (1996a, 1996b) and Janos (1996).

In contrast, agricultural practices negatively affected ectomycorrhizal inoculum potential for *Tetraberlinia*, but not for *Afzelia*. The most likely explanation of differential behaviour of both caesalps is that both taxa differ in their specificity towards ectomycorrhizal fungi. The issue of host plant specificity of ectomycorrhizal fungi has been repeatedly discussed. Smits (1994) emphasised specificity of ectomycorrhizal fungi in dipterocarp forests in Kalimantan (Indonesia) and implied that tropical ectomycorrhizal fungi were different in that respect from ectomycorrhizal fungi from temperate areas. Th. W. Kuyper (unpublished observations) revised the ectomycorrhizal fungi from these Kalimantan forests and concluded that ectomycorrhizal specificity was not different between tropical and temperate forests. Brundrett et al. (1996b) explained some negative results, when assessing ectomycorrhizal inoculum potential by bio-assays, by host specificity of ectomycorrhizal fungi. The hypothesis that *Afzelia* (a member of the almost exclusively arbuscular mycorrhizal tribe Detarieae) and *Tetraberlinia* (a member of the exclusively ectomycorrhizal tribe Amherstieae) do not share many ectomycorrhizal fungi is consistent with observations that *Afzelia* is absent from the late-successional ectomycorrhizal clumps. Thoen & Bâ (1989) observed large differences in ectomycorrhizal fungal species composition under *Afzelia africana* and *Uapaca guineensis*, but felt that it was untimely to attribute these differences to host specificity of the fungi, suggesting that edaphic differences (large physico-chemical differences in the upper soil and humus profile under both trees at the sites where both species co-occurred were observed) might have been responsible for the differences in species composition. No data are presently available about the degree of host specificity of ectomycorrhizal fungi of *Afzelia* compared to those of tribe Amherstieae. It is therefore not clear in how far established trees of *Afzelia* could serve as focal points for regeneration of ectomycorrhizal caesalps that are characteristic for clumps. We did not test whether *Tetraberlinia* would be rapidly colonised by ectomycorrhizal fungi from the rhizosphere of *Afzelia*. If many fungi are compatible with members of Amherstieae and *Afzelia*, the ability of the latter species to become rapidly colonised with ectomycorrhizal fungi suggests that it might be a key-stone species for the maintenance of ectomycorrhizal tree and fungal diversity in Africa's forests. However, lack of ectomycorrhizal colonisation of *Tetraberlinia* in soils from agricultural fields and fallow seems to suggest that the level of compatibility might be too low for *Afzelia* to serve as an ectomycorrhizal stepping stone.

The question remains how *Afzelia* can be so rapidly colonised by ectomycorrhizal fungi and survive as ectomycorrhizal islands in a sea of arbuscular mycorrhizal trees. It has been suggested that in the absence of ectomycorrhizal inoculum, plant species that are dual mycorrhizal could establish and survive with arbuscular mycorrhiza. Under that view, dual mycorrhizal taxa such as *Afzelia* and *Uapaca* species (CHAPTER 2) could link the ectomycorrhizal and arbuscular mycorrhizal tree guilds. Several observations would argue against this view, however. First, arbuscular mycorrhizal colonisation did not improve

seedling biomass, whereas ectomycorrhizal colonisation did result in more heavy seedlings. Second, the amount of arbuscular mycorrhizal colonisation was independent of the amount of ectomycorrhizal colonisation. Third, dual mycorrhiza might be more widespread. Arbuscular mycorrhizas have been observed in a large number of trees of tribe Amherstieae (Moyersoen & Fitter, 1999) and it is possible that all ectomycorrhizal trees have (primitively) retained the ability to become colonised by arbuscular mycorrhizal fungi (Van der Heijden, 2000). Finally, the dual mycorrhizal *Uapaca* does not easily establish in disturbed sites and is most common in the ectomycorrhizal clumps. It is therefore unlikely that the possible key role of *Afzelia* is related to its dual mycorrhizal habit. However, for the Malaysian rain forest tree *Intsia palembanica* (Caesalpiniaceae, Detarieae), Alexander et al. (1992) noted that seedlings with arbuscular mycorrhizas (with colonisation levels between 40% and 60%) grew better than those with ectomycorrhizas (with colonisation levels between 70% and 80%), and in this case the dual mycorrhizal habit might play a key role in its survival.

While maintenance of *Afzelia* seems possible under shifting cultivation, at least if heavy seed predation can be controlled, survival of *Tetraberlinia* is more crucial. Only in fallows with the ectomycorrhizal liana *Gnetum* did seedlings of *Tetraberlinia* become ectomycorrhizal. Whereas the dominant ectomycorrhizal fungus of *Gnetum*, *Scleroderma sinnamariense*, easily recognisable because of its bright yellow ectomycorrhizas and rhizomorphs, has not been observed on seedlings of *Tetraberlinia* in the greenhouse, other ectomycorrhizal species of *Gnetum* apparently show less host specificity. A number of other mycorrhizal types (black, reddish, whitish) have occasionally been encountered and the host specificity of these fungi merits further research. Next to its ecological importance as a potential stepping stone for establishment of ectomycorrhizal trees, *Gnetum* is also a very highly priced agricultural product. Local subsistence farmers, however, are unable to cultivate the plant, possibly due to neglect of its mycorrhizal requirements. Overharvesting of plants for selling on the wealthier markets in the larger towns can lead to a local decline in *Gnetum* abundance, which could ultimately have a long-term negative impact on the maintenance of these ectomycorrhizal caesalps.

Co-occurrence of ectomycorrhizal and arbuscular mycorrhizal trees in tropical rain forests has been differently interpreted. For African rain forests, where ectomycorrhizal clumps occur side by side in an arbuscular mycorrhizal forest, some edaphic specialisation had been suggested by Newbery et al. (1988). Newbery et al. (1997, 1998) noted in Korup National Park, Cameroon higher phosphorus and nitrogen flux rates in a site where ectomycorrhizal trees abounded than in a site where these trees were rare or absent. Moyersoen et al. (1998a), however, observed that the ectomycorrhizal tree *Tetraberlinia moreliana* (actually *T. korupensis*, see Wieringa, 1999) and the arbuscular mycorrhizal tree *Oubanguia alata* were equally efficient in extracting phosphorus from these soils, and Högberg & Alexander (1995) failed to find differences in nitrogen utilisation patterns between trees of both mycorrhizal types in that forest. A subsequent study by Moyersoen et al. (1998b) did not yield spatial separation of ectomycorrhizal and arbuscular mycorrhizal roots. It seems therefore likely that edaphic specialisation is not a major factor in niche differentiation between ectomycorrhizal and arbuscular mycorrhizal trees. The large differences in climate and soils between Korup (with a very strongly seasonal climate and sandy soils) and the eastern part of the TCP area

(with a more equitable climate and very highly clayey soils) with a similar importance of ectomycorrhizal caesalps (to a large extent even the same tree species) also suggest that habitat specialisation of ectomycorrhizal trees is not very important.

Our data provide better support for the hypothesis that ectomycorrhizal and arbuscular mycorrhizal trees differ in their regeneration niche (Grubb, 1977). In a separate study (CHAPTER 4) it was found that inoculum potential of arbuscular mycorrhizal fungi was about twice as high in early successional forest as in late successional forest. In that study the effects of ectomycorrhizal clumps on inoculum potential of arbuscular mycorrhizal fungi had not been addressed. Mycorrhizal colonisation of *Vigna unguiculata* indicated that in ectomycorrhizal clumps the inoculum potential of arbuscular mycorrhizal fungi is very low. Apparently, the build up of ectomycorrhizal inoculum is accompanied by the decline of arbuscular mycorrhizal inoculum. Direct competitive interactions in the soil between ectomycorrhizal and arbuscular mycorrhizal fungi have also been postulated by Moyersoen et al. (1998b). However, in individual plants there was no negative correlation between ectomycorrhizal and arbuscular mycorrhizal fungi, consistent with similar observations by Van der Heijden (2000) on the dual mycorrhizal plant *Salix repens*. Inoculum potential of ectomycorrhizal fungi in forested sites was highest in Nyangong and lowest in Ebom. These data are consistent with the relative contribution of ectomycorrhizal trees to basal area (CHAPTER 2) and to inventories of ectomycorrhizal fungi (CHAPTER 3), where Nyangong was also highest and Ebom lowest.

In the absence of edaphic niche differentiation, physico-chemical differences in soils under ectomycorrhizal and arbuscular mycorrhizal trees are likely due to mycorrhizal effects on soils. However, whereas Newbery et al. (1997) noted higher amounts of plant available nitrogen and phosphorus in ectomycorrhizal clumps compared to sites where ectomycorrhizal trees were rare, our data do not support these observations. Statements made by local subsistence agriculturalists even suggest that soils under ectomycorrhizal trees (e.g. a large clump with *Gilbertiodendron dewevrei* very close to the village of Ebom) are less suitable for growing crops.

As a consequence of differences in the regeneration niche of ectomycorrhizal and arbuscular mycorrhizal trees, maintenance of ectomycorrhizal trees that commonly occur in clumps could be a critical issue. The build up of ectomycorrhizal inoculum o*f Tetraberlin*ia, which occurs gradually, suggests that members of Amherstieae after establishment do serve as focal points for subsequent establishment of related ectomycorrhizal caesalps. Members of this tribe possess a number of attributes that are conducive for clumping. The trees produce pods that explode, resulting in dispersal up to 60 m (Van de Burgt, 1997), although most seeds will fall close to the tree, where survival chances are higher as seedlings might become integrated in the ectomycorrhizal network (Simard et al., 1997a, b; Robinson & Fitter, 1999). The trees also produce large seeds with substantial carbohydrate reserves, which allow rapid colonisation by ectomycorrhizal fungi in the subterranean stage, before any leaves are developed, and long-term survival under low-light conditions (Ernst, 1988; Chidumayo, 1991; Munyanziza, 1994). Consequently, the relation between fractional ectomycorrhizal colonisation and seedling weight would be less straightforward than for arbuscular mycorrhizal trees (CHAPTER 7).

In conclusion, our results demonstrate that if most ectomycorrhizal tree species behave

similarly to *Tetraberlin*ia, conservation of forest patches and clumps where these trees occur is urgently needed. In the framework of sustainable management of tropical rain forests it would be important to assess whether addition of soil with ectomycorrhizal inoculum to sites where selective logging has occurred would increase chances for seedlings of ectomycorrhizal caesalps to become mycorrhizal and hence contribute to the maintenance of the diversity of ectomycorrhizal trees and fungi.

**CHAPTER 6**

# IMPORTANCE OF THE ECTOMYCORRHIZAL NETWORK FOR ECTOMYCORRHIZA FORMATION AND SEEDLING SURVIVAL IN RAIN FORESTS OF SOUTH CAMEROON


## ABSTRACT

*An investigation was undertaken in two sites within the Tropenbos Cameroon Programme in South Cameroon to assess the importance of living roots of adult trees as sources of inoculum for survival, ectomycorrhizal colonisation, and seedling growth. Beneath each of four adult tree species (*Brachystegia cynometroides*,* Afzelia bipindensis, Tetraberlinia bifoliolata*, and* Paraberlinia bifoliolata*) one month-old seedlings of* Paraberlinia bifoliolata*, isolated from or in contact with roots of adult target trees, were transplanted in concentric circles at 5, 10, 15, and 30 m away from the stem base. After 4 and 8 months, survival, ectomycorrhiza formation, and seedling height were measured; plant biomass was determined after 8 months. After 4 months ectomycorrhiza formation was higher in seedlings that were in contact with roots from adult trees than in seedlings that were isolated; however, there was no difference in seedling survival. After 8 months, both seedling survival and ectomycorrhiza formation were higher in seedlings that were in contact with roots of adult trees than in seedlings that were not. Effects of site, tree species, and planting distance were also significant. The fraction of surviving seedlings that were ectomycorrhizal declined monotonically towards the edge of the crown projection. Seedling height and biomass did not yield significant differences due to treatment, possibly because of differences in light conditions. Ectomycorrhiza formation indicated some degree of host-tree selectivity with seedlings of* Afzelia *showing the lowest ectomycorrhizal colonisation. The results are discussed in terms of sustainable management of the ectomycorrhizal component of Cameroon's rain forests.*


## INTRODUCTION

Regeneration and recovery of tropical rain forests depends on the survival and establishment rates of young plants. As almost all trees of the tropical rain forest form mycorrhizas, mutualistic symbioses with root-inhabiting fungi, survival and establishment rates of seedlings depend on the rate with which they become mycorrhizal (Janos, 1980, 1996). The amount and activity of mycorrhizal inoculum that is able to withstand disturbance will then affect recovery rates. An important component of mycorrhizal inoculum is the intact mycorrhizal network (Smith & Read, 1997). In the presence of a mycorrhizal network, by which plants of the same or different species are interlinked, seedling establishment can be enhanced if seedlings become more rapidly colonised. Whether species can be integrated in an existing mycorrhizal network depends on the degree of compatibility between mycorrhizal fungus and mycorrhizal plant. If plants become connected in a mycelial network that is larger than they could maintain on their own, such networks could be highly beneficial (Newman, 1988). It has even been suggested that this rapid and early integration of seedlings into adult mycorrhizal root systems has potential benefits in terms of carbon transfer from donor to receiver plants. Carbon transfer to seedlings of subordinate plants could maintain species diversity (Grime et al., 1986) or enhance seedling survival under mother trees. Yasman (1995) suggested that such

mother trees could effectively function as nurse trees by enabling seedling maintenance under conditions that were below the compensation point light intensity. However, the issue of carbon transport in ecologically significant amounts from donor to receiver plants has remained highly controversial (Robinson & Fitter, 1999).

The rain forest of south Cameroon consists predominantly of arbuscular mycorrhizal trees, but local patches that are dominated by ectomycorrhizal trees occur regularly (Newbery et al., 1988; CHAPTER 2). These patches probably originate due to the very low dispersal capacity of the ectomycorrhizal symbiosis, as the seeds do not possess a dormancy mechanism and the fungal propagules have no capacity to survive saprotrophically. Establishment of seedlings of ectomycorrhizal trees in such patches could be due to the presence of the ectomycorrhizal network in which the seedlings become integrated. However, studies on the functioning of the ectomycorrhizal network have very seldomly been carried out in tropical rain forest areas. Alexander et al. (1992) observed that the dual mycorrhizal tree *Intsia palembanica* (Caesalpiniaceae) became more rapidly mycorrhizal if in direct contact with the roots of mature *Shorea leprosula* (Dipterocarpaceae) than if isolated from mature trees and concluded that early infection of seedlings would be much enhanced if seedlings were in contact with living ectomycorrhizal roots, a point that should be recognised in sustainable forest management programmes. If this phenomenon is widespread in the rhizosphere of trees of rain forests, then it is another argument for conservation of undisturbed forest patches as refugia.

The objective of this investigation was to assess the importance of living roots of adult trees as sources of inoculum for seedling survival and ectomycorrhiza formation in rain forests of south Cameroon. By growing seedlings in concentric circles at increasing distances from the stem base of conspecific or confamilial ectomycorrhizal adult trees, this information would allow to determine the distribution of ectomycorrhizal inoculum around the stem base and the optimum distance to trail logs without disturbing or destroying ectomycorrhizal mycelia and to assess the role of ectomycorrhizal specificity in maintaining these networks.

## MATERIALS AND METHODS

### *Study site*

Two field sites of the Tropenbos Cameroon Programme (TCP) research area were selected in Ebimimbang and Ebom. Both sites differ in soil surface texture, pH, available phosphorus, and abundance and species composition of clumps of ectomycorrhizal trees (Van Gemerden & Hazeu, 1999; CHAPTER 2). Ebimimbang (3°02’N; 10°28’E) is located about 80 km east of Kribi, Cameroon. It is found in the southwest lowlands (80 – 200 m a.s.l.) of the TCP area, with sandy clay loam surface soils. Ebom (3°06’N; 10°44’E) is situated 34 km farther northeast, on a rolling landscape (350 – 500 m a.s.l.). Surface soils are strongly acid and highly clayey. In both sites, forests are under intense human influence, both shifting agriculture and selective logging.

### *Field bioassays*

In the Ebom site, one large specimen of *Afzelia bipindensis* and another of *Brachystegia cynometroides*, and in the Ebimimbang site, one large tree of *Tetraberlinia bifoliolata* and

another of *Paraberlinia bifoliolata* were selected on flat terrain. In this paper the generic names will be used. All tree genera belong to the Caesalpiniaceae; *Brachystegia*, *Paraberlinia*, and *Tetraberlinia* belong to the exclusively ectomycorrhizal tribe Amherstieae, and the dual mycorrhizal tree *Afzelia* (like *Intsia*) belongs to the predominantly arbuscular mycorrhizal tribe Detarieae. The adult trees were found in old successional forest stands, except *Afzelia*, which was located in an early successional forest stand. Around each test tree, four concentric circles were laid out at 5, 10, 15, and 30 m distance away from the stem base. On each circle, 40 one-month old seedlings of *Paraberlinia bifoliolata* were transplanted beneath each target adult tree, either isolated by a 15 cm diameter by 45 cm long, at the bottom open PVC tube or in contact with roots of selected adult trees. Isolated seedlings and those in contact with roots were planted about 50 cm apart. Seedlings were raised from seeds collected under a seed-bearer tree in Ebimimbang, in sterilised washed sea sand in a greenhouse in Kribi, Cameroon (2°57'N; 9°59'E). Before transplanting, all seedlings were checked for the presence of ectomycorrhizal colonisation with a hand lens (10x), and none was colonised. Seedlings were transplanted in the period November 26 to December 2, 1998, which corresponds to the end of the long rainy season. Therefore, from January to April 1999, seedlings were watered twice a week. There were two harvests, on April 7 – 9, 1999 and August 7 – 9, 1999. At each harvest, the survival rate was determined by counting the number of living seedlings. Ten isolated seedlings and ten others in contact with roots of adult trees were harvested. Pairs of seedlings (one in contact with, one isolated from an adult tree) were randomly selected at the start of the experiment. In the field, height was measured from the stem collar to the point of insertion of the youngest fully expanded leaf. The entire root system was separated from the shoots. At the second harvest shoot dry weight was determined after drying the shoots at 70° C for 72 hours. Root samples were washed free of soil debris, placed in a large glass dish in water, and examined for the presence or absence of ectomycorrhizal colonisation of root tips under a dissecting microscope at 40x and under a microscope at 100 – 400x after mounting in water between slides and cover glass and squashing five selected root tips.

*Statistics*

Effects of treatment (seedlings in contact with or isolated from roots of mother trees), sites (Ebimimbang, Ebom), tree species (*Brachystegia*, *Paraberlinia*, *Tetraberlinia*, *Afzelia*), and distance (5, 10, 15, 30 m) were tested with the G-test (Sokal & Rohlf, 1995). The various factors are not independent (different tree species at both sites) and light conditions under the various adult trees might not have been identical.

**RESULTS**

Four months after outplanting there was a significant effect of distance (survival of seedlings at 30 m was much lower), but not of treatment, sites, or tree species. Ectomycorrhiza formation was significantly affected by treatment (seedlings in contact with roots of the adult tree had twice as often ectomycorrhizas as isolated seedlings), tree species (highest under *Brachystegia* and lowest under *Afzelia*), site, and distance (highest at 15 m). The fraction of surviving plants with ectomycorrhiza was also highest at 15 m, viz. 75% (Table 6-1).

**Table 6-1:** Effect of treatment, site, tree species, and distance on survival and ectomycorrhiza formation of seedlings of *Paraberlinia bifoliolata* 4 months after transplantation. *: 0.01<p<0.05; **: 0.001<p<0.01; ***: p < 0.001; n.s.: not significant

| | Survival | Ectomycorrhiza |
|---|---|---|
| Treatment (d.f. = 1) | | |
| contact | 116 | 85 |
| isolated | 129 | 42 |
| G | 0.69 n.s. | 10.80 ** |
| Site (d.f. = 1) | | |
| Ebimimbang | 115 | 52 |
| Ebom | 130 | 75 |
| G | 0.92 n.s. | 4.19 * |
| Tree (d.f. = 3) | | |
| *Paraberlinia* | 60 | 30 |
| *Tetraberlinia* | 55 | 22 |
| *Brachystegia* | 70 | 60 |
| *Afzelia* | 60 | 15 |
| G | 1.90 n.s. | 34.34 *** |
| Distance (d.f. = 3) | | |
| 5 m | 80 | 30 |
| 10 m | 60 | 20 |
| 15 m | 72 | 54 |
| 30 m | 33 | 23 |
| G | 22.72 *** | 20.64 *** |

Eight months after outplanting survival of plants that were in contact with roots of the adult tree was significantly higher than that of plants that were isolated (Table 6-2).

Effects of site (more seedlings survived in Ebom), tree species (lower survival under *Tetraberlinia* and higher survival under *Brachystegia*), and distance (higher survival at 10 m and lower survival at 15 m distance) were also significant. The effects of treatment, sites, tree species, and distance on ectomycorrhiza formation were all significant too. Again, seedlings in contact showed higher ectomycorrhiza formation. Ectomycorrhiza formation was highest under *Brachystegia* and lowest under *Afzelia*, and much higher at 5 and 10 m than at 15 and 30 m. The fraction of surviving plants with mycorrhiza declined strongly with distance. It was 100% at 5 m, 66% at 10 m, 34% at 15 m, and only 11% at 30 m. The fraction of surviving seedlings with ectomycorrhiza was also dependent on the tree species: it was highest under *Brachystegia* (85%) and lowest under *Afzelia* (25%) (Table 6-2).

Surviving seedlings in contact with roots of the adult plant were on average 35% heavier than surviving plants that were isolated in PVC tubes (average plant weight 1.87 ± 0.70 versus 1.39 ± 0.39 gram; T = 5.68; p < 0.001). Surviving plants under *Paraberlinia* and *Tetraberlinia* had on average a larger weight than surviving plants under *Brachystegia* and *Afzelia* (Table 6-3).

**Table 6-2:** Effect of treatment, site, tree species, and distance on survival and ectomycorrhiza formation of seedlings of *Paraberlinia bifoliolata* 8 months after transplantation. *: 0.01<p<0.05; **: 0.001<p<0.01; ***: p < 0.001; n.s.: not significant

| | Survival | Ectomycorrhiza |
|---|---|---|
| Treatment (d.f. = 1) | | |
| contact | 107 | 63 |
| isolated | 59 | 25 |
| G | 14.08 *** | 16.96 *** |
| Site (d.f. = 1) | | |
| Ebimimbang | 69 | 34 |
| Ebom | 83 | 54 |
| G | 4.75 * | 4.59 * |
| Tree (d.f. = 3) | | |
| *Paraberlinia* | 44 | 19 |
| *Tetraberlinia* | 25 | 15 |
| *Brachystegia* | 55 | 40 |
| *Afzelia* | 42 | 14 |
| G | 11.93 ** | 18.11 *** |
| Distance (d.f. = 3) | | |
| 5 m | 35 | 35 |
| 10 m | 58 | 38 |
| 15 m | 29 | 10 |
| 30 m | 44 | 5 |
| G | 12.26 ** | 43.45 *** |

**Table 6-3:** Biomass (g) of surviving seedlings of *Paraberlinia bifoliolata* in contact with or isolated from the root system of different adult tree species after 8 months. *: significant difference (p < 0.05) according to a T-test.

| | Contact | Isolated | Significance |
|---|---|---|---|
| *Paraberlinia* | 2.43 ± 0.81 | 1.52 ± 0.47 | * |
| *Tetraberlinia* | 2.19 ± 0.57 | 1.78 ± 0.08 | * |
| *Brachystegia* | 1.48 ± 0.50 | 1.24 ± 0.32 | * |
| *Afzelia* | 1.59 ± 0.36 | 1.16 ± 0.25 | * |

However, these differences did not depend on whether surviving plants were or were not ectomycorrhizal (data not shown). Due to the large variation in plant height within replicates, there were no significant effects of treatment, site, tree species, and distance (data not shown). At the second harvest the relationship between average seedling height and biomass was also not significant ($r = 0.15$, $n = 20$, $p > 0.05$).

**DISCUSSION**

More seedlings in contact with roots of mother trees survived and formed ectomycorrhizas than those isolated from roots. This effect tended to become stronger in time. Our data

confirm the conclusion of Alexander et al. (1992) about the importance of the ectomycorrhizal network in the tropical rain forest for the regeneration of ectomycorrhizal trees. They noted that with *Intsia palembanica* 6 months after outplanting half of the plants that were isolated from the adult tree had remained without ectomycorrhiza, which is slightly lower than our data (after 4 and 8 months around 65% of seedlings remained uninfected). Whereas in the investigations of Alexander et al. (1992) seedlings and the adult trees belonged to different families, our experiment also allowed an independent evaluation of the importance of host tree specificity. Earlier observations (CHAPTER 5) had shown that trees of *Afzelia* and *Tetraberlinia* experience different ectomycorrhizal inoculum potentials in the same soil, probably as a consequence of host tree selectivity of the ectomycorrhizal fungi. The data reported here are consistent with these earlier observations, as the number of ectomycorrhizal seedlings (and the fraction of surviving seedlings with ectomycorrhiza) were lower for seedlings planted under *Afzelia* than under the three other genera. Surprisingly, the number of ectomycorrhizal seedlings (and the fraction of surviving seedlings with ectomycorrhiza) were not highest under a conspecific tree (*Paraberlinia*) but under a different species that belongs to the same tribe (*Brachystegia*). Differential survival could be due to differences in the light environment under the various trees. We did not measure light availabilities under the mature trees, but the large differences in plant biomass under different trees and at different distances, and the large variation between replicates in the same circle do suggest that light availability could have been an important factor.

Alternatively, lower survival of seedlings under a mature conspecific tree could be related to the Janzen-Connell model of tree spacing. Under this model tree seedling survival is less under parent trees because of host-specific predation and parasitism which results in a disproportionally high mortality under conspecifics (Janzen, 1970; Connell, 1971; Clark & Clark, 1984). Under such a model lower survival under conspecifics is a mechanism that could maintain high tree species diversity. For the time being, we cannot evaluate both alternative explanations. However, we note that both models have different implications for the importance of ectomycorrhizal networks for carbon transport between adult and juvenile plants. If the Janzen-Connell model applies, there is a larger chance that carbon flows to non-conspecific trees than to conspecific seedlings (or even seedlings that are direct progeny of the adult tree), implying that there are larger chances for cheaters (i.e. seedlings that take carbon in the seedling stage without donating carbon when adult).

After 8 months the fraction of tree seedlings that have become ectomycorrhizal declined monotonically with distance from the stem base. Seedling survival on the other hand did not show such a consistent pattern with distance from the stem base (Table 6-2). It is possible that seedling survival depends on the amount of ectomycorrhizal mycelium (being highest close to the tree) on the one hand and on the intensity of competition (both root density and light conditions are apparently more favourable for survival at the edges of the crown projection). Seedling survival and ectomycorrhiza formation at 30 m distance might also have been affected by neighbouring trees. Around these trees, we observed other ectomycorrhizal tree species. Around the adult tree of *Paraberlinia*, a mature *Tetraberlinia* was noted, around the adult *Tetraberlinia* a mature *Uapaca* and a mature *Afzelia*, and around the mature tree of

*Brachystegia* a mature tree of *Berlinia*. These surrounding trees could explain the colonisation of some seedlings at 30 m distance from the stem base.

Smits (1994) outplanted non-mycorrhizal seedlings *of Shorea assamica* at various distances of mature trees of various dipterocarps (both congeneric and of different genera). He noted that survival was independent of distance to the stem base. Surprisingly, after one year none of these surviving plants possessed any ectomycorrhizas. Smits concluded that there must exist a high degree of ectomycorrhizal specificity for certain host trees as seeds of a conspecific tree became very rapidly ectomycorrhizal. Our results, like those of Alexander et al. (1992) do not confirm his observations on host tree specificity. The question of host tree specificity has potentially large practical implications for sustainable management of the various ectomycorrhizal timber species. If host tree specificity is high, regeneration should be carried out under conspecific parent trees. In the case of *Afzelia*, which yields a highly priced timber, this question needs future attention.

Earlier workers observed delays in colonisation of seedlings isolated from roots of parent trees, of for example 5 – 6 months after germination (Alexander et al., 1992). The length of the PVC tubes made it unlikely that colonisation of seedlings that were isolated took place after the seedlings escaped from the bottom of the tube, as most ectomycorrhizas are located in the uppermost 5 cm of the soil. The diameter of our PVC tubes was large enough to keep isolated ECM fungal propagules infective for a period sufficiently long to initiate colonisation of young seedlings. Additionally, fruitbody formation at the end of the rainy season and rain splashes could have contaminated some tubes by introducing fungal propagules. Fleming (1983, 1984) and Simard et al. (1997c) showed that after trenching seedlings isolated from roots acquired different mycorrhizal fungi from those in contact with living roots. In this study the identity of the ectomycorrhizal morphotypes has not been determined, so the question whether seedlings in isolation become colonised by different ectomycorrhizal fungi cannot be addressed.

For dipterocarps Smith (1994) concluded that the root system did not extend much beyond the crown projection. However, Ostertag (1998) warned that the analogy between canopy gaps and root gaps should not be extended too far; an opening overhead does not imply a root gap directly below and vice versa. In general root gaps are not complete holes but more like sites of lower root biomass. However, around solitary ectomycorrhizal trees protection of the ectomycorrhizal inoculum could still be achieved by conserving an area that equals that of the crown projection.

**CHAPTER 7**

# GROWTH RESPONSE OF THREE NATIVE TIMBER SPECIES TO SOILS WITH DIFFERENT ARBUSCULAR MYCORRHIZAL INOCULUM POTENTIALS IN SOUTH CAMEROON. I. INDIGENOUS INOCULUM AND EFFECT OF ADDITION OF GRASS INOCULUM


## ABSTRACT

*After tropical forest disturbance mycorrhizal inoculum could be insufficient and increasing mycorrhizal density through inoculum addition is then crucial for the successful regeneration of deforested lands. Greenhouse bioassays were set up to determine the effectiveness of native arbuscular mycorrhizal fungi in soils from different disturbance stages on the growth of three important timber species,* Terminalia superba, Distemonanthus benthamianus, *and* Entandrophragma utile. *Soils were collected from late and early successional forest stands, fields of food crops, fallow of* Chromolaena odorata, *skid trails, bare soil landings, and landings with the pioneer tree* Musanga cecropioides. *These soils were used to grow seedlings without or with addition of a grass inoculum. The extent to which seedlings responded to indigenous fungal inoculum and inoculum addition varied with tree species and with mycorrhizal inoculum potential. After inoculum addition,* Terminalia *strongly increased root colonisation with a small increase in shoot dry weight and* Distemonanthus *hardly increased root colonisation but showed a strong increase in shoot dry weight.* Entandrophragma *increased both root colonisation and shoot dry weight. Plant biomass was low in soils with low inoculum potential such as late successional stands, skid trails, and both kinds of landings; the mycorrhizal inoculation effect was then large. Plant biomass was high in agricultural fields and fallow; mycorrhizal inoculation effect was sometimes even negative. These data indicate that low inoculum might limit plant reestablishment after disturbance and that mycorrhizal inoculation has a potential for improving seedling establishment on deforested land.*


## INTRODUCTION

The roots of the majority of tree species of tropical rain forests throughout the world harbour arbuscular mycorrhizal fungi (Brundrett, 1991; Janos, 1996). This mutualistic symbiosis between tree root and fungus is the most widespread nutrient absorbing organ in vascular plants. The fungus is completely dependent on this symbiosis, having no capacity for growth and reproduction in the absence of the plant. In many cases, the trees are equally dependent on the symbiosis, being unable to complete their life cycle under normal conditions in the absence of arbuscular mycorrhizal fungi (Gianinazzi-Pearson & Diem, 1982; Janos 1980a, 1996). In the rain forest of south Cameroon the majority of tree species are also arbuscular mycorrhizal. Of 97 tree species investigated, including all major actual and potential timber species, 74 were arbuscular mycorrhizal, with the remaining 23 species being either ectomycorrhizal or dual mycorrhizal (CHAPTER 2).

Land use practices such as logging and shifting cultivation could affect the abundance of arbuscular mycorrhizal fungi and consequently the ability of trees to become established or to

regenerate after disturbance could change. While agricultural practices in general did not have a negative impact on the abundance of arbuscular mycorrhizal fungal propagules, several authors reported a negative effect of logging practices, such as manual and mechanical clearing, creation of forest roads, skid trails, and landings, and erosion and soil compaction (Alexander et al., 1992; Musoko et al., 1994). Similar effects have been noted for the rain forest of south Cameroon, where arbuscular mycorrhizal inoculum potential was higher in agricultural fields, fallows of the exotic weed *Chromolaena odorata* (Asteraceae), and early-successional forests than in the undisturbed, late successional forest, but lower in skid trails and landings compared to undisturbed forest (CHAPTER 4). As arbuscular mycorrhizal inoculum potential was assessed by different methods, these conclusions are likely robust.

However, the relationship between propagule abundance and plant growth response is almost certainly not linear. It has been observed that soils with the highest inoculum potential did not always yield the highest plant response (Asbjornsen & Montagnini, 1994). This could be due to fungal species-specific differences in sporulation and effectiveness or to plant costs rising more sharply than plant benefits with increasing colonisation. It is therefore important to include plant response to different inoculum densities in an assessment of sustainability of land use practices. We determined how soils that differed in arbuscular mycorrhizal inoculum potential due to selective logging and shifting cultivation affected plant performance. As absence or scarcity of mycorrhizal inoculum might impede plant functioning, we also addressed whether addition of mycorrhizal inoculum could improve plant performance. If indeed seedling growth and nutrient uptake could be improved, boosting mycorrhizal inoculum might be a viable option to increase sustainability of forestry practices (Michelsen, 1993; Cuenca et al., 1998; Munro et al., 1999). Expected benefits from mycorrhizal inoculation are likely to depend on both abundance (quantity) and on efficiency (quality) of fungal populations. Therefore, even soils with high propagule numbers are not necessarily non-responsive to arbuscular mycorrhizal inoculation.

The objectives of this investigation were: (1) to determine how soils with varying mycorrhizal inoculum potential after logging and shifting cultivation affect growth of seedlings of three commercially important timber species and (2) to assess tree seedling response in those soils to inoculation with arbuscular mycorrhizal fungi from a grass vegetation.

## MATERIALS AND METHODS

### *Site and vegetation description*

The soils used for the bioassays belonged to the Ebom series. Ebom (3°06'N; 10°41'E) is one of three field sites of the Tropenbos Cameroon Programme (TCP), located 114 km east of Kribi. Elevation ranges from 350 – 500 m a.s.l. Annual rainfall was 2082 mm in 1998, with two maxima in May and October. The soils are derived from Precambrian metamorphic rocks and old volcanic intrusions; they are deep, well-drained (Xanthic Ferralsol) with low to medium organic matter content, moderately heavy clayey, and generally strongly acid (Van Gemerden & Hazeu, 1999).

Seven vegetation types, considered as disturbance stages, were selected, *viz.* late successional forest stands, early successional forest stands, agricultural fields of food crops, fallows of *C. odorata*, 17 months old skid trails, and landings. Two types of landings were included, bare

soil and revegetated ones with *Musanga cecropioides* (Moraceae), an early successional tree species that is facultatively arbuscular mycorrhizal. Late successional stands are floristically the most diverse with an overwhelming majority of adult woody plants. Early successional stands are very dense due to abundance of climbers, young saplings and juveniles, and undergrowth vegetation. Agricultural fields were included as disturbance stage after slashing the undergrowth vegetation, felling the existing trees, removing of surface debris, and burning. Well-vegetated fallows of *C. odorata* (hereafter referred to as fallow) of three to four years old were chosen.

In all vegetation types, indigenous mycorrhizal inoculum was collected from three intact soil cores (surface soil, 0-20 cm) that were bulked in one composite sample. The arbuscular mycorrhizal inoculum used for additional inoculation was a surface soil (0 – 15 cm) with fine roots collected from a 2 $m^2$ (2 m x 1m) pure stand of *Paspalum conjugatum* (Poaceae), hereafter referred to as grass inoculum. The grass inoculum was chosen to test whether an early successional plant, which is commonly observed in heavily disturbed sites, could increase mycorrhizal effectiveness. As the species is abundant, it easily allows collection of sufficient inoculum for practical purposes. Collected inoculum was placed in air-filled polyethylene bags, taken to Kribi, and kept in the greenhouse before use. Data on inoculum potential of the various vegetation types and of the grass sod are presented in Table 7-1.

**Table 7-1:** Inoculum potential of the various vegetation types (disturbance stages) and the grass sod used for inoculum addition.

| Vegetation type | Spore number[1] | Most Probable Number[2] | Root colonisation[3] |
|---|---|---|---|
| Late Successional Forest | 12 | 19 | 18 |
| Agricultural Field | 28 | 29 | 31 |
| Fallow | 44 | 55 | 39 |
| Early Successional Forest | 21 | 16 | 23 |
| Skid trails | 9 | 0 | 16 |
| Bare Landings | 5 | 0 | 3 |
| Landings with *Musanga* | 8 | 6 | 25 |
| *Paspalum* sod | 160 | 253 | ND |

ND: Not Determined

1. Number of spores.$g^{-1}$ dry soil.
2. Number of infective propagules.$g^{-1}$ dry soil. Method according to Porter (1979). Four-fold dilution series with 5 replicates. Bait plant a local variety of Cowpea (*Vigna unguiculata* (Fabaceae)).
3. Root colonisation (% root length colonised) of a local variety of Cowpea in intact soil cores.

*Test timber species*

Three native timber species of national economic importance were chosen. Frake, *Terminalia superba* (Combretaceae), is a light white wood widely used in national regeneration programmes. It ranks third in export from Kribi Port that amounted to 4.4% of logs exported (Anonymus, 1995). Its seeds are flat (12 – 18 mm long and 35 mm broad) across the stuffy,

papery wings. The seedlings have a coarsely branching root system almost devoid of root hairs. Movingui, *Distemonanthus benthamianus* (Caesalpiniaceae), is also a commercially popular light yellow hardwood that ranks fifth in export from Kribi Port that amounted to 3.5% of logs exported (Anonymus, 1995). It is a legume with elliptic (7 – 10 cm long by 3.5 cm broad) pods with up to 5 seeds. The species is non-nodulating and develops a finely branched root system with short segments and narrow intercellular spaces. Sipo, *Entandrophragma utile* (Meliaceae), ranks sixth in export from Kribi Port with 3.4% of logs exported (Anonymus, 1995). It has fruits 17-28 cm long, containing 10 - 12 seeds. In terms of national exports from Cameroon *Terminalia* ranks third, *Distemonanth*us sixth, a*nd Entandrophrag*ma seventh. In this paper the generic names will be used.

Seeds were collected around seed-bearing mother trees. Seeds o*f Terminal*ia a*nd Entandrophrag*ma needed no pregermination treatment and those o*f Distemonanth*us were manually scarified. All seeds were sterilised in 70% alcohol and rinsed three times with sterile water. Pretreated seeds o*f Distemonanth*us were placed on wet filter paper while those o*f Terminal*ia a*nd Entandrophrag*ma were germinated in sterilised washed sea sand. One pregerminated seed was planted per bag. The treatments were arranged on greenhouse benches in a randomised complete block design with three replicates per treatment. The plants were grown without nutrient addition under natural light in a greenhouse in Kribi (02°57'N; 09°55'E), Cameroon. *Terminal*ia seedlings were grown from October 20, 1998 to March 21, 1999, *Distemonanth*us seedlings from November 7, 1998 to April 8, 1999, and *Entandrophrag*ma seedlings from September 26, 1998 to February 27, 1999. Water was added as needed to maintain soils at water holding capacity.

Seedlings were grown in five kg (dry weight basis) portions of fresh soils transferred to black plastic bags. Half of the bags were thoroughly mixed with 50 g portions of fresh grass inoculum.$kg^{-1}$ test soil. Non-inoculated soils received similar amounts of steam sterilised inocum and 40 ml of filtrated inoculum leachings to ensure similar microbial flora (Hetrick et al., 1988). Steam sterilisation was achieved in an oven at 100° C for 1 hour. The sterilised soil was left to stand for five days on greenhouse benches before use.

*Assessment of plant growth and mycorrhizal colonisation and data analysis*

Plant height was measured every month from the stem collar to the point of insertion of the youngest fully expanded leaf. After 5 months plants were harvested, dried at 70° C for 72 hours, and shoot dry weight determined. Fractional arbuscular mycorrhizal colonisation was estimated by scoring under a dissecting microscope the presence or absence of mycorrhizal fungal structures in at least 100 intersection points between root fragments and grid lines in a Petri dish after clearing, bleaching, and staining.

The response to mycorrhizal inoculation was calculated as the mycorrhizal inoculation effect (MIE): the difference in shoot dry weight between plants grown in inoculated and uninoculated soils divided by shoot dry weight of plants grown in inoculated soils (Bagyaraj, 1994; Munyanziza et al., 1997). This formula is structurally similar to that of plant responsiveness to mycorrhiza (Plenchette et al., 1983). Positive values of MIE indicate that either the amount or the quality of the indigenous inoculum is insufficient for maximal plant growth response.

Statistical analyses were performed using the SPSS package (SPSS Inc., 1993). All data were tested for normality and homogeneity of variances using the Levene test in the one-way analysis of variance (ANOVA). As variances were unequal, fractional colonisation data were arcsin square root transformed, and shoot dry weight square root transformed. Average means were separated by Duncan's Multiple Range Test. Mycorrhizal inoculation effect was based on average shoot dry weight of plants with indigenous inoculum and plants with inoculum added, hence no statistical tests of MIE were executed. Spearman's rank correlation coefficients between inoculum quantity and plant biomass per species were calculated.

**RESULTS**

In the presence of indigenous inoculum shoot biomass of *Terminalia* was highest in soils from fallow and lowest in soils from late successional stands and skid trails. Inoculum addition significantly increased shoot dry weight in soils from late successional stands, fallow, skid trails, and landings with *Musanga*, whereas in soils from early successional forest and agricultural fields shoot dry weight was significantly reduced. With inoculum addition soils from fallow still yielded highest shoot biomass and those from late successional stands, skid trails, and bare landings yielded lowest shoot biomass (Fig. 7-1). Plant growth followed the same pattern, with seedlings growing in soils from fallow with inoculum added being largest and seedlings growing in soils from skid trails with original inoculum being smallest. As plant height was more variable that shoot dry weight, inoculum addition did not show many significant effects: addition of inoculum only significantly increased growth in soils from skid trails and decreased growth in soils from early successional forest (data not shown). Mycorrhizal colonisation by original inoculum was very high in soils from early successional forest, and (almost) absent in soils from late successional forest, skid trails, and bare landings. Inoculum addition substantially increased root colonisation, except in soils from early successional forest and fallow. Root colonisation was low and was unaffected by inoculum addition in soils from fallow (Fig. 7-2), although plants growing in these soils performed best.

In the presence of indigenous inoculum shoot biomass of *Distemonanthus* was highest in soils from fallow and lowest in soils from skid trails and bare landings. After inoculum addition shoot dry weight was substantially increased except for soils from fallow. Seedlings on soils from bare landings still had the lowest weight (Fig. 7-3). Plant growth was not significantly affected by either soil type or inoculum addition (data not shown). Root colonisation ranged from 15-42% with indigenous inoculum and was only slightly increased after inoculum addition. Root colonisation only significantly increased after inoculum addition in soils from skid trails (Fig. 7-4).

In the presence of indigenous inoculum shoot biomass of *Entandrophragma* was highest in soils from agricultural fields and lowest in soils from late successional forest, early successional forest, skid trails, bare landings, and landings with *Musanga*. Addition of grass inoculum significantly increased shoot biomass of seedlings in soils from late successional forest, agricultural fields, early successional forest, skid trails, and landings with *Musanga* (Fig. 7-5). Root colonisation with original inoculum was highest in soils from early successional forest, agricultural fields, and landings with *Musanga*, and lowest in soils from skid trails and bare landings. Addition of grass inoculum significantly increased mycorrhizal

colonisation in soils from late successional forest, skid trails, bare landings, and landings with *Musanga* (Fig. 7-6).

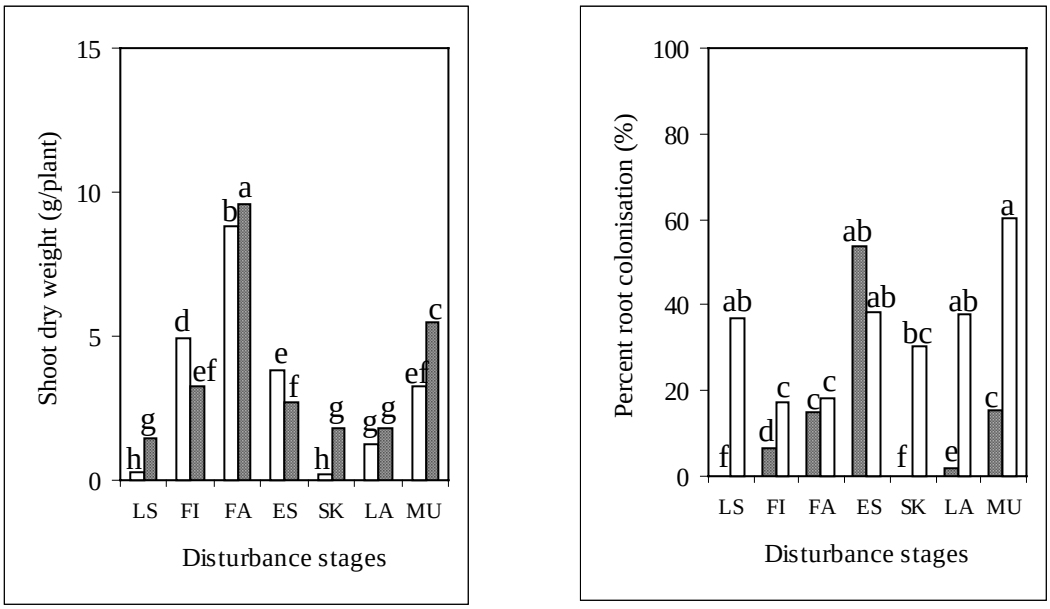



**Fig. 7-1:** Shoot dry weight of *Terminalia* after 5 months. Open bars indicate original inoculum, shaded bars inoculum addition. Abbreviations: LS: Late successional forest; FI: Agricultural field; FA: Fallow; ES: Early successional forest; SK: Skid trails; LA: Bare landings; MU: Landings with *Musanga*. Different letters indicate significant differences based on Duncan's Multiple Range Test ($p < 0.05$).

**Fig. 7-2:** Root colonisation of *Terminalia* after 5 months. See Fig. 7-1 for explanations.

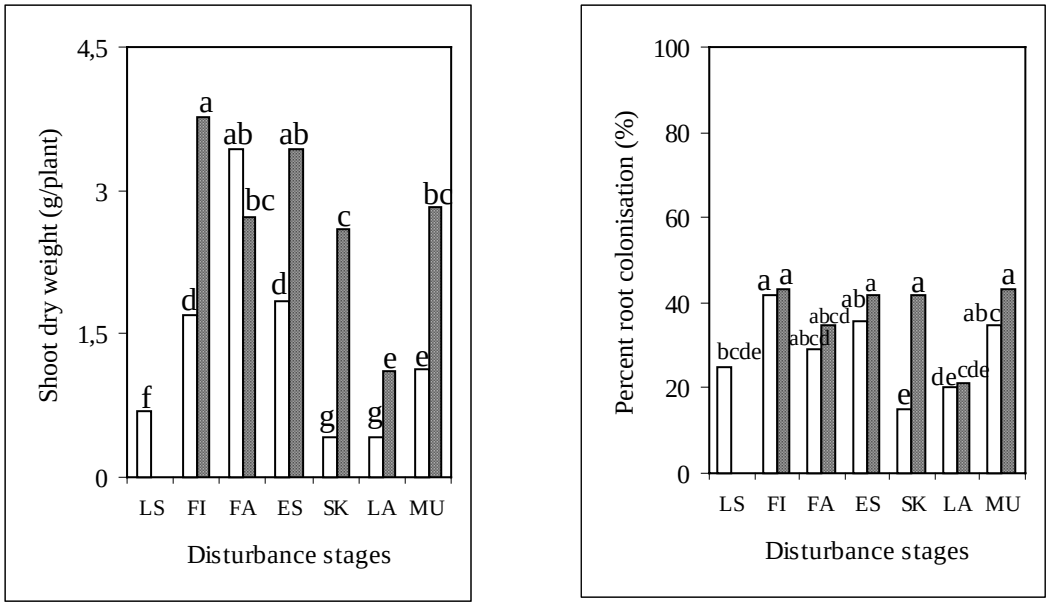



**Fig. 7-3:** Shoot dry weight of *Distemonanthus* after 5 months. See Fig. 7-1 for explanations.

**Fig. 7-4:** Root colonisation of *Distemonanthus* after 5 months. See Fig. 7-1 for explanations.

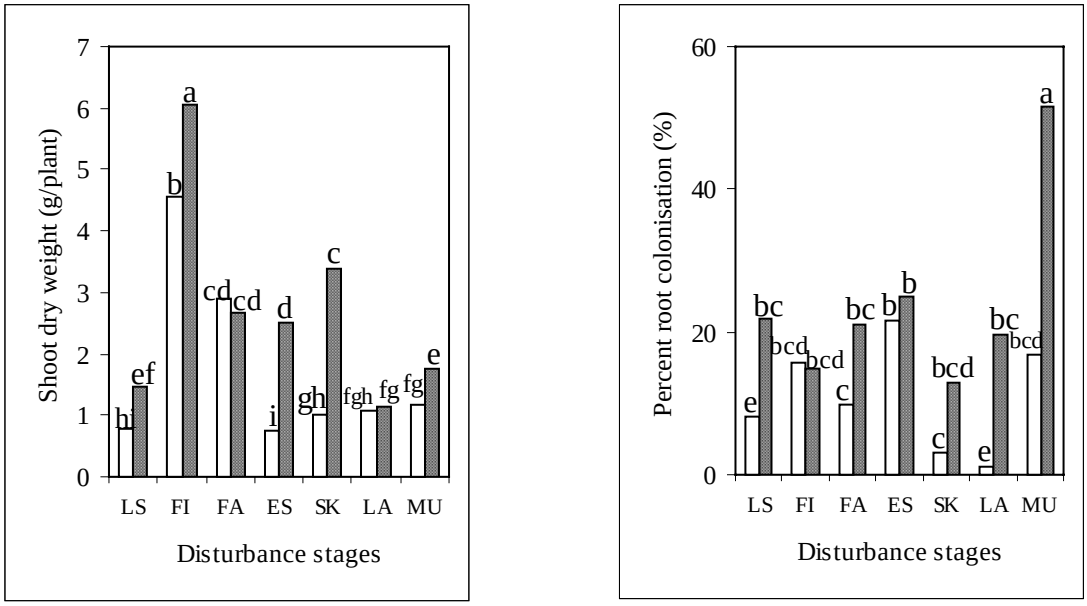



**Fig. 7-5:** Shoot dry weight of *Entandrophragma* after 5 months. See Fig. 7-1 for explanations.
**Fig. 7-6:** Root colonisation of *Entandrophragma* after 5 months. See Fig. 7-1 for explanations.

Mycorrhizal Inoculum Effect (MIE) is given in Table 7-2. MIE was close to zero in soils from fallow and usually highly positive in soils from skid trails, bare landings, landings with *Musanga*, and late successional forest. MIE was different between species. Effect of inoculum addition was on average lowest with *Terminalia* and highest with *Distemonanthus*.

**Table 7-2:** Mycorrhizal Inoculation Effect (MIE) in the various vegetation types based on shoot dry weight.

| | Timber species | | |
|---|---|---|---|
| Vegetation type | Frake | Movingui | Sipo |
| Late Successional Forest | **+0.88** | ND | **+0.46** |
| Agricultural Field | **- 0.51** | **+0.55** | +0.17 |
| Fallow | **+0.08** | - 0.26 | - 0.09 |
| Early Successional Forest | **- 0.36** | **+0.46** | **+0.72** |
| Skid trails | **+0.90** | **+0.84** | **+0.68** |
| Bare landings | +0.27 | **+0.65** | +0.06 |
| Landings with *Musanga* | **+0.41** | **+0.59** | **+0.34** |

ND: Not determined
Values in bold indicate significant differences (at $p < 0.05$) in shoot dry weight between soils with grass inoculum added and without addition of grass inoculum (according to Duncan's Multiple Range Test).

**DISCUSSION**

Addition of grass inoculum resulted in most cases in substantially larger plants. This implies that addition of grass inoculum could be a way to increase plant performance on disturbed sites. Restoration of degraded tropical savannas in Venezuela, where there was an insufficiency of autochthonous inoculum, after addition of mycorrhizal inoculum from *Brachiaria decumbens* (Poaceae) has been described by Cuenca et al. (1998), who stated that rehabilitation of these degraded lands without mycorrhizal inoculum would have been impossible. Insufficiency of indigenous mycorrhizal inoculum at disturbed sites has also been reported from the arid and semi-arid regions of Africa. Michelsen (1992) surveyed a large number of tree species in nurseries in northeastern Africa and provided evidence that naturally occurring mycorrhizal fungi were scarce, leading to bad performance of trees after outplanting. Beneficial effects to tree seedlings of inoculation in unsterile soils have been reported for *Hevea brasiliensis* (Ikram et al., 1992), *Theobroma cacao* (Chulan & Ragu, 1986), and several species of *Acacia* (Michelsen, 1993; Munro et al., 1999).

In our study plant response to the soils with indigenous inoculum was quite variable and for that reason the interpretation of MIE should be done with caution. Different plant responses to the various soils can be due to initial differences in inoculum density and to differences in soil fertility. Compared to undisturbed, late successional forests, both agricultural field and fallows have a higher soil fertility and harbour a larger amount of mycorrhizal propagules. Although increased nutrient availability often depresses mycorrhizal functioning, a synergism between mycorrhizal inoculation and nutrient availability might exist in soils that are extremely phosphorus-deficient (Bolan et al., 1984). Soils in the Ebom area are heavily clayey and very low in extractable phosphorus.

The importance of inoculum quantity in determining plant response can be concluded from a significantly positive rank correlation between plant growth with indigenous inoculum and inoculum potential for *Terminalia* ($r = 0.83$) and *Distemonanthus* ($r = 0.92$), but not for *Entandrophragma* ($r = 0.39$; n.s.). Inoculum quantity seemed also a major factor in determining growth response of *Acacia tortilis* (Mimosaceae) to various sources of inoculum (Munro et al., 1999). Mycorrhizal fungal species composition (inoculum quality) could play a role as well, although this factor seems less important than that of inoculum quantity. Addition of allochthonous inoculum could result in competition with indigenous mycorrhizal fungi. Although inoculum addition never resulted in decreased fractional colonisation, significantly negative plant responses to inoculum addition (*Terminalia* in soils from agricultural fields and early successional forest) might suggest that allochthonous inoculum might not always be beneficial (Johnson, 1993; Janos, 1996).

Earlier investigations (Blal & Gianinazzi-Pearson, 1992) have also reported that *Terminalia* is very sensitive to inoculum quality, with mycorrhizal fungi from some sites being very beneficial and from other sites hardly favourable to that species. *Distemonanthus* on the other hand did not show higher root colonisation after inoculum addition in most soils, even though plants responded significantly positively to the allochthonous inoculum. We did not attempt to identify the mycorrhizal fungal species present in the indigenous inoculum or in the grass inoculum. However, visual inspection of spore types after extraction indicated that our soils contained a diversity of spores.

Compared to single-species additions, application of mixed inoculum might be a form of risk spreading (Van der Heijden et al., 1998b). However, mixed inocula might not always be most beneficial for plant growth. Although arbuscular mycorrhizal fungi have long been considered as not host specific, recent investigations have demonstrated that the effects of mycorrhizal colonisation on plant species can show a large degree of selectivity (McGonigle & Fitter, 1990; Dhillion, 1992; Bever et al., 1996; Zhu et al., 2000). Experiments with mycorrhizal inoculum, isolated from the rhizosphere of mother trees, are needed to determine the importance of host plant selectivity for regeneration of tropical rain forest trees (CHAPTER 8).

Janos (1992, 1996) defined mycorrhizal inoculum potential as the capacity of inocula existing at a site to produce mycorrhiza sufficient to affect plant performance (positively or negatively). This concept might be a suitable way to understand how different tree species experience different mycorrhizal inoculum potentials. *Terminalia*, an early-successional tree with small seeds and minor seed reserves, responded to inoculum addition by increased mycorrhizal colonisation (+166%), even though MIE was relatively minor (+0.10, excluding soils from undisturbed forest). *Distemonanthus*, a late successional tree with large seeds and substantial seed reserves, did only slightly increase mycorrhizal colonisation (+28%), but showed a large MIE (+0.46). *Entandrophragma*, also a tree with large seeds, showed an intermediary response. Leaf phosphorus status showed the same pattern as root colonisation: as a consequence of inoculum addition leaf P of *Terminalia* increased, whereas that of *Distemonanthus* was unaffected (data not shown).

In general, MIE was large in soils with a low inoculum potential such as late successional forest, skid trails, and both types of landings, while MIE was low (and sometimes even negative) in soils with a high inoculum potential such as agricultural fields and fallows. Growth depression was observed for *Terminalia* seedlings grown in inoculated soils of agricultural fields and early successional stands. Agricultural field and fallow soils were shown to have high mycorrhizal inoculum potential across various sites in south Cameroon (CHAPTER 4). Good growth of seedlings with indigenous inoculum on soils from agricultural fields and fallow suggests that these soils maintain the capacity to revert to forest. Very good seedling growth was observed in soils from fallow. It is possible that fallowing and establishment of the exotic weed *C. odorata* increases the availability of nutrients such as phosphorus or potassium, thus explaining the observation of local agriculturalists that productivity of peanut (*Arachis hypogaea*) is increased after fallowing. Litter of *C. odorata* is easily degradable, due to its low lignin content, and much larger quantities of potassium are released than in litter of several bush fallow trees (Kanmegne et al., 1999). Relatively slow growth and large response to inoculum addition in soils of undisturbed forests is somewhat surprising. This slow response may result from disturbance of hyphal networks under our experimental conditions, since other sources of mycorrhizal propagules could be scanty in undisturbed sites. However, spore numbers in undisturbed forest were high compared to values reported for other rain forests, making this explanation unlikely (CHAPTER 4). Janos (1996) suggested that mycorrhizal dependency and responsiveness could be different for seedlings and adults of the same species, with seedlings being more responsive than (slow-growing) adults.

If adequacy of mycorrhizal inoculum is especially important in the establishment phase of trees, slow plant growth on sites of forestry practices and a large MIE suggest that boosting inoculum potential is necessary to have adequate regeneration after selective logging. Establishment on landings by *M. cecropioides* usually occurs after about five to ten years and mycorrhizal colonisation and plant performance were significantly higher in landings with this pioneer tree than in landings without it. Establishment of facultative mycorrhizal trees such as *M. cecropioid*es might increase recovery rates. However, inoculum addition still had a large effect for the various seedlings. The build up of sufficient mycorrhizal inoculum on landings might be slow and establishment of obligate mycorrhizal trees, if they need a high inoculum density, might be retarded. This might explain the failure of tree species lik*e Lophira alat*a (Ochnaceae) and the nitrogen-fixin*g Pterocarpus soyaux*ii (Fabaceae) to establish; although many seedlings of these species were observed on landings, they all died within a few years. In Ebom about 5% of the forested area was impacted by these forestry practices. However, that site had only sparsely been logged and under normal selective logging practices the area affected by forest roads, skid trails, and landings varies between 10 and 18%.

Although inoculum addition led to substantially improved plant performance on soils that have been affected by forestry practices, it is not clear whether inoculum addition in the field is a feasible strategy. Plant response to mycorrhizal inoculum on skid trails and landings might, next to insufficiency of mycorrhizal inoculum, be constrained by soil compaction. On sites that are impacted by heavy trafficking, bulk density was about 20% higher. Soil compaction had a negative effect on arbuscular mycorrhizal colonisation in varies agricultural crops (Entry et al., 1996; Kothari & Singh, 1996; Nadian et al., 1996).

**CHAPTER 8**

# GROWTH RESPONSE OF TWO NATIVE TIMBER SPECIES TO SOILS WITH DIFFERENT ARBUSCULAR MYCORRHIZAL INOCULUM POTENTIALS IN SOUTH CAMEROON. II. INDIGENOUS INOCULUM AND ADDITION OF HOST TREE SPECIFIC INOCULUM


## ABSTRACT

*Deficiency in mycorrhizal inoculum in soils can be corrected by inoculum addition. Effects of inoculum addition depend on both the quantity of inoculum applied (as observed by changes in fractional root colonisation) and by inoculum quality, with host tree specific inoculum being considered as quality inoculum. A greenhouse bioassay was carried out to determine the effect of quality inoculum addition to soils from different vegetation types on growth response and mycorrhizal colonisation of seedlings of two commercial timber species,* Pterocarpus soyauxii *and* Lophira alata*. Soils were collected from six vegetation types: early successional forest stands, agricultural fields of food crops, fallow of* Chromolaena odorata*, skid trails, bare soil landings, and landings revegetated by the pioneer tree* Musanga cecropioides*. The Mycorrhizal Inoculation Effect (MIE) was subdivided in effects of Inoculum Quality and Inoculum Quantity. Both plant species markedly differed in growth response and mycorrhizal colonisation in the presence of indigenous inoculum and to addition of host tree specific inoculum. In the presence of indigenous inoculum, seedlings of* Pterocarpus *performed poorly, while those of* Lophira *performed poorly only in soils from forestry practices. Seedlings of* Pterocarpus *were mainly colonised by members of the Glomaceae, those of* Lophira *by members of the Gigasporaceae. Inoculation with host tree specific inoculum from* Pterocarpus *significantly increased plant biomass and favoured nodulation of seedlings, resulting in a large and positive MIE; for* Lophira *the MIE was low. Fractional colonisation of* Pterocarpus *was significantly affected by inoculum addition and was positively correlated with plant biomass; fractional colonisation of* Lophira *was hardly affected by inoculum addition and was not correlated with plant biomass. Our results demonstrate that seedling responses of timber species to mycorrhizal inoculum could depend on the quality of that inoculum, implying that successful regeneration of important tropical timber species requires knowledge on the significance of host tree specific mycorrhizal fungi.*


## INTRODUCTION

The current rates of deforestation and timber exploitation in the tropics suggest that sustained production of highly priced timbers will require alternative management strategies. With the present pressure on tropical rain forests, conservation of pristine forests and sustainable forestry are not combinable. Alternative management strategies include plantation forestry and artificial regeneration through quality seedlings in enrichment planting. In the strategy of enrichment planting, tree species composition is regulated by preferentially stimulating establishment and subsequent growth of desirable species. However, the effect of this strategy with regard to the biological value of such forests very much depends on the number of species that are ultimately commercialised. Maintenance of tree species that are predominantly valuable through their non-timber forest products and, more generally,

maintenance of the overwhelming tree species richness is not necessarily guaranteed through such management strategies (Eba'a, 2000).

Seedlings of virtually all rain forest trees are mycorrhizal and many of them highly mycotrophic, i.e. dependent on and responsive to mycorrhizal fungi (Janos, 1980; CHAPTER 2). However, the availability of mycorrhizal fungal propagules might not always be sufficient to guarantee optimal plant response under the prevailing soil physical, chemical, and biological conditions. Therefore, fitness of seedlings could be enhanced by adequate mycorrhizal fungal propagules in nursery substrates. Field application of mycorrhizal biotechnology has been constrained by difficulties in producing large quantities of effective inoculum of mycorrhizal fungi. This impediment is likely to be partly alleviated if small quantities of soil could harbour adequate mycorrhizal inoculum to enhance growth and establishment of seedlings. Such low-input technology, successfully applied in Africa in nursery inoculation of exotic, ectomycorrhizal pines with natural soil and humus from established plantations (Mikola, 1973), could also be very valuable for silviculture of indigenous tree species, irrespective of whether they form ectomycorrhiza or arbuscular mycorrhiza. Application of field soils with roots might not be without problems, however. Energy stored in roots could be rapidly exhausted after severing roots from live plants, thus quickly rendering such inoculum ineffective (Janos, 1996). Decaying root material, while ultimately a source of mineral nutrients, might initially depress nitrogen availability because of immobilisation by microorganisms decomposing woody tissue.

Michelsen (1992, 1993) and Munro et al. (1999) showed that application of field soil with plant roots with arbuscular mycorrhizal fungi was successful for several species of *Acacia*. Revegetation of denuded sites in the Venezuelan savanna was enhanced by addition of arbuscular mycorrhizal inoculum (Cuenca et al., 1998). For rain forest trees from the south of Cameroon, CHAPTER 7 showed that addition of field inoculum to soils deficient in arbuscular mycorrhizal fungal inoculum increased root colonisation and seedling growth of three timber species to different extent. The field inoculum applied was taken from a field where the grass *Paspalum conjugatum* abundantly grew. These data from Cameroon strongly suggested that inoculum quantity was the main factor contributing to improved plant performance.

However, host tree selectivity by arbuscular mycorrhizal fungi has been observed in the growth response to mycorrhizal colonisation by earlier workers (McGonigle & Fitter, 1990; Dhillion, 1992; Bever et al., 1996; Zhu et al., 2000). Van der Heijden et al. (1998a, 1999) made the general claim that different plant species benefit from different species of arbuscular mycorrhizal fungi, as shown by significant plant species x fungal species interactions. Therefore, inoculum quality may be critical for effective mycorrhizal colonisation and growth of mycorrhiza dependent seedlings. No such information is available for seedlings of timber species in tropical rain forests.

The aims of this investigation were (1) to determine how soils with different inherent inoculum potential of arbuscular mycorrhizal fungi caused by selective logging and shifting cultivation practices affect mycorrhizal colonisation and growth of seedlings of two important timber species; and (2) to assess the response to inoculation of soils with host tree specific inoculum taken from the root zones of target mature tree species.

## MATERIALS AND METHODS

*Site and vegetation description*

The soils used for the bioassays belonged to the Ebom series. Ebom (3º06'N;10º41'E) is one of the three field sites of the Tropenbos Cameroon Programme (TCP), located 114 km east of Kribi. Elevation ranges from 350 – 500 m a.s.l. Annual rainfall was 2038 mm in 1998, with two maxima in May and October. Soils are moderately heavy clayey, generally strongly acid, deep and well drained, with low to medium organic matter content. They are classified in the FAO system as Xanthic Ferralsol (Van Gemerden & Hazeu, 1999). Within the Ebom area, primary forests are rare but old secondary stands are widespread. Old secondary vegetation, dominated by members of the Myristicaceae, has been affected by human activities, especially subsistence agriculture for food crops and selective logging.

Six vegetation types, considered as disturbance stages, were selected, *viz.* early successional forest stands, agricultural fields of food crops, fallows of *Chromolaena odorata* (Asteraceae), skid trails, bare landings, and landings recolonised by the pioneer, facultatively mycorrhizal tree *Musanga cecropioides* (Moraceae). Early successional forest stands are very dense due to abundance of climbers, young saplings and juveniles, and undergrowth vegetation. Agricultural fields were included as disturbance stages after slashing the undergrowth vegetation, felling the existing trees, removing surface debris and burning dried biomass. Well-vegetated fallows of 3 – 5 m high shrubs of *C. odorata* (hereafter referred to as fallow) of three to four years old were chosen.

*Timber species*

Two native large tree species, which provide highly valued timber and which are among the most frequently harvested trees nationwide, were chosen: Padouk, *Pterocarpus soyauxii* (Fabaceae) and Azobe, *Lophira alata* (Ochnaceae). In this chapter, the generic names will be used. *Pterocarpus* is commercially popular, being harvested for exports in Gabon, Equatorial Guinea, and Cameroon; in the latter country, it is well-renowned for the local wood transformation industry. It ranked eighth in national exports in 1998 with about 2 % but locally it constituted 8 % of volume exports in both Kribi and Campo ports (Anonymus, 1999). 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 nodules. *Lophira* is harvested in Ghana, Gabon, Equatorial Guinea, and Cameroon; in the latter country, it ranked fourth in total national exports in 1998. Locally the most renowned timber, *Lophira* constituted 60 % of extracted wood volume at the Kribi port (Anonymus, 1999). Its seeds are bulging and elongated, weighing about 1.0 g. Seedlings grow rapidly and produce abundant dark red roots that are coarsely branched and devoid of root hairs.

Seeds were collected around seed-bearer mother trees in Ebom. *Lophira* seeds were soaked overnight in cool water before manual scarification. Seeds of both plants were sterilised in 70 % alcohol for 1 min, rinsed three times with sterile water, and placed in washed and sterilised sea sand. One one-week-old pregerminated seed was placed in five kg (dry weight basis) portions of fresh soils from the six vegetation types transferred to black plastic bags. Half of the bags received only the indigenous mycorrhizal inoculum from that disturbance stage, the other half received an addition of quality inoculum.

*Mycorrhizal inoculum*

In all vegetation types, indigenous mycorrhizal inoculum was collected from three intact soil cores (surface soil, 0 – 20 cm) that were bulked into one composite sample. The arbuscular mycorrhizal inoculum used for quality inoculation was a mixture of surface soil and fine root samples (0 – 15 cm) taken at four points around, and in the vicinity of the stem base of five widely spaced adult trees of *Pterocarpus* and *Lophira*. These inocula will hereafter be referred to as *Pterocarpus* and *Lophira* inoculum, respectively. Collected inocula were placed in air-filled polyethylene bags, taken to Kribi, Cameroon, and kept under greenhouse benches. Data on inoculum potential of the various vegetation types are presented in Table 8-1.

Half of the bags were thoroughly mixed with 50 g portions $kg^{-1}$ soil of either *Pterocarpus* or *Lophira* inoculum. Non-inoculated unsterile soils received similar amounts of steam sterilised soil inoculum and 40 ml of filtered inoculum leachings to insure similar microbial activity (Hetrick et al., 1988). Steam sterilisation was achieved at 100° C for 1 hour. The sterilised 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 disturbance stages and two inoculation treatments with three replicates. In addition, there were three replicates for the assessment of the host tree specific inoculum. These treatments were placed on greenhouse benches in a randomised complete block design. Plants were grown without nutrient amendment. Water was added as needed to maintain the soils at water holding capacity. Plants were raised under natural light in a greenhouse in Kribi. *Pterocarpus* seedlings were grown from October 16, 1997 to April 17, 1998 and those of *Lophira* from April 10, 1999 to October 17, 1999. Due to irregular seed production and differences in the timing of seed set of both species, it was not possible to carry out both bioassays during the same period.

**Table 8-1:** Inoculum potential of the various vegetation types (disturbance stages).

| Vegetation type | Spore number[1] | Most probable Number[2] | Root colonisation[3] |
|---|---|---|---|
| Agricultural Field | 28 | 29 | 31 |
| Fallow | 44 | 55 | 39 |
| Early Successional Forest | 21 | 16 | 23 |
| Skid Trails | 9 | 0 | 16 |
| Bare landings | 5 | 0 | 3 |
| Landings with *Musanga* | 8 | 6 | 25 |

1. Number of spores.$g^{-1}$ dry soil
2. Number of infective propagules.$g^{-1}$ dry soil. Method according to Porter (1979). Four-fold dilution series with 5 replicates. Bait plant a local variety of cowpea (*Vigna unguiculata* (Fabaceae)).
3. Root colonisation (% root length colonised) of a local variety of Cowpea in intact soil cores.

*Assessment of plant growth and mycorrhizal colonisation*

Plant height was assessed on a monthly basis from the stem collar to the point of insertion of the youngest fully expanded leaf. At harvest, the above-ground part of the plant was removed

and dried at 70°C for 72 hours for determination of shoot dry weight. The nodules on roots of *Pterocarpus* seedlings were counted and grouped in three categories: category 1: 1 - 5 nodules, 2: 6 – 10; 3: above 10. Fractional colonisation by arbuscular mycorrhizal fungi was estimated by scoring under a dissecting microscope the presence or absence of mycorrhizal fungal structures in at least 100 intersection points between root fragments and gridlines in a Petri dish after clearing, bleaching, and staining with acid fuchsin.

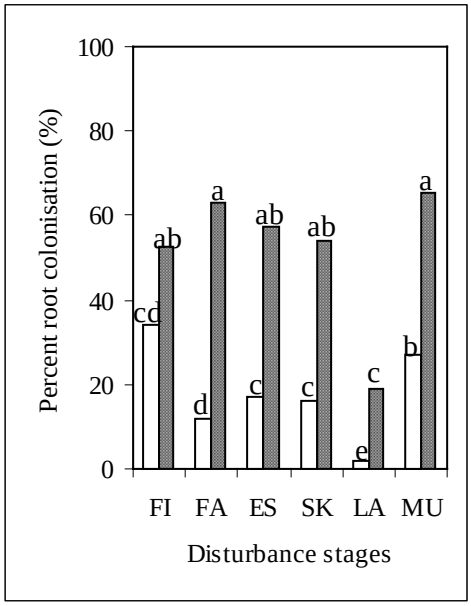
The response to mycorrhizal inoculation was calculated as the mycorrhizal inoculation effect (MIE): the difference in shoot dry weight between plants grown in inoculated and uninoculated soils divided by shoot dry weight of plants grown in inoculated soils (Bagyaraj, 1994; Munyanziza et al., 1997). This formula is structurally similar to that of plant responsiveness to mycorrhiza (Plenchette et al., 1983). The mycorrhizal inoculation effect depends both on inoculum quantity (assessed by fractional root colonisation) and quality. If root colonisation does not change after inoculum addition, inoculum quantity (IQN) does not contribute to MIE and MIE can then be equated with the effect of inoculum quality (IQL). Inoculum quality is expressed as: IQL = MIE x Square Root (Fractional colonisation of plants growing in uninoculated soil divided by fractional colonisation of plants growing in inoculated soil). Inoculum quantity is expressed as: IQN = MIE – IQL.

Statistical analyses were performed using the SPSS package (SPSS Inc., 1993). All data were tested for normality and homogeneity of variances using the Levene test in the one-way analysis of variance (ANOVA). As variances were unequal, fractional colonisation data were arcsin square root transformed and shoot dry weight square root transformed. A general factorial ANOVA was done with inoculation and disturbance stages as variables. Average means were separated by Duncan's multiple range test. No statistical tests of MIE, IQL, and IQN were executed as calculations were based on average shoot dry weights. Pearson's correlation coefficients were calculated between fractional colonisation and shoot dry weight for each plant species.

**RESULTS**

Initial evidence of host specific colonisation of both tree species was provided by morphological characteristics of the arbuscular mycorrhizal fungi. Root colonisation of *Pterocarpus* seedlings showed abundant intraradical hyphae, variously shaped vesicles, some arbuscules and hyphal coils. Few auxiliary bodies on external hyphae were observed in soils from some disturbance stages. Root colonisation of *Lophira* seedlings was mainly extraradical with large and thick-walled hyphae frequently carrying numerous spiny auxiliary cells; internal colonisation was sparse without arbuscules, vesicles, and hyphal coils. These colonisation patterns were consistent in *Pterocarpus* roots and inconsistent in those of *Lophira*.

Mycorrhizal colonisation of *Pterocarpus* seedlings was significantly affected by disturbance stages and inoculum addition. The interaction disturbance stage x inoculum addition was not significant (Table 8-2). Before inoculation, mycorrhizal colonisation was the highest in soils from landings with *Musanga* and agricultural fields, and low in all other disturbance stages. Substantial increase in fractional colonisation after inoculum addition was observed in soils from all disturbance stages. After inoculum addition, fractional colonisation in soils from bare



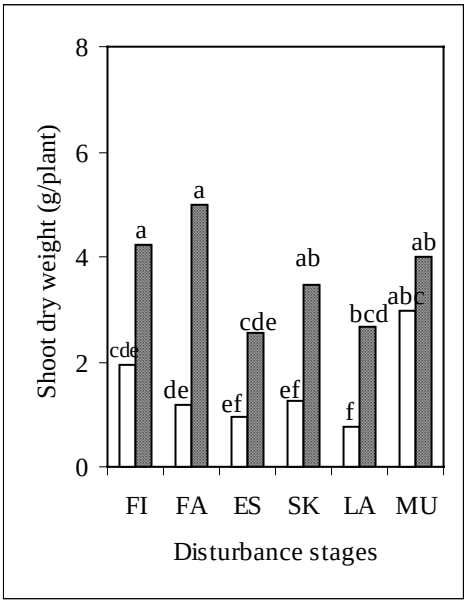




**Fig. 8-1:** Fractional colonisation of *Pterocarpus*. Open bars indicate original inoculum, shaded bars inoculum addition. Abbreviations: FI: Agricultural field; FA: Fallow; ES: Early successional forest; SK: Skid trails; LA: Bare landings; MU: Landings with *Musanga*. Different letters indicate significant differences based on Duncan's Multiple Range Test ($p < 0.05$).
**Fig. 8-2:** Shoot dry weight of *Pterocarpus*. See Fig. 8-1 for explanations.

landings was still low (Fig. 8-1). In the presence of indigenous inoculum, nodules were generally scarce (or even absent on seedlings grown in soils from skid trails and bare landings). Mycorrhizal inoculation increased the number of nodules in all soils (Table 8-3).

**Table 8-2:** General factorial analysis of variance of fractional colonisation of *Pterocarpus* seedlings in relation to disturbance stages and addition of host tree specific inoculum.

| Source of variation | Df | F | p |
|---|---|---|---|
| Inoculum | 1 | 66.6 | 0.000 |
| Disturbance stage | 5 | 9.32 | 0.000 |
| Inoculum x Disturbance stage | 5 | 1.26 | 0.306 |

**Table 8-3:** Number of nodules of seedlings of *Pterocarpus*. 1: 1-5 nodules per seedling; 2: 6-10 nodules per seedling; 3: more than 10 nodules per seedling.

| | Indigenous inoculum | Host tree specific inoculum |
|---|---|---|
| Agricultural Field | 1 | 3 |
| Fallow | 1 | 2 |
| Early successional Forest | 1 | 2 |
| Skid trails | 0 | 2 |
| Bare landings | 0 | 2 |
| Landings with *Musanga* | 2 | 3 |

Shoot dry weight of *Pterocarpus* seedlings was significantly affected by disturbance stages and by inoculum addition. The interaction between disturbance stage and inoculum addition was not significant (Table 8-4).

**Table 8-4:** General factorial analysis of variance of shoot dry weight of *Pterocarpus* seedlings in relation to disturbance stages and addition of host tree specific inoculum.

| Source of Variation | Df | F | p |
|---|---|---|---|
| Inoculum | 1 | 45.6 | 0.000 |
| Disturbance stage | 5 | 3.55 | 0.015 |
| Inoculum x Disturbance stage | 5 | 1.20 | 0.341 |

Shoot dry weight of seedlings grown in soils taken under *Pterocarpus* trees was 7.6 g. In the presence of indigenous inoculum, shoot biomass of *Pterocarpus* seedlings was low in soils from all disturbance stages, except in soils from landings with *Musanga*; the lowest shoot biomass was measured in soils from bare soil landings. Inoculum addition with Padouk inoculum significantly increased shoot biomass in soils from all disturbance stages, except in soils from landings with *Musanga*. With inoculum addition, soils from fallow and agricultural fields produced highest shoot dry weight and those from early successional forest stands and bare landings yielded lowest shoot biomass (Fig. 8-2). Mycorrhizal colonisation and shoot dry weight were positively significantly correlated ($r = 0.89$, $n = 12$; $p < 0.001$). In the presence of indigenous inoculum, plant growth rate of *Pterocarpus* seedlings was low in all soils, except in soils from landings with *Musanga*. With the addition of Padouk inoculum, growth rate increased in all cases. After 4 months seedlings in soil with inoculum added were on average twice as high as seedlings in soils with indigenous inoculum, except for the soils from the landing with *Musanga* (Fig. 8-3).

Mycorrhizal colonisation of *Lophira* was neither affected by disturbance stages, nor by inoculum addition; the interaction was also not significant (Table 8-5). Mycorrhizal colonisation by indigenous inoculum was very high in soils from agricultural soils. Addition of quality inoculum resulted in a very significant drop in colonisation in soils from agricultural fields, whereas in the other soils the effects were only minor (Fig. 8-4).

Shoot dry weight of *Lophira* seedlings was significantly influenced by disturbance stages and inoculum addition; the interaction was also significant (Table 8-6). In the presence of indigenous inoculum, shoot biomass of *Lophira* was lowest in soils from forestry practices (skid trails, bare landings, and landings with *Musanga*) and highest in soils from agricultural practices (fields and fallow). Inoculum addition significantly increased shoot biomass in soils from forestry practices, and had no significant effect in the other soils (Fig. 8-5). Fractional colonisation did not show a significant correlation with shoot biomass ($r = 0.04$, $n = 12$; $p > 0.1$). Plant growth rate of *Lophira* seedlings was constant and was not affected by inoculation in all soils (Fig. 8-6).

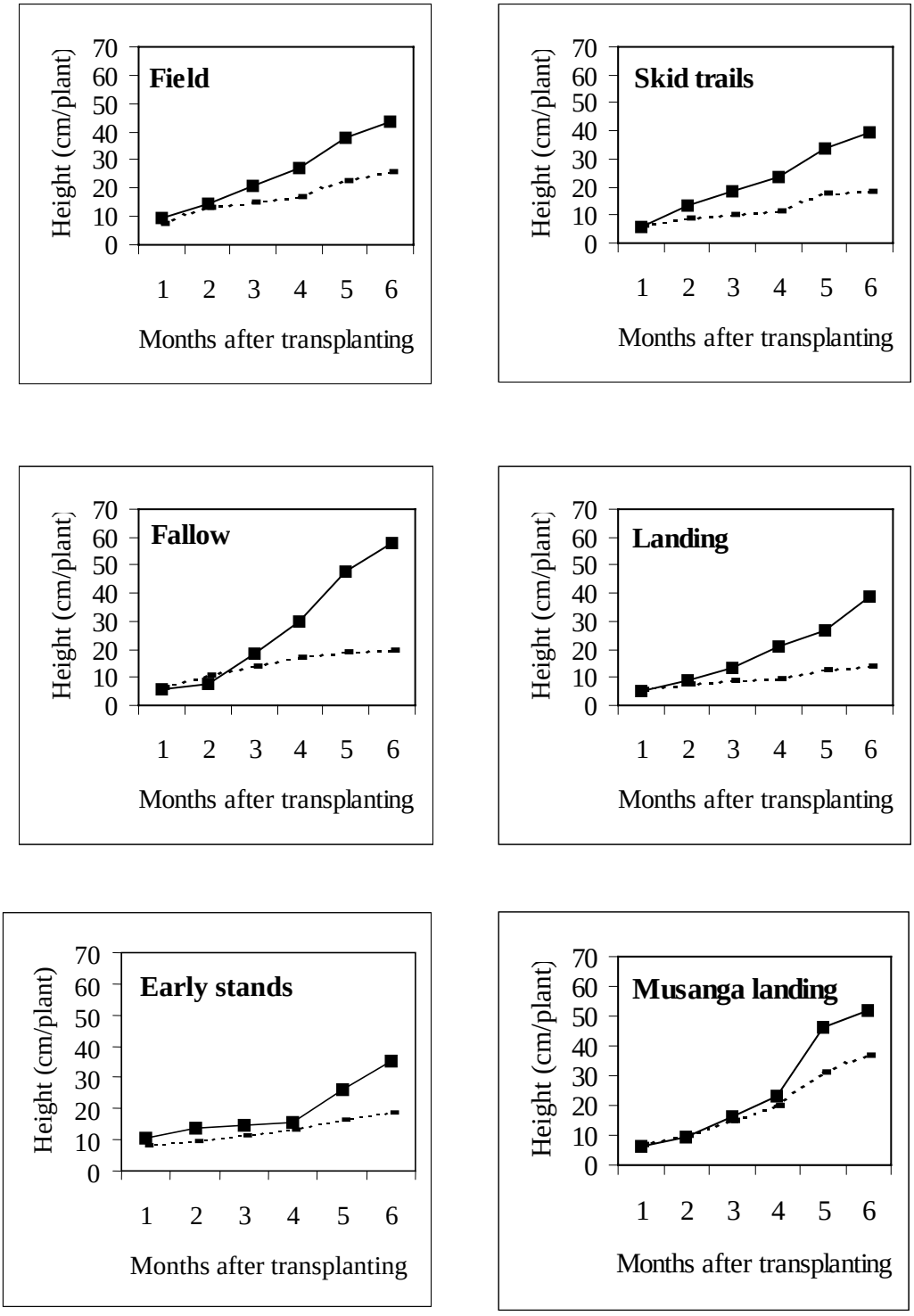


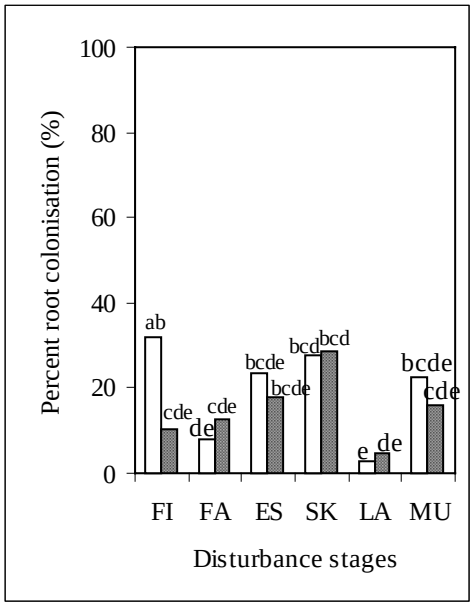

**Fig. 8-3:** Plant growth rate of seedlings of *Pterocarpus*. Continuous lines indicate inoculum addition, dotted lines original inoculum.

**Table 8-5:** General factorial analysis of variance of fractional root colonisation of *Lophira* seedlings in relation to disturbance stages and addition of host tree specific inoculum.

| Source of variation | Df | F | p |
|---|---|---|---|
| Inoculum | 1 | 1.14 | 0.296 |
| Disturbance stage | 5 | 2.49 | 0.059 |
| Inoculum x Disturbance stage | 5 | 0.59 | 0.708 |

**Table 8-6:** General factorial analysis of variance of shoot dry weight of *Lophira* seedlings in relation to disturbance stages and addition of host tree specific inoculum.

| Source of Variation | Df | F | p |
|---|---|---|---|
| Inoculum | 1 | 21.3 | 0.000 |
| Disturbance stage | 5 | 22.6 | 0.000 |
| Inoculum x Disturbance stage | 5 | 6.12 | 0.001 |



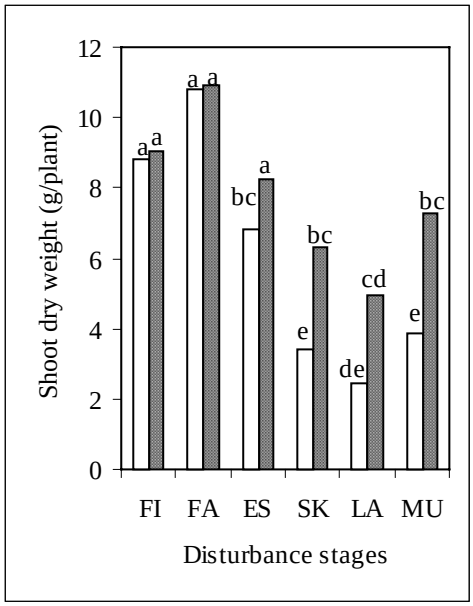




**Fig. 8-4:** Fractional colonisation of *Lophira*. Open bars indicate original inoculum, shaded bars inoculum addition. Abbreviations: FI: Agricultural field; FA: Fallow; ES: Early successional forest; SK: Skid trails; LA: Bare landings; MU: Landings with *Musanga*. Different letters indicate significant differences based on Duncan's Multiple Range Test ($p < 0.05$).
**Fig. 8-5:** Shoot dry weight of *Lophira*. See Fig. 8-4 for explanations.

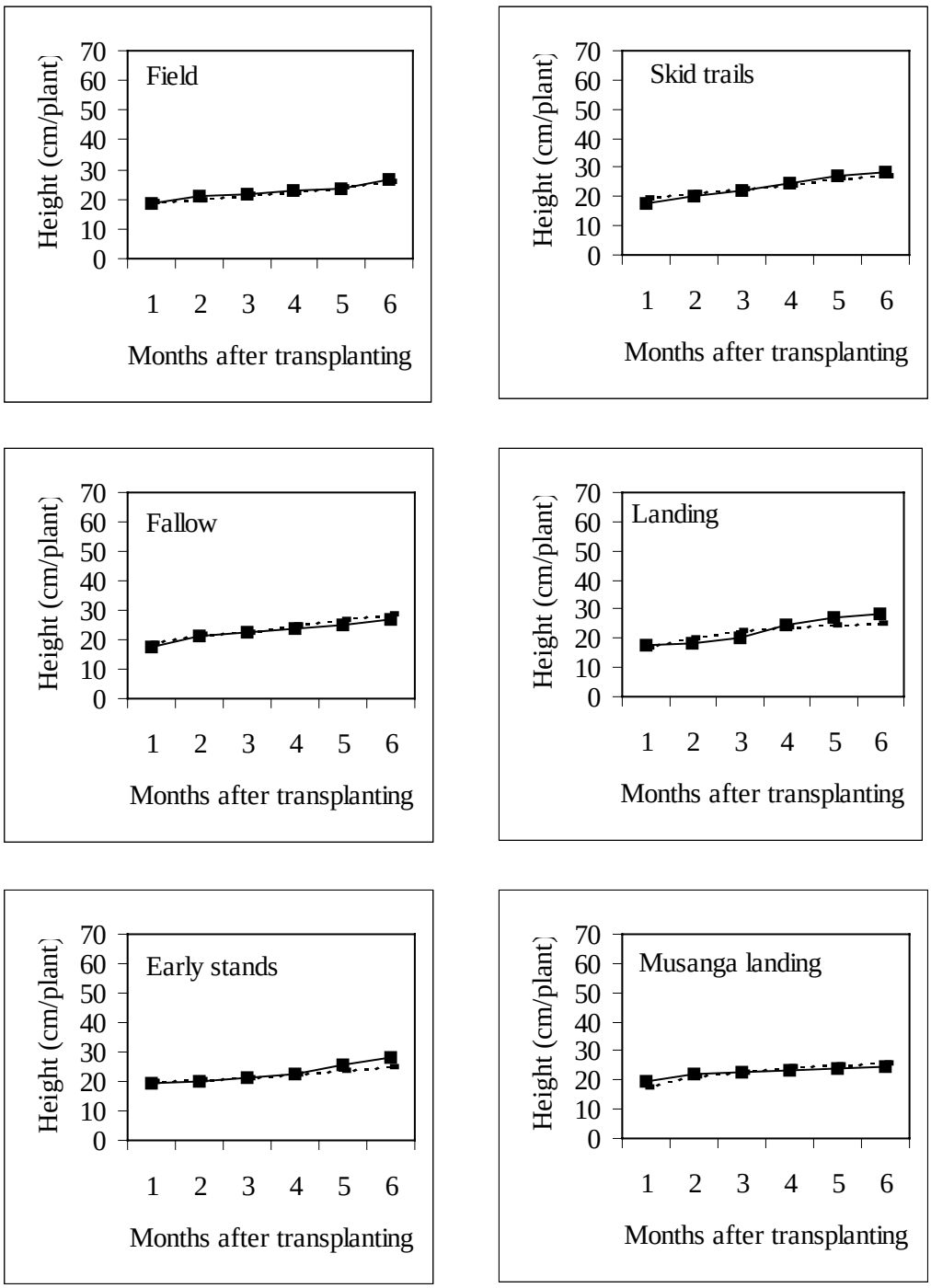



**Fig. 8-6:** Plant growth rate of seedlings of *Lophira*. See Fig. 8-3 for explanations.

The mycorrhizal inoculation effect is presented in Table 8-7. MIE was generally high in *Pterocarpus* (except in soil from landings with *Musanga*), whereas with *Lophira* the effects were small, except in soils of forestry practices. In *Pterocarpus*, where inoculum addition resulted in increased fractional colonisation, both inoculum quality and inoculum quantity played an important role. As in *Lophira* differences in fractional colonisation with indigenous inoculum and quality inoculum were rather low, the quality aspects of mycorrhizal inoculum addition prevailed over inoculum quantity.

**Table 8-7:** Mycorrhizal inoculation effect (MIE) and effect of inoculum quality (IQL) and inoculum quantity (IQN) in the various vegetation types, based on shoot dry weight. See text for explanation. Values in bold indicate significant differences (at $p < 0.05$) in shoot dry weight between soils with indigenous inoculum and soils with host tree specific inoculum added (according to Duncan's Multiple Range Test).

*Pterocarpus*

| Vegetation type | MIE | IQL | IQN |
|---|---|---|---|
| Agricultural Field | **+ 0.54** | +0.44 | +0.10 |
| Fallow | **+0.79** | +0.35 | +0.44 |
| Early Successional Forest | **+0.63** | +0.34 | +0.29 |
| Skid Trails | **+0.64** | +0.35 | +0.29 |
| Bare landings | **+0.83** | +0.27 | +0.56 |
| Landings with *Musanga* | +0.25 | +0.19 | +0.06 |

*Lophira*

| Vegetation type | MIE | IQL | IQN |
|---|---|---|---|
| Agricultural Field | +0.02 | +0.04 | - 0.02 |
| Fallow | +0.01 | +0.01 | 0.00 |
| Early Successional Forest | +0.17 | +0.20 | - 0.03 |
| Skid Trails | **+0.55** | +0.54 | +0.01 |
| Bare landings | **+0.52** | +0.40 | +0.12 |
| Landings with *Musanga* | **+0.63** | +0.76 | - 0.13 |

**DISCUSSION**

Morphology of the arbuscular mycorrhizal fungi colonising *Pterocarpus* and *Lophira* seedlings markedly differed. *Pterocarpus* seedlings showed typical arbuscular mycorrhizal internal colonisation of the *Arum*-type (Smith & Smith, 1997), with vesicle shapes suggesting that these mycobionts belong to the Glomaceae, while that of *Lophira* seedlings was chiefly extraradical with large hyphae harbouring numerous auxiliary cells, indicating colonisation by the Gigasporaceae. Although no attempt was made to isolate and identify spores from host tree specific inocula and to determine their contribution to inoculum potential, it seems that in the same rain forest ecosystem, communities of different arbuscular mycorrhizal fungal taxa could associate preferentially with different tree species. Host tree selectivity of arbuscular mycorrhizal fungi has been observed for Tali (*Erythrophloeum ivorense*, Caesalpiniaceae), where members of the Gigasporaceae also abounded (C. Schmitt & N.A. Onguene,

unpublished observations). In more than ten years old fields of cacao (*Theobroma cacao*, Sterculiaceae), cocoa roots were associated more with Gigasporaceae (N.A.Onguene & C.S.O. Onana, unpublished observations) than Glomaceae, confirming an earlier report by Laycock (1945).

Similar observations on host tree specificity or selectivity have been made in the Guyanan rain forest. Inoculation of seedlings of *Dicorynia guianensis* and of *Eperua falcata* (both members of the Caesalpiniaceae) with roots of mature trees of the former tree species increased growth of only the former plant (Béreau et al., 1998). If preferential associations of arbuscular mycorrhizal fungi with certain tree species are widespread in tropical rain forests, it could to some extent contribute to the explanation for the lack of success of artificial regeneration attempts of indigenous timber species even in the presence of seemingly adequate mycorrhizal inoculum.

The different morphologies and evolutionary histories of the various families of the Glomales has led to the hypothesis of functional differentiation, with species of Glomaceae predominantly enhancing uptake of immobile elements such as phosphorus and Gigasporaceae contributing to soil stability (Boddington & Dodd, 1998, 1999; Wright & Upadhyaya, 1998). The common occurrence of members of the Glomaceae in *Pterocarpus*, coupled with the increase in nodulation, which depends on an adequate phosphorus supply, after addition of host tree specific inoculum in all soils would be consistent with this hypothesised role. Members of the Glomaceae and Gigasporaceae also differ in phosphorus transfer mechanisms, with members of the Glomaceae transferring far more phosphorus to the plant than members of the Gigasporaceae in exchange for the same amount of carbon (Pearson & Jakobsen, 1993). Lack of growth response of *Lophira* seedlings, compared to the significant growth response of Padouk seedlings, would be consistent with the hypothesised differences in phosphorus exchange efficiency.

Our results clearly demonstrate that addition of host tree specific inoculum, just like addition of grass inoculum can produce substantially larger seedlings of timber tree species in the rain forest of south Cameroon (CHAPTER 7). The present study for the first time tried to separate the effects of inoculum quantity and inoculum quality. These calculations suggest that growth of *Pterocarpus* is equally limited by inoculum quality and quantity, and that *Lophira* is only limited by inoculum quality. The importance of inoculum quality also might be due to the fact that intensification of land use practices could select for fungi that are less beneficial for the plant. Johnson (1993) noted that fertilisation of soils might lead to less mutualistic mycorrhizal fungi, and Egerton-Warburton & Allen (2000) observed that nitrogen enrichment changed arbuscular mycorrhizal fungal community structure by selecting for less effective mycobionts. In the latter study the altered mycorrhizal community structure favoured grasses over shrubs leading to changes in vegetation structure. As both agricultural fields and fallow have an increased nutrient status (M. Yemefack & L. Nounamo, unpublished observations), similar changes in mycorrhizal fungal community composition and a decline in the quality (effectiveness) of the mycorrhizal inoculum could constrain secondary succession in tropical areas.

Differences between tree species in dependency on and responsiveness to arbuscular mycorrhizal fungi has been attributed to various plant characteristics. Janos (1996) proposed

that early successional species are generally less dependent on mycorrhizas than late successional and climax forest tree species. The higher responsiveness of *Pterocarpus* to inoculum addition than that of *Lophira* is consistent with this suggestion. Root features have also been implicated as a determinant of mycorrhizal dependency and responsiveness. Incidence of root mass appears to be more important than other root features such as root diameter, root density, root hair incidence, and root hair length (Manjunath & Habte, 1991b). Species with a higher root production are predicted to be less dependent on mycorrhizas. In this study, *Pterocarpus* and *Lophira* markedly differed in root production but were similar in their lack of root hairs. *Pterocarpus* seedlings had a low root production and *Lophira* seedlings yielded abundant roots. Differences in MIE are also consistent with these patterns of root production. Finally Janos (1980) suggested that non-mycotrophic and facultatively mycotrophic species have lighter seeds than obligately mycorrhizal species. Our data do not conform to this suggestion, as *Pterocarpus* was more responsive to mycorrhizal inoculum addition than *Lophira*. The phosphorus requirements of a nodulating legume might make such species more mycotrophic, independent of seed size. However, in an earlier study with three timber species (CHAPTER 7), the heavy-seeded *Distemonanthus benthamianus* (Caesalpiniaceae) had a larger MIE than the light-seeded (*Terminalia superba* (Combretaceae).

Poor growth of both seedlings along skid trails and bare landings may arise from negative impacts on physical and chemical soil surface properties in addition to reduction in inoculum potential. A substantial increase in bulk density and soil compaction has been recorded along skid trails and landings, which might be responsible for bad performance of seedlings on soils of forestry practices. We regularly observed seedlings of both species on landings, but they all died within a few years. However, in our experiment *Pterocarpus* seedlings grew very well in soils from landings with *Musanga* in the presence of indigenous inoculum.

In conclusion, our results demonstrate that seedlings of timber species do not always form effective mycorrhizal associations with all arbuscular mycorrhizal fungal communities, confirming the hypothesis that both quantity and quality of the mycorrhizal propagules in soils determine seedling fitness. For sustainable timber management and conservation, there is a need for the evaluation of inoculum quality and quantity for the effective and rapid mycorrhizal formation of seedlings of important timber species. Both aspects need to be determined for the performance of the fifteen most highly appreciated timber species of Cameroon.

# CHAPTER 9

# GENERAL DISCUSSION

*"Before we fully appreciated the vital role that mycorrhizal symbiosis plays in the lives of many plants, what kind of value would we have assigned to the tiny, threadlike fungi in the soil that make those relationships possible?"* (Ehrenfeld, 1988)

## INTEGRATION OF MYCORRHIZAL KNOWLEDGE IN SUSTAINABLE FOREST MANAGEMENT

Sustainable forest management has become the major paradigm for the use of tropical rain forests. Sustainable management is based on understanding and maintaining the components, processes, and interactions of ecological systems, while simultaneously producing timbers, non-timber forest products, and services for the community. Next to the ecological component of sustainability, economic and social aspects of sustainability are equally important, however they are not discussed here (see Eba'a, 2000; Van Dijk, 1999).

Mycorrhizal fungi perform key roles in the functioning of tropical rain forest ecosystems and for that reason constitute important components in forest management. Therefore, successful management of forest ecosystems requires the integration of knowledge of functional and taxonomic aspects of biodiversity of mycorrhizal fungi in future management plans. In most cases, management plans are preceded by floristic inventories (or at least inventories of the major timber tree species) for forest management units. This inventory should result in a classification of forest stands. It is clear from this thesis that in such classifications the mycorrhizal habit of trees in these forest management units should not be neglected. Emphasis should not be put on establishing whether trees are mycorrhizal, as in the rain forest of South Cameroon (almost) all tree species are mycorrhizal (CHAPTER 2), but on the kinds of mycorrhizal associations as both ectomycorrhizal and arbuscular mycorrhizal trees occur in the same forest stands (CHAPTER 2). While occurrence of arbuscular mycorrhizal trees in tropical rain forests is well known, the presence of ectomycorrhizal trees in such stands has received less attention. Therefore, within the Tropenbos Cameroon Programme (TCP) area, the forest inventory should account specifically for ectomycorrhizal trees, which usually occur in clumps. This recommendation to give special emphasis for clumps of ectomycorrhizal trees should improve upon the actual procedure on floristic inventories in Cameroon. The present ONADEF procedure does not include knowledge of the mycorrhizal habit of the trees and hence could neglect the ecologically sensible ectomycorrhizal component of such forests. Debroux (1988) suggested that in forest areas where ectomycorrhizal trees are widespread, such as in the Dja Faunal Reserve (Southeast Cameroon), such clumps could be mapped on the basis of aerial photographs.

Giving recognition, and, by implication, special management status, to ectomycorrhizal forest communities contributes not only to the preservation of forest refuges of southern Cameroon with several endemic species (Letouzey, 1985), but also to the preservation of the biodiversity of Africa's rainforest ectomycorrhizal fungi, whose inventories have only recently begun (CHAPTER 3). Maintenance of such ectomycorrhizal forest communities with their associated

mushrooms such as chanterelles also helps preserving a valuable source of an alternative protein-rich diet for local communities who depend on the forest (Malaisse, 1997). The percentage of protected areas (limited only to national parks, natural reserves, and few sanctuaries) in Cameroon is still very low in comparison with recommended standards by international organisations. Currently, only 4.41% (20,974 $km^2$) out of 475,000 $km^2$ (IUCN, 1999) are under a certain form of protection, suggesting that much effort is still needed to achieve the recommended 10 % level. Increasing the percentage of protected areas should especially be helpful for maintenance of the mixed arbuscular mycorrhizal – ectomycorrhizal forest.

Another argument that strongly supports this conservation measure is that that approach to natural silviculture techniques of ectomycorrhizal trees and forest clumps may require different techniques from those currently in practice, viz. removal of dominant or co-dominant trees to favour stems of the future, using either arboricides or girdling. The results from these studies indicate that such techniques could reduce or even eliminate the ectomycorrhizal fungal and tree diversity. Hence, research is urgently needed for ecologically sound natural silvicultural procedures to safeguard ectomycorrhizal associations.

Several authors (Oates, 1999; Terborgh, 1999) have expressed pessimism about the combinability of biodiversity conservation and sustainable forest management. The work done by Eba'a (2000) lends support to the suggestion that there is a potential conflict between biodiversity conservation and sustainable forestry. However, the scope of that conflict crucially depends on the number of tree species that are included in the forestry programme. The model calculations of Eba'a (2000) indicated that on a 30-years rotational scheme 117 $m^3.ha^{-1}$ could be harvested; however, the most commercialised species contributed only slightly over 10% of that volume. Enrichment planting could increase the fraction that is harvested but inevitably results in a reduction of tree species diversity. Increasing the number of species that can be commercialised, while accepting a lower monetary reward because some of these species are less valuable, could help in reconciling conservation and management goals. Including ectomycorrhizal trees, hitherto mostly considered as potentially exploitable timber species, could be part of such a strategy. The challenge, then, is to find ways in which ectomycorrhizal trees could be harvested without jeopardising their regeneration and subsequent establishment, as their clumped behaviour suggests that their dispersal and recolonisation capacities are restricted. This question on sustainable management of ectomycorrhizal trees cannot be addressed yet.

**STABILITY OF ECTOMYCORRHIZAL AND ARBUSCULAR MYCORRHIZAL FORESTS**

Co-occurrence of ectomycorrhizal and arbuscular mycorrhizal trees in the same tropical forest area, where islands of ectomycorrhizal trees occur in a larger matrix of arbuscular mycorrhizal trees, has intrigued ecologists. Connell & Lowman (1989) suggested that clumping was causally related to the ectomycorrhizal habit, due to the more limited dispersal ability of ectomycorrhizal trees and fungi on the one hand and competitive superiority of ectomycorrhizal trees on the other. Competitive superiority was hypothesised to be due to the production of litter with a lower decomposition and mineralisation rate, conferring benefits to ectomycorrhizal fungi with the ability to take up organic nitrogen and phosphorus. However,

such clumping behaviour in Africa's rain forest is neither unique to ectomycorrhizal caesalps nor to caesalps in general. In Cameroon, monospecific stands of arbuscular mycorrhizal trees have been observed in *Garcinia lucida* (Clusiaceae) within the TCP area (N.A. Onguene & N. Guedje unpublished observation), and on the hill summits of Mbam Minkom, near Yaoundé (Achoundong, 1996), *Lophira alata* (Ochnaceae) in the Campo-Ma'an National Park (R. M. Bibani & J. E. Etoundi, pers. comm.), and *Coula edulis* (Olacacaceae) in the Dja Faunal Reserve (Seme, 1998). In Gabon, near pure stands of the arbuscular mycorrhizal tree *Aucoumea klaineana* (Burseraceae) are widely distributed in its natural growing area (Fuhr et al., 1998; N.A. Onguene & J. E. Etoundi, unpublished observation). Evidence for competitive superiority of ectomycorrhizal trees could not be obtained in a comparison of the ectomycorrhizal *Tetraberlinia korupensis* (Caesalpiniaceae) and *Oubangia alata* (Scytopetalaceae) in Korup National Park, Cameroon (Moyersoen et al., 1998a) and a comparison of $^{15}N$ patterns of ectomycorrhizal and arbuscular mycorrhizal trees did not suggest differential access to nitrogen sources (Högberg & Alexander, 1995). A comparison between the TCP area and Korup National Park made clear that both sites are equally characterised by an abundance of ectomycorrhizal trees, whereas they largely differ in soil and climate characteristics (CHAPTER 2). Edaphic specialisation of ectomycorrhizal trees seems therefore implausible. It is likely that arbuscular mycorrhizal and ectomycorrhizal trees do not differ in their edaphic niches, but in their regeneration niches.

As seeds of ectomycorrhizal caesalps disperse only over a short distance and do not have dormancy, and as spores of ectomycorrhizal fungi, in contrast to those of arbuscular mycorrhizal fungi, have extremely limited reserves, it is likely that establishment of ectomycorrhizal trees is a chance event and that subsequent recruitment of seedlings of the same or different species in such islands is facilitated by their integration into the ectomycorrhizal network. The data presented in CHAPTER 6 provide support for the importance of the ectomycorrhizal network. For sustainable exploitation of ectomycorrhizal trees removal of the canopy emergents might hamper regeneration of the seedlings as they might not possess the photosynthetic capacity to sustain the large ectomycorrhizal biomass. Low dispersal of such ectomycorrhizal islands is also suggested by the present-day distribution of these ectomycorrhizal caesalps, which possibly reflect Pleistocene refugia (Sosef, 1994).

The limited dispersal and hence colonisation capacities of ectomycorrhizal trees could be offset by their higher longevity. Data on longevity of tropical trees are scarce (Martínez-Ramos & Alvarez-Buylla, 1999). For caesalp trees, the ectomycorrhizal species *Dicymbe altsonii* was calculated to have a mean age at which newborns produce offspring of 467 years and a life span of 567 years (Zagt, 1997). On the basis of palaeoclimatological criteria Newbery et al. (1998) suggested that in Korup National Park the ectomycorrhizal species *Microberlinia bisulcata* established in the second half of the 18$^{th}$ century (between 1765 and 1799). Data on management cycles for forestry practices, now ranging from 15 to 80 years, must include data on tree life span, as cutting cycles that are too short will have a negative impact on tree species diversity. If ectomycorrhizal caesalps are at a higher risk at short cutting cycles, their extinction might lead to further extinctions involving not only the ectomycorrhizal fungi. Newbery et al. (1998) observed mass flowering and mast fruiting

(similar to mast fruiting of the ectomycorrhizal Dipterocarpaceae in south-east Asia: Ashton et al., 1988) in a three-year cycle for *Microberlinia bisulcata*. Such cycles of abundance and fasting could well constrain the population dynamics of frugivorous animals (Onguene et al., 2000).

**DIVERSITY AND REDUNDANCY OF ECTOMYCORRHIZAL FUNGI**

Assessment of inoculum potential of ectomycorrhizal fungi yielded large differences between disturbance stages and smaller differences between sites (CHAPTER 5). Selective logging practices resulted in a complete disappearance of ectomycorrhizal inoculum, indicating that sustainable management and maintenance of the ectomycorrhizal component of Cameroon's rain forests needs special attention. The effects of agricultural practices on the inoculum potential were dependent on the ectomycorrhizal host tree used for baiting. Different ectomycorrhizal host trees also perceived different inoculum potentials in early and late successional forests, and, even more surprisingly, in ectomycorrhizal clumps (CHAPTER 5). While *Tetraberlinia bifoliolata*, a species characteristic for ectomycorrhizal clumps, indicated an increase of ectomycorrhizal inoculum during succession, *Afzelia bipindensis*, a tree species, which usually established as individuals in earlier successional stages and was not observed in these clumps, indicated a relatively high ectomycorrhizal inoculum in agricultural fields and fallow, and a much lower inoculum in (late) successional forests. Different ectomycorrhizal inoculum potentials for both species made clear that the specificity of the ectomycorrhizal symbiosis is important. However, the causes for this host tree specificity have not been understood. Specificity could have a basis in the taxonomy of the host trees. *Tetraberlinia* is a member of the exclusively ectomycorrhizal tribe Amherstieae, while *Afzelia* is a member of the predominantly arbuscular mycorrhizal tribe Detarieae (Alexander, 1989b). Assuming that the ectomycorrhizal habit evolved independently in both tribes, it is possible that different fungi associated with both groups. Host plant specificity has been observed for the liana *Gnetum*, where the dominant species *Scleroderma sinnamariense* has never been observed with other trees. Even though soils taken under *Gnetum* yielded ectomycorrhizal colonisation of *Tetraberlinia* seedlings, the conspicuous bright yellow mycorrhizas of *S. sinnamariense* were absent. Apparently other ectomycorrhizal fungi of *Gnetum* show less specificity, but these fungi have not yet been identified, as no fruitbodies of other ectomycorrhizal species where found associated with the liana. Host tree specificity is unlikely for the genus *Uapaca* (Uapacaceae), which occurs regularly in the ectomycorrhizal clumps, where observations on fruitbodies of ectomycorrhizal fungi indicated a very large similarity with the species composition under ectomycorrhizal caesalps. Data by Thoen & Bâ (1989) on different species composition of ectomycorrhizal fungi associated with *Uapaca guineensis* and *Afzelia africana* are consistent both with a hypothesis that host tree specificity has a taxonomic basis and an ecological basis, as both trees had different edaphic requirements. Under the hypothesis that host tree specificity has a taxonomic basis, all members of the tribe Amherstieae should have the potential to be interlinked in a common ectomycorrhizal network. However, not all members of that tribe occur regularly in these clumps. Members of the genus *Berlinia* and *Anthonota* occurred usually solitarily in younger

forests. The way in which these species perceive ectomycorrhizal inoculum potential in various vegetation types has not been studied.

Mycofloristic inventories could contribute to the assessment of the specificity of the ectomycorrhizal inoculum. Whereas the ectomycorrhizal clumps were very rich in ectomycorrhizal fungi (CHAPTER 3), hardly any ectomycorrhizal fungi have been encountered in secondary successional stands with isolated ectomycorrhizal trees. Within the ectomycorrhizal clumps it was virtually impossible to determine which fungi were associated with which trees. Morphotyping and molecular identification of the ectomycorrhizas will be needed to answer the question about the identity and host specificity of the various ectomycorrhizal fungi.

In the absence of reliable data on specificity of the ectomycorrhizal fungi, it is premature to establish whether certain fungal species are keystone species as originally defined by Paine (1966), i.e. species whose losses cause subsequent extinctions cascading through the biotic community. Even though it is widely accepted that different mycorrhizal fungi fulfil different roles, the presence of a large number of congeneric species (*Amanita*: 30 species; *Russula*: 29 species) will more likely show redundancy, making these communities more resilient. However, there is no doubt that the ectomycorrhizal fungi as a group constitute a keystone guild, fulfilling key roles in the maintenance of tree species diversity.

A key role has been suggested for tree species that form dual mycorrhizas, functional symbioses with both ectomycorrhizal and arbuscular mycorrhizal fungi. Consistent dual mycorrhizas have been noted for members of the genus *Afzelia* (CHAPTER 2). Dual mycorrhizal plants could be hypothesised to connect the arbuscular mycorrhizal and ectomycorrhizal components of forest succession. However, for the TCP area such a key role seems less likely now, as (1) most (if not all) ectomycorrhizal trees can be effectively dual mycorrhizal (Moyersoen & Fitter, 1999; Van der Heijden, 2000); (2) the presence or amount of arbuscular mycorrhizal colonisation did not affect seedling performance in *Afzelia* (CHAPTER 5); (3) ectomycorrhizal fungi of *Afzelia* and the clump-forming caesalps showed only a limited degree of compatibility. However, the compatibility between *Gnetum* mycorrhizas and those of *Tetraberlinia*, suggests that maintenance of *Gnetum*, next to its importance as an important dietary component, could be important for regeneration of ectomycorrhizal clumps.

**DIVERSITY AND REDUNDANCY OF ARBUSCULAR MYCORRHIZAL FUNGI**

Within the TCP area, the number of spores of arbuscular mycorrhizal fungi in secondary and primary rain forests was quite high (CHAPTER 4). In fact, spore numbers were much higher than suggested by Janos (1992, 1996) on the basis of data from Central and South America. This discrepancy has not been explained to date. The degree of colonisation of bait plants in intact and disturbed soil cores was consistent with the observation that spores formed a substantial part of the mycorrhizal inoculum, with a smaller role for the intact mycorrhizal network. An important consequence of high spore inoculum of arbuscular mycorrhizal fungi is that such forests are quite resilient to small-scale disturbances. However, it does not follow from these data that the forests would remain resilient against larger-scale disturbances.

Creation of skid trails and landings always resulted in drastic decrease of population density and effectiveness of mycorrhizal fungi with slow recovery, thus leading to diminished plant biomass. Destructive soil surface features such as removal of litter, erosion, and compaction were observed on logged-over sites and these could persist for more than ten years. Substantial increases in bulk density of 29 % and 38 % were recorded from undisturbed forest stand (1.09 $g.cm^{-3}$) to skid trail and bare soil landings, respectively; penetrometer resistance, compared to undisturbed forest, increased between 17 % along skid trails to 36 % in landings (P.A. Nguengang & N.A. Onguene, unpublished observation). Consequently, such negative impacts on soil surface properties, mycorrhizal fungi, and plant growth performance call for a reduced impact logging. Such techniques, essential to reduce deleterious effects of logging operations on the forest canopy and soil environment, include reduction of the area impacted, such as reduction of trafficking, of length of skid trails, and of the number of landings. There is also a need to minimise soil erosion. Most roots, and hence, the largest part of the arbuscular mycorrhizal inoculum, are located in the uppermost 5 cm (Bellgard, 1993; Brundrett, 1991). The consequences of lack of sufficient inoculum could be grave. Janos & Hartshorn (1996) reduced arbuscular mycorrhizal fungus inocula in Costa Rica by clear cutting and subsequent soil fumigation and noted that after 20 years of secondary succession tree density, basal area, and tree species richness were significantly lower than on non-fumigated plots. Data from the TCP area support the claim that strong reduction of mycorrhizal inocula on sites of forestry practices could constrain subsequent regrowth of the forest. The data in CHAPTERS 7 and 8 indicate that inoculum addition to soils of forestry practices had a very large positive effect on colonisation and biomass increment of various seedlings of major economic importance.

Interestingly, inoculum potential of arbuscular mycorrhizal fungi was also very low in ectomycorrhizal clumps (CHAPTER 5). How rapidly would arbuscular mycorrhizal fungi invade such clumps after disturbance? It would be important to address this question in future studies. If recolonisation by arbuscular mycorrhizal fungi is slow, the scarcity of inoculum could well constrain agricultural activities on such sites. Local farmers suggested that sites of ectomycorrhizal clumps have an inherent lower soil fertility and are less suited for agriculture after slashing and burning than sites with arbuscular mycorrhizal trees. This suggestion was surprising, as data from Korup National Park suggested that phosphorus and nitrogen cycling rates under ectomycorrhizal clumps were around 50% higher than in stands without ectomycorrhizal trees (Newbery et al., 1997).

Spore number and colonisation of bait plants in soil cores were always high in agricultural fields and *Chromolaena* fallow (CHAPTER 4). Plant growth was always high, and addition of inoculum usually did not result in improved performance of seedlings of various timber trees (CHAPTER 7 and 8). In late successional forest plant growth could often be improved after inoculum addition, even though spore numbers were relatively high.

While spore numbers (and colonisation of bait plants) were highest in agricultural fields and *Chromolaena* fallow, it does not follow that having higher amounts of inoculum is automatically better. It has been observed that soils with a higher mycorrhizal inoculum potential are not always most effective in enhancing plant growth (Asbjornsen & Montagnini, 1994). This could be due to selection for heavily sporulating fungal species, which do not

necessarily increase benefits, in agricultural fields and fallow. Data by Johnson (1993) and Egerton-Warburton & Allen (2000) provided evidence that increased fertiliser use could select for less mutualistic mycorrhizal fungi. If the same phenomenon occurs in tropical sites (and some evidence for it was observed in agricultural fields and fallow, where inoculum addition sometimes resulted in a lower colonisation or seedling biomass), species selection for revegetation purposes could be paramount (Herrera et al., 1997). CHAPTERS 7 and 8 compared the effects of indigenous inoculum and inoculum addition on seedling colonisation and performance. It was clear from the data with five different timber species, both characteristic for younger and more mature forests, that both the quantity of inoculum and the quality of the inoculum have to be considered in reforestation practices.

Inoculum quality is related to the identity of the fungal species and the specificity of those fungal species for certain host trees. It was not possible during these studies to identify the arbuscular mycorrhizal fungi in the various vegetation types. However, observations on spore types after extraction indicated that a large number of different types were present. In undisturbed rain forest in Cameroon around Mbalmayo 17 species of arbuscular mycorrhizal fungi were observed, and in Ivory Coast in undisturbed forest 16 species were present (Mason et al., 1992; Wilson et al., 1992). In the latter study species richness of disturbed sites was also included. In plantations of *Terminalia superba* 41 different species were observed. This number represent around 25% of the biodiversity of arbuscular mycorrhizal fungi known worldwide! Clearly, an inventory of species diversity of arbuscular mycorrhizal fungi in the TCP area remains a top priority for future research. Such an inventory should also be followed by an assessment of the various roles that the arbuscular mycorrhizal fungi could play, as it has become increasingly clear that the arbuscular mycorrhizal symbiosis shows multifunctionality in various respects (Newsham et al., 1995): the same fungus having different effects on different plants, depending on root characteristics of the plant; and different fungi having different effects on the same plant, related to their ecological roles such as phosphorus uptake, protection against biotic stresses (pathogens), and soil aggregate stabilisation. Both aspects of multifunctionality could also provide a basis for specificity of the arbuscular mycorrhizal symbiosis. This question has not been systematically addressed, however, it was observed that roots of *Pterocarpus soyauxii* and *Musanga cecropioides* were mainly colonised by members of the Glomaceae, and *Lophira alata, Erythrophloeum ivorense,* and *Theobroma cacao* by members of the Gigasporaceae (CHAPTER 8). Assessment of host tree specificity of a larger range of economically important timber species would also remain an important future project. Failure of artificial regeneration of indigenous tree species in plantation forestry in the tropics might well bear a causal relationship to the issue of ecological specificity. Separate assessment of the roles of inoculum quantity and inoculum quality, for which a provisional formula was proposed in CHAPTER 8, would help in understanding under what conditions the identity of arbuscular mycorrhizal inoculum (Herrera et al., 1997) is more and less important. The data for 5 timber tree species would suggest that the importance of inoculum quality and quantity is different for trees that are characteristic for relatively young forests (and hence are indicators of human impact on the forest, such as *Lophira alata*) and for undisturbed forest (such as several members of the arbuscular mycorrhizal Caesalpiniaceae).

**RESEARCH NEEDS IN TROPICAL MYCORRHIZAL TECHNOLOGY AND SCIENCE**

The results of this study indicate that current agricultural practices are not detrimental to inoculum potential of arbuscular mycorrhizal fungi and that productivity of food crops is still maintained. The regeneration potential of fallow land with *Chromolaena odorata* seems also sufficiently high, if instead of agriculture plantation forestry should be planned. On the other hand, most ectomycorrhizal host trees cannot find a suitable mycobiont in agricultural fields and fallow. Additionally, it would be interesting to determine whether abandoning ectomycorrhizal tree species in plots opened for cropping just like it is done for trees with social importance to local populations could maintain the diversity and abundance of ectomycorrhizal associations in agricultural land.

The staggering rate of deforestation in the tropics ultimately argues for plantation forestry and artificial regeneration. Ideally, it should pay off to replant native tree species and to maintain the original level of biodiversity. The results of this study show that different sources of mycorrhizal inocula potentially elicit different host responses. Thus, selection of the most appropriate inocula will become a must for successful attempts to artificial regeneration. For almost completely denuded sites of forestry practices, the simple saying that any arbuscular mycorrhizal inoculum is better than none, holds, and addition of grass inoculum could be successful for revegetation (Cuenca et al., 1998). Native and host tree specific inoculum shows promises for the regeneration of more demanding species (CHAPTER 8). But a huge research effort is required for the development of an inoculation programme for reforestation of degraded forest lands with indigenous tree species, notably the fifteen most harvested timber species, using native mycorrhizal inoculum. A collection of cultures of both indigenous arbuscular mycorrhizal and ectomycorrhizal fungi, maintained in Cameroon (or somewhere else in Africa, provided that there is efficient access for Cameroonian researchers) will be an essential tool for that programme.

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**SUMMARY**

This thesis deals with mycorrhizal associations in rain forests of south Cameroon under various disturbance regimes and stages in order to provide essential information on the roles of mycorrhizas in tree establishment and forest recovery. Western Africa's rain forest ecosystems contain representatives of the two major kinds of mycorrhizal association, viz. arbuscular mycorrhiza and ectomycorrhiza. However, knowledge how both mycorrhizal associations interact and how these different kinds of mycorrhizal forest communities function is fragmentary. It is therefore unclear how disturbances as a consequences of forestry practices and shifting cultivation will affect inoculum potential of arbuscular mycorrhizal and ectomycorrhizal fungi, and regeneration and growth of seedlings of arbuscular mycorrhizal and ectomycorrhizal trees. It is also unknown how these various disturbances affect the biodiversity of arbuscular and ectomycorrhizal fungi. Knowledge on the various kinds of mycorrhizal associations, their diversity, and their dynamics is an important prerequisite for sustainable management of below-ground ecosystems, and consequently for the sustainable management of forest ecosystems. The research approach adopted in these investigations involved several steps:

1. Investigation in undisturbed forest stands of the mycorrhizal status of selected timber species of economical, social, and ecological significance, and its relation with distribution and abundance of mycorrhizal associations.
2. Inventory of species diversity of ectomycorrhizal fungi.
3. Study of changes in populations and activities of mycorrhizal fungal propagules in relation to stages of soil disturbance induced by practices of shifting cultivation and selective commercial logging.
4. Assessment of the importance of intact ectomycorrhizal networks as a source of inoculum for ectomycorrhizal formation and establishment of seedlings around timber species.
5. Evaluation of the regeneration potential and growth response of seedlings of two native ectomycorrhizal and five native arbuscular mycorrhizal timber species in soils with indigenous inoculum potential. For the arbuscular mycorrhizal trees the effect of inoculum addition, either of inoculum derived from a grass field or inoculum derived under mother trees, was also studied to assess the importance of inoculum quantity and inoculum quality.

Mycorrhizal root colonisation of selected tree and liana species, and contribution of arbuscular mycorrhizal and ectomycorrhizal trees to stem number and basal area in undisturbed forest stands are described in CHAPTER 2. All one hundred plant species investigated (belonging to seventy-seven genera and twenty-nine families) were mycorrhizal. Seventy-four tree species formed only arbuscular mycorrhizas; twenty-three tree and three liana species (belonging to thirteen genera and three families: Caesalpiniaceae, Uapacaceae, Gnetaceae) formed ectomycorrhizas of which five (dual mycorrhizal species) also harboured arbuscular mycorrhizal fungal structures but to a different extent. Among the thirteen ectomycorrhizal genera, eleven belonged to the Caesalpiniaceae (ten genera of tribe Amherstieae, one genus of tribe Detarieae). Most species of tribe Amherstieae are locally known to timber prospectors as

Ekop. Contribution of ectomycorrhizal trees to basal area of forest stands varied with sites; it was lowest in Ebom (the youngest stand) and was highest (about 70 % basal area) in a nearly pristine rain forest in Bityili.

Both major types of mycorrhizal associations occurred in all three sites, however, ectomycorrhizal trees usually formed distinct patches due to their clumped behaviour. Arbuscular mycorrhizal associations dominated most vegetation stands, with the exception of these ectomycorrhizal clumps. Some ectomycorrhizal species occurred also in low stem numbers in arbuscular mycorrhizal forest stands, including species of *Afzelia, Anthonotha, Berlinia,* and *Uapaca*. *Afzelia* was never encountered in ectomycorrhizal clumps. In fallow of the exotic weed *Chromolaena odorata*, the only ectomycorrhizal taxon was *Gnetum*. Co-occurrence of arbuscular mycorrhizal and ectomycorrhizal forest communities raises questions about their coexistence under disturbance regimes (climatic changes, human-induced disturbance) and about the importance of edaphic or regeneration niche differentiation.

In CHAPTERS 2 and 3, the species richness of arbuscular mycorrhizal fungi and ectomycorrhizal fungi is treated. Root examination revealed that all three families of the Glomales (the fungi forming the arbuscular mycorrhizal symbiosis) were present: Glomaceae (*Glomus*), Acaulosporaceae (*Acaulospora*), and Gigasporaceae (*Gigaspora*, *Scutellospora*). Root colonisation patterns of different tree species suggested that certain fungi preferentially colonised certain trees. Spore morphology indicated that a number of different fungal taxa were present, however these fungi have not yet been identified and an inventory of arbuscular mycorrhizal species diversity remains a high priority for Cameroon. More than 125 species of ectomycorrhizal fungi were identified, mainly from near pristine forests in ectomycorrhizal clumps, either near the stem base o*f Uapa*ca species or those of the caesalps, while one further fungal species was found in association wit*h Gnet*um. Among the ectomycorrhizal fungi, the Amanitaceae, Russulaceae, Boletaceae, and Cantharellaceae were (very) well represented, whereas only a few species of Cortinariaceae, Sclerodermataceae, Gomphaceae, Clavulinaceae, and Hymenochaetaceae were observed (CHAPTER 3). Species composition was largely similar among sites, suggesting a homogeneous species composition of ectomycorrhizal fungi within the TCP area. The same ectomycorrhizal fungi (and the same ectomycorrhizal trees) also occur in Korup National Park, Cameroon, even though soils and climate are very different between Korup and the forests of the TCP area.

Differences in inoculum potential of arbuscular mycorrhizal fungi (CHAPTER 4) and of ectomycorrhizal fungi (CHAPTER 5) in vegetation types that belong to different stages of disturbance as a consequence of shifting cultivation and selective logging practices were described. Spores of arbuscular mycorrhizal fungi mainly contributed to inoculum potential of soils of the TCP area, as shown by the significantly positive correlation between spore numbers and colonisation of bait plants. Apparently, the arbuscular mycorrhizal network was of minor significance for the inoculum potential. Spore numbers and colonisation rates were similar across sites, again indicating that the TCP area is homogeneous, despite differences in soil texture, pH, and phosphorus availability. Spores of arbuscular mycorrhizal fungi were relatively abundant in late stage forests (compared to similar forests in Central and South America), however in ectomycorrhizal clumps inoculum potential of arbuscular mycorrhizal

fungi was very low. Inoculum potential of arbuscular mycorrhizal fungi was much higher in agricultural fields and fallow. After secondary succession starts and fallow reverts to early and late successional forest, spore numbers again drop. Inoculum potential was (very) considerably decreased by forestry practices compared with primary forest, and this negative impact could persist for over a decade. Such low mycorrhizal inoculum potential raised the question whether tree seedling establishment and growth was hampered by lack of inoculum, and whether seedling performance could be boosted by mycorrhizal inoculum addition.

In contrast, inoculum potential of ectomycorrhizal fungi was more dependent on the intact ectomycorrhizal network. Inoculum potential of ectomycorrhizal fungi was different for two ectomycorrhizal tree species, suggesting an important effect of host tree specificity of ectomycorrhizal fungi. When *Tetraberlinia bifoliolata* (a clump-forming caesalp) was used as a bait plant, ectomycorrhizal inoculum was present in fallow with *Gnetum* and in various forest disturbance stages, increasing to highest amounts in ectomycorrhizal clumps. When *Afzelia bipindensis* (a solitarily growing caesalp) was used, inoculum was highest in agricultural fields and declined during succession towards old-growth forest. In sites of forestry practices (skid trails, bare and vegetated landings) ectomycorrhizal inoculum was absent, indicating that current days forestry practices might not guarantee the maintenance of the ectomycorrhizal forest component. Seedling growth in soils from various land use practices correlated well with the amount of ectomycorrhizal inoculum present. A key role of dual mycorrhizal plants such as *Afzelia bipindensis* for forest regeneration could not be demonstrated (CHAPTER 5). Data from CHAPTERS 4 and 5 did not provide evidence for edaphic niche specialisation of arbuscular mycorrhizal and ectomycorrhizal trees, suggesting that the maintenance and coexistence of both mycorrhizal forest communities depends on differences in their regeneration niche.

In CHAPTER 6 the importance of the ectomycorrhizal network for formation of ectomycorrhiza and plant performance was assessed. Seedlings of the ectomycorrhizal caesalp *Paraberlinia bifoliolata* in contact with roots of adult trees proved more efficient in enhancing ectomycorrhiza formation and establishment than seedlings that were isolated from the roots of a mature tree. The area of high ectomycorrhizal inoculum potential coincided more or less with that of the crown projection of the ectomycorrhizal tree. Rate of ectomycorrhiza formation was also influenced by the degree of host specificity of the ectomycorrhizal inoculum, confirming results from CHAPTER 5. The management implication of CHAPTERS 5 and 6 is that management of the ectomyorrhizal forest communities needs special attention, considering the importance of the mycelial network and the limited dispersal abilities of both ectomycorrhizal fungus and ectomycorrhizal tree with large seeds.

In CHAPTER 7, arbuscular mycorrhizal colonisation and growth response of three major timber tree species *Terminalia superba, Distemonanthus benthamianus,* and *Entandrophragma utile* to indigenous inoculum potential and addition of grass inoculum were studied in soils collected under various vegetation types that correspond with disturbance stages. Both fungal colonisation and plant response depended on the timber tree, inoculum potential of the vegetation type, and inoculum addition. Mycorrhizal colonisation and seedling growth was low in soils with low inoculum potential such as skid trails, landings, and late successional stands; simultaneously the mycorrhizal inoculation effect was large. In soils from agricultural

fields and fallow inoculum potential was high, colonisation rate high and plant response good, and mycorrhizal inoculation effect was low or even negative. *Terminalia superba* was only slightly responsive with a strong colonisation increase, whereas with *Distemonanthus benthamianus* the opposite pattern was noted*; Entandrophragma utile* showed an intermediate response. The data from this chapter confirm that arbuscular mycorrhizal inoculum potential is a good predictor of mycorrhizal colonisation and subsequent seedling growth. They also show that in sites of low mycorrhizal inoculum, inoculum addition can lead to more successful regeneration of timber trees. Although lack of sufficient mycorrhizal inoculum in some sites indicates that inoculum addition is potentially successful, it should not be forgotten that host specificity of arbuscular mycorrhizal fungi for certain tree species could also play a role. While the adage that any inoculum is better than none holds for severely depauperated disturbance stages due to forestry practices, successful regeneration of some timber trees could be better if host tree specific, high-quality inoculum is applied. In CHAPTER 8, mycorrhizal colonisation and growth response of *Pterocarpus soyauxii* and *Lophira alata* were assessed in soils from various disturbance stages in relation to indigenous inoculum potential and addition of host tree specific inoculum that was taken from the rhizosphere of a conspecific tree. An attempt was made to assess the effect of inoculum quantity and inoculum quality separately. Results indicated that inoculum quality could also be a major determinant of the mycorrhizal inoculation effect, especially for the late successional, nitrogen-fixing timber tree *Pterocarpus soyauxii*. For *Lophira alata*, the effect of inoculum quality was much lower, except in sites of forestry practices.

CHAPTER 9 integrates the results of the previous chapters. It draws attention to the relationship between mycorrhizal inoculum potential and plant response, and to the ways in which human-induced forest disturbance as a consequence of shifting cultivation and forestry practices affect possibilities for sustainable management. It also demonstrates that there are large differences between arbuscular mycorrhizal and ectomycorrhizal inoculum, and hence in ways in which disturbances could affect the maintenance of arbuscular mycorrhizal and ectomycorrhizal tree species diversity. Considering the high rates of deforestation in the tropics, it is unlikely that sustainable management of rain forests is possible without jeopardising tree species diversity. Enrichment planting and plantation forestry could be sustainable although a trade-off with tree species diversity is likely. Considering the differences in regeneration niches between arbuscular mycorrhizal and ectomycorrhizal trees, sustainable management should take mycorrhizal associations of tropical trees into account. As the ectomycorrhizal forest communities seem more vulnerable to various forms of disturbance, they are in need of special conservation status. Because of differences in fungal specificity for ectomycorrhizal trees that usually grow solitarily and that commonly grow in clumps, both tree species and forest types need to be carefully managed. For *Afzelia* (and other) trees occurring in isolation, small forest refugia should be created around them in forests opened to logging. Such refugia can also serve for wildlife and avifauna, and could constitute the seed reservoir for the recolonisation of degraded forest lands. Ectomycorrhizal forest clumps should be integrally protected. The sampling method used in Cameroon in management inventory should be modified to include ectomycorrhizal forest clumps. The first step should aim at mapping ectomycorrhizal clumps so that they are clearly delineated and

conserved. Ectomycorrhizal forests also deserve a special conservation status as fruitbodies of ectomycorrhizal fungi could make a valuable contribution to the protein intake of forest dwelling human communities. *Gnetum*, another important food item, should only be harvested in small amounts. As the liana cannot be cultured as a vegetable, possibly due to neglect of its ectomycorrhizal requirements, overharvesting the plant in the wild could easily lead to its demise. Local populations should be made aware of the importance of the ectomycorrhizal symbiosis by explaining to them the necessity to protect these trees in the same way as fruit trees and other socially important tree species during forest clearing for the preparation of agricultural fields.

**RESUMÉ**

Cette thèse traite des associations mycorhiziennes en forêts humides du sud Cameroun sous divers régimes et stades de perturbation en vue d'apporter des informations essentielles des rôles des mycorhizes sur la mise en place des arbres et la rétablissement de la forêt. Les écosystèmes des forêts humides d'Afrique occidentale renferment les représentants des deux types majeurs d'associations mycorhiziennes, à savoir, mycorhize arbusculaire et ectomycorhize. Cependant, les connaissances comment les deux associations mycorhiziennes interagissent et comment les différentes sortes de communautés de forêts mycorhiziennes fonctionnent sont fragmentaires. Il reste par conséquent peu clair comment les perturbations résultant de pratiques forestières et d'agriculture itinérante affecteront le potentiel infectieux des champignons mycorhiziens arbusculaires et ectomycorhiziens, et la régénération et la croissance de plants d'arbres à mycorhizes arbusculaires et ectomycorhiziens. Il est aussi peu connu comment ces diverses perturbations affectent la biodiversité des champignons arbusculaires et ectomycorhiziens. Les connaissances sur les divers types d'associations mycorhiziennes, leur diversité, et leur dynamique sont des conditions préalables en aménagement durable des écosystèmes souterrains, et conséquemment en aménagement durable des écosystèmes forestiers. L'approche de recherche adoptée pour ces investigations a consisté en plusieurs étapes:

1. Investigation dans des peuplements forestiers non perturbés du statut mycorhizien d'espèces d'essences sélectionnées d'importance économique, sociale et écologique, en relation avec la distribution et l'abondance des associations mycorhiziennes.
2. Inventaire de la diversité en espèces de champignons ectomycorhiziens.
3. Etude des variations en populations et activités des propagules mycorhiziens infectieux en relation avec les stades de perturbation des sols induits par les pratiques d'agriculture itinérante et d'exploitation forestière, sélective et commerciale.
4. Estimation de l'importance des réseaux ectomycorhiziens intacts comme source d'inoculum pour la formation ectomycorhizienne et la mise en place de jeunes plants autour d'espèces d'essences.
5. Evaluation du potentiel de régénération et de la réponse en croissance de jeunes plants de deux espèces locales d'essences ectomycorhiziennes et de cinq espèces locales d'essences mycorhiziennes arbusculaires dans des sols à potentiel mycorhizien infectieux indigène. Pour les arbres mycorhiziens arbusculaires, l'effet de l'addition d'inoculum, soit d'inoculum dérivé d'un champ de graminées soit d'inoculum dérivé de l'arbre-mère, a été aussi étudié afin d'estimer l'importance de la quantité et de la qualité de l'inoculum.

La colonisation mycorhizienne des racines d'espèces d'arbres et de lianes sélectionnées, et la contribution des arbres mycorhiziens arbusculaires et ectomycorhiziens au nombre de tiges et à la surface terrière dans les peuplements forestiers non perturbés ont été décrits au CHAPITRE 2. Toute la centaine d'espèces examinées (appartenant à soixante-dix-sept genres et vingt-neuf familles) portent des mycorhizes. Soixante-quatorze espèces d'arbres forment uniquement des mycorhizes arbusculaires; vingt-trois espèces d'arbres et trois espèces de lianes (appartenant à treize genres et trois familles: Caesalpiniaceae, Uapacaceae, Gnetaceae)

forment des ectomycorhizes dont cinq (espèces mycorhiziennes doubles) portent aussi des structures mycorhiziennes arbusculaires mais d'importance variable. Parmi les treize genres ectomycorhiziens, onze appartiennent à la famille des Caesalpiniaceae (dix genres de la tribu Amherstieae, un genre de la tribu Detarieae). La plupart des espèces de la tribu Amherstieae sont localement connues des prospecteurs d'essences forestières comme Ekop. La contribution des arbres ectomycorhiziens à la surface terrière des peuplements forestiers varie avec les sites ; il est bas à Ebom (le plus jeune peuplement) et le plus élevé (environ 70 % de la surface terrière) en forêt presque primaire de Bityili.

Les deux types majeurs d'associations mycorhiziennes se rencontrent dans tous les trois sites, cependant, les arbres ectomycorhiziens forment habituellement des peuplements distincts en raison de leur caractère grégaire. Les associations mycorhiziennes arbusculaires dominent la plupart des formations végétales, à l'exception des peuplements ectomycorhiziens. Certaines espèces ectomycorhiziennes apparaissent aussi en nombre de tiges bas au sein des formations forestières à mycorhizes arbusculaires, consistant en espèces d'*Afzelia, Anthonotha, Berlinia* et *Uapaca*. *Afzelia* n'a jamais été rencontré dans les peuplements ectomycorhiziens. Dans la jachère de l'herbe exotique *Chromolaena odorata*, le seul taxon ectomycorhizien est *Gnetum*. La co-occurrence des communautés de forêts mycorhiziennes arbusculaires et ectomycorhiziennes soulève des questions sur leur coexistence sous des régimes de perturbation (changements climatiques, perturbations anthropiques) et sur l'importance de la différentiation édaphique ou de la niche de régénération.

Aux CHAPITRES 2 et 3, la richesse en espèces de champignons mycorhiziens arbusculaires et ectomycorhiziens est discutée. L'examen racinaire révèle que toutes les trois familles des Glomales (les champignons formant la symbiose mycorhizienne arbusculaire) sont présentes: Glomaceae (*Glomus*), Acaulosporaceae (*Acaulospora*), et Gigasporaceae (*Gigaspora, Scutellospora*). Les modèles de colonisation racinaire de différentes espèces d'arbres suggèrent que certains champignons colonisent préférentiellement certains arbres. La morphologie des spores indique qu'un certain nombre de différents taxons fongiques est présent, cependant ces champignons n'ont pas encore été identifiés et l'inventaire d'espèces mycorhiziennes arbusculaires reste une grande priorité pour le Cameroun. Plus de 125 espèces de champignons ectomycorhiziens ont été identifiées, principalement dans les forêts presque primaires à peuplements ectomycorhiziens, soit au voisinage de pieds d'espèces d*e Uapa*ca soit de celles de césalpiniacées, alors qu'une autre espèce fongique n'a été rencontrée qu'en association ave*c Gnet*um. Parmi les champignons ectomycorhiziens, les Amanitaceae, Russulaceae, Boletaceae, et Cantharellaceae sont (très) bien représentées, alors que seulement quelques espèces de Cortinariaceae, Sclerodermataceae, Gomphaceae, Clavulinaceae, et Hymenochaetaceae ont été observées (CHAPITRE 3). La composition en espèces est largement similaire entre les sites, suggérant une composition homogène en espèces de champignons ectomycorhiziens à travers le site du Programme Tropenbos Cameroun (PTC). Les mêmes champignons ectomycorhiziens (et les mêmes arbres ectomycorhiziens) apparaissent aussi au Parc National de Korup, Cameroun, malgré que les sols et le climat sont très différents entre Korup et les forêts de site du PTC.

Les différences en potentiel mycorhizien infectieux des champignons mycorhiziens arbusculaires (CHAPITRE 4) et des champignons ectomycorhiziens (CHAPITRE 5) dans les

formations végétales qui appartiennent à différents stades de perturbation résultant des pratiques d'agriculture itinérante et d'exploitation forestière sélective sont décrites. Les spores des champignons mycorhiziens arbusculaires contribuent principalement au potentiel mycorhizien infectieux des sols du site du PTC, comme démontré par les corrélations positives et significatives entre les nombres de spores et la colonisation des plantes-tests. Apparemment, le réseau mycorhizien arbusculaire a une signification mineure sur le potentiel mycorhizien infectieux. Les nombres de spores et les taux de colonisation sont similaires à travers les sites, indiquant de nouveau que le site du PTC est homogène, en dépit des différences de texture du sol, pH, et de disponibilité en phosphore. Les spores de champignons mycorhiziens arbusculaires sont relativement abondantes dans les vieilles formations forestières (comparé aux forêts similaires d'Amérique Centrale et du Sud), cependant dans les peuplements ectomycorhiziens le potentiel mycorhizien infectieux des champignons mycorhiziens arbusculaires est très bas. Le potentiel mycorhizien infectieux des champignons mycorhiziens arbusculaires est très élevé dans les champs agricoles et jachères. Après le début de la succession secondaire et la conversion de la jachère en stades de succession forestière récents et avancés, les nombres de spores baissent de nouveau. Le potentiel mycorhizien infectieux est (très) considérablement réduit par les pratiques forestières comparé à la forêt primaire, et cet impact négatif persiste pendant plus d'une décade. Un tel potentiel mycorhizien infectieux bas pose la question si la mise en place et la croissance de jeunes plants d'arbres sont entravées par l'absence d'inoculum, et si la performance de jeunes plants pourrait être accrue par l'addition d'inoculum mycorhizien.

Par contraste, le potentiel mycorhizien infectieux des champignons ectomycorhiziens est plus dépendant du réseau ectomycorhizien intact. Le potentiel mycorhizien infectieux des champignons ectomycorhiziens a été différent pour deux espèces d'arbres ectomycorhiziens, suggérant un important effet de la spécificité de l'arbre-hôte pour les champignons ectomycorhiziens. Lorsque *Tetraberlinia bifoliolata* (espèce de césalpiniacées grégaire) a été utilisé comme plante-test, l'inoculum ectomycorhizien a été présent dans les jachères à *Gnetum* et dans des forêts à stades de perturbation variés, augmentant le plus dans les peuplements ectomycorhiziens. Lorsque *Afzelia bipindensis* (espèce de césalpiniacées croissant en solitaire) a été utilisé, l'inoculum a été le plus élevé dans les champs agricoles et a été réduit pendant la succession vers les vieilles forêts. Dans les sites d'exploitation forestière (pistes de débardage, parcs-à-bois nus et recolonisés), l'inoculum ectomycorhizien est absent, indiquant que les pratiques forestières actuelles ne garantiraient pas la maintenance de la composante ectomycorhizienne de la forêt. La croissance de jeunes plants dans des sols sous diverses pratiques d'utilisation est bien corrélée avec la quantité d'inoculum ectomycorhizien présent. Un rôle clé des plantes mycorhiziennes doubles comme *Afzelia bipindensis* en régénération forestière n'a pu être démontré (CHAPITRE 5). Les données des CHAPITRES 4 et 5 n'ont pas apporté d'évidence de spécialisation en niche édaphique des arbres mycorhiziens arbusculaires et ectomycorhiziens, suggérant que la maintenance et la coexistence des deux communautés de forêts mycorhiziennes dépendent des différences en leur niche de régénération.

Au CHAPITRE 6, l'importance du réseau ectomycorhizien pour la formation des ectomycorhizes et la performance des plantes ont été estimées. Le contact des plants de la

césalpiniacée *Paraberlinia bifoliolata* avec les racines d'arbres adultes dans l'amélioration de la formation ectomycorhizienne et la mise en place a prouvé plus efficace que pour les plants isolés des racines de l'arbre adulte. La zone à fort potentiel infectieux ectomycorhizien a coïncidé plus ou moins avec celle de la projection de la couronne de l'arbre ectomycorhizien. Le taux de formation ectomycorhizienne a été aussi influencé par le degré de spécificité de l'hôte pour l'inoculum ectomycorhizien, confirmant les résultats du CHAPITRE 5. L'implication en aménagement des CHAPITRES 5 et 6 est que l'aménagement des communautés de forêts ectomycorhiziennes requiert une attention spéciale, au regard de l'importance du réseau mycélien et des capacités de dispersion limitées des deux le champignon ectomycorhizien et l'arbre ectomycorhizien avec de larges graines. Au CHAPITRE 7, la colonisation mycorhizienne arbusculaire et la réponse en croissance de trois espèces d'essences forestières majeures, *Terminalia superba*, *Distemonanthus benthamianus*, et *Entandrophragma utile* au potentiel mycorhizien infectueux indigène et à l'addition d'inoculum graminéenne ont été étudiées dans des sols collectés sous diverses formations végétales correspondant aux stades de perturbation. La colonisation fongique et la réponse de la plante dépendent de l'essence forestière, du potentiel mycorhizien infectieux, de la formation végétale, et de l'addition de l'inoculum. La colonisation mycorhizienne et la croissance de jeunes plants ont été faibles dans des sols à potentiel mycorhizien infectieux bas; simultanément l'effet de l'inoculation mycorhizienne a été large. Dans les sols de champs agricoles et de jachères, le potentiel mycorhizien infectieux a été large, le taux de colonisation élevé et la réponse de la plante-hôte bonne, et l'effet de l'inoculation mycorhizienne a été bas ou même négatif. La réponse en croissance de *Terminalia superba* a été légère avec une forte augmentation de la colonisation, tandis qu'avec *Distemonanthus benthamianus*, le modèle opposé a été observé; la réponse en croissance d'*Entandrophragma utile* a été intermédiaire. Les données de ce chapitre confirment que le potentiel mycorhizien infectieux est un bon prédicteur de la colonisation mycorhizienne et de la croissance subséquente de jeunes plants. Elles montrent aussi que dans les sites à inoculum mycorhizien bas, l'addition d'inoculum peut conduire à une régénération plus réussie d'essences forestières. Bien que le manque d'inoculum mycorhizien suffisant dans certains sites indique que l'addition d'inoculum peut potentiellement être couronnée de succès, il ne doit pas être oublié que la spécificité pour l'hôte de certains champignons mycorhiziens arbusculaires pour certaines espèces d'arbres pourrait aussi jouer un rôle. Quoique l'adage que tout inoculum vaut mieux que rien s'applique pour des stades de perturbation sévèrement appauvris, la régénération avec succès de certaines essences forestières ne pourrait être meilleure que si un inoculum de haute qualité, spécifique à l'arbre-hôte est appliquée. Au CHAPITRE 8, la colonisation mycorhizienne et la réponse en croissance de *Pterocarpus soyauxii* et *Lophira alata* ont été évaluées dans des sols à divers stades de perturbation en relation avec le potentiel mycorhizien infectieux indigène et l'addition d'inoculum spécifique à l'arbre-hôte collecté sous la rhizosphère de l'arbre conspécifique. Un essai a été réalisé pour estimer séparément l'effet de la qualité et de la quantité de l'inoculum. Les résultats indiquent que la qualité de l'inoculum pourrait aussi être un déterminant majeur de l'effet de l'inoculation mycorhizienne, spécialement pour l'essence de succession avancée, fixatrice d'Azote,

*Pterocarpus soyauxii*. Pour *Lophira alata*, l'effet de la qualité de l'inoculum a été plus bas, à l'exception des sites de pratiques forestières.

Le CHAPITRE 9 intègre les résultats des chapitres précédents. Il attire l'attention sur la relation entre le potentiel mycorhizien infectieux et la réponse de la plante et aux moyens par lesquels la perturbation induite par l'homme conséquente des pratiques d'agriculture itinérante et d'exploitation forestière affectent les possibilités en aménagement durable. Il démontre aussi qu'il y a de larges différences entre l'inoculum mycorhizien arbusculaire et ectomycorhizien, et donc dans les manières que les perturbations pourraient affecter la maintenance de la diversité d'espèces d'arbres mycorhiziens arbusculaires et ectomycorhiziens. Considérant les taux de déforestation élevés sous les tropiques, il est improbable que l'aménagement durable des forêts humides soit possible sans mettre en péril la diversité d'espèces d'arbres. L'enrichissement et la plantation des arbres pourraient être durables malgré que le changement en diversité d'espèces d'arbres est probable. Considérant les différences en niche de régénération entre les arbres mycorhiziens arbusculaires et ectomycorhiziens, l'aménagement durable doit prendre en compte les associations mycorhiziennes des arbres tropicaux. Comme les communautés de forêts ectomycorhiziennes apparaissent plus vulnérables aux diverses formes de perturbation, elles ont en fait besoin d'un statut spécial de conservation. En raison de différences en spécificité fongique des arbres ectomycorhiziens qui ordinairement poussent en solitaires et apparaissent généralement en peuplements, les deux espèces d'arbres et de types de forêts doivent être prudemment aménagés. Pour *Afzelia* (et autres) arbres apparaissant isolés, de petites forêts-refuges doivent être crées autour d'eux dans les forêts ouvertes à l'exploitation forestière. De tels refuges peuvent aussi servir à la faune sauvage et à l'avifaune, et pourraient constituer un réservoir à semences pour la recolonisation des terres forestières dégradées. Les peuplements forestiers ectomycorhiziens doivent être intégralement protégées. La méthode d'échantillonnage utilisée au Cameroun pour l'inventaire d'aménagement doit être modifiée afin d'inclure les peuplements forestiers ectomycorhiziens. La première étape doit viser à cartographier les peuplements ectomycorhiziens afin qu'ils soient clairement délimités et conservés. Les forêts ectomycorhiziennes méritent aussi un statut spécial de conservation comme les carpophores de champignons ectomycorhiziens pourraient faire une contribution précieuse pour la ration protéique des communautés humaines riveraines des forêts. Le *Gnetum*, un autre aliment important, doit être récolté seulement en petites quantités. Comme la liane ne peut être cultivée comme légume, possiblement en raison de la négligence de ses exigences ectomycorhiziennes, la sur-récolte de la plante à l'état sauvage pourrait facilement conduire à sa ruine. Les populations locales doivent prendre conscience de l'importance de la symbiose ectomycorhizienne en leur expliquant la nécessité de protéger ces arbres de la même manière que les arbres fruitiers et autres espèces d'arbres socialement importantes lors du défrichement pour la préparation des champs agricoles.

**SAMENVATTING**

Mycorrhiza's zijn samenlevingsvormen tussen wortels van hogere planten en bepaalde schimmels. Beide partners hebben baat bij deze verbinding en beide hebben niet het vermogen om onder natuurlijke omstandigheden hun levenscyclus te voltooien. Dit proefschrift behandelt mycorrhiza's in de regenbossen van zuid Kameroen in relatie tot verschillende vormen van verstoring en verschaft informatie over de rollen van mycorrhiza bij de vestiging van bomen en herstel van het regenbos. Uit eerder onderzoek was reeds bekend dat in de regenbossen van westelijk Afrika vertegenwoordigers van de twee voornaamste typen van mycorrhiza voorkomen, namelijk arbusculaire mycorrhiza en ectomycorrhiza. Kennis over de interactie tussen beide mycorrhizatypen en over de verschillende bosgemeenschappen met een verschillend mycorrhizatype was echter zeer beperkt. Daardoor was het onbekend hoe verstoringen van het regenbos ten gevolge van bosbouwpraktijken en zwerflandbouw invloed zouden hebben op de inoculumpotentiaal van arbusculaire-mycorrhiza- en ectomycorrhiza-schimmels, en op de regeneratie en groei van zaailingen van bomen met arbusculaire mycorrhiza en ectomycorrhiza. Het was eveneens onbekend hoe deze verschillende vormen van verstoring de soortenrijkdom (biodiversiteit) van arbusculaire-mycorrhiza- en ectomycorrhiza-schimmels zouden beïnvloeden. Kennis van de verschillende typen van mycorrhiza, hun diversiteit en hun dynamiek is een wezenlijke voorwaarde voor het duurzame beheer van het ondergrondse deel van ecosystemen en daarmee voor het duurzame beheer van bos-ecosystemen. Het hier beschreven onderzoek omvatte de volgende stappen:

1. Onderzoek in ongestoorde bosopstanden naar het vóórkomen en naar het type van mycorrhiza van een groot aantal boomsoorten met economische, sociale of ecologische betekenis, en het verband met de verdeling en talrijkheid van beide mycorrhizatypen.
2. Inventarisatie van de soortenrijkdom van ectomycorrhizaschimmels.
3. Onderzoek naar de veranderingen in de populaties en de aktiviteit van het inoculum van mycorrhizaschimmels in relatie tot stadia van bodemverstoring veroorzaakt door zwerflandbouw en commerciële selectieve houtkap.
4. Bepalen van de betekenis van ongestoorde ectomycorrhiza-netwerken als een bron van inoculum voor de vorming van ectomycorrhiza en de vestiging van zaailingen in de directe omgeving van volwassen bomen.
5. Evaluatie van het herstelvermogen en de groei van zaailingen van twee inheemse boomsoorten met ectomycorrhiza en vijf inheemse boomsoorten met arbusculaire mycorrhiza. Voor de bomen met arbusculaire mycorrhiza werd ook het effect van het toevoegen van inoculum, hetzij inoculum afkomstig van een grasvegetatie, hetzij inoculum afkomstig onder volwassen bomen van die soort, bestudeerd om het belang te kunnen schatten van zowel de hoeveelheid inoculum als de kwaliteit van dat inoculum.

De kolonisatie van wortels van een aantal soorten bomen en lianen door mycorrhiza-schimmels, en de relatieve bijdrage van bomen met arbusculaire mycorrhiza en ecto-mycorrhiza aan stamtal en grondvlak in ongestoorde bossen worden beschreven in Hoofdstuk

2. Alle honderd bestudeerde plantensoorten, behorende tot zevenenzeventig geslachten en negenentwintig families, vormden mycorrhiza. Vierenzeventig boomsoorten vormden uitsluitend arbusculaire mycorrhiza; drieëntwintig boomsoorten en drie soorten lianen (behorende tot dertien geslachten en drie families: Caesalpiniaceae, Upacaceae, Gnetaceae) vormden ectomycorrhiza, en vijf van deze soorten bevatten eveneens arbusculaire mycorrhiza, zij het in verschillende mate. Deze vijf soorten vormden dubbele mycorrhiza. Van de dertien geslachten met ectomycorrhiza behoorden er elf tot de Caesalpiniaceae (tien geslachten tot de tribus Amherstieae, één geslacht tot de tribus Detarieae). De meeste soorten van de tribus Amherstieae waren locaal bekend bij boomprospectors als Ekop. De bijdrage van bomen met ectomycorrhiza aan het grondvlak varieerde per locatie en was het laagste in Ebom (in een relatief jonge opstand) en het hoogste (vrijwel 70%) in een vrijwel ongestoord regenbos in Bityili. Beide typen mycorrhiza kwamen op de drie belangrijkste onderzoekslocaties voor. Ectomycorrhizavormende bomen vormden gewoonlijk eigen plekken ten gevolge van hun geaggregeerde voorkomen. In de meeste vegetatietypen domineerden bomen met arbusculaire mycorrhiza, met uitzondering van deze clusters met dominantie door bomen met ectomycorrhiza. Sommige boomsoorten met ectomycorrhiza kwamen ook in laag stamtal voor in opstanden met arbusculaire mycorrhiza, zoals soorten van *Afzelia*, *Anthonota*, *Berlinia* en *Uapaca*. *Afzelia* werd nooit waargenomen in deze ectomycorrhiza-clusters. In braakland, overgroeid met het niet-inheemse onkruid *Chromolaena odorata*, waren lianen van het geslacht *Gnetum* de enige vertegenwoordigers met ectomycorrhiza. Het samen vóórkomen van bosecosystemen met arbusculaire mycorrhiza en ectomycorrhiza roept vragen op naar de mogelijkheid tot gemeenschappelijk voortbestaan na verstoringen (klimaatsveranderingen, door de mens veroorzaakte verstoringen) en naar het belang van nisdifferentiatie wat betreft bodemeigenschappen of regeneratiemogelijkheden.

In Hoofdstuk 2 en 3 wordt de soortenrijkdom van arbusculaire-mycorrhiza- en ectomycorrhizaschimmels behandeld. Onderzoek van boomwortels toonde aan dat de drie families van de Glomales (de schimmels die arbusculaire mycorrhiza vormen) alle aanwezig waren: Glomaceae (*Glomus*), Acaulosporaceae (*Acaulospora*) en Gigasporaceae (*Gigaspora*, *Scutellospora*). De patronen van wortelkolonisatie van verschillende boomsoorten leken erop te wijzen dat bepaalde schimmels bij voorkeur bepaalde boomsoorten koloniseren. De morfologie van de sporen van de arbusculaire-mycorrhizaschimmels gaf voorts aan dat een aantal verschillende schimmelsoorten aanwezig was. Deze soorten zijn echter nog niet op naam gebracht en een inventarisatie van de soortenrijkdom van de arbusculaire-mycorrhizaschimmels is een belangrijke prioriteit in het mycorrhiza-onderzoek in Kameroen. Meer dan 125 soorten ectomycorrhizaschimmels werden op naam gebracht. Deze soorten werden hoofdzakelijk gevonden in vrijwel ongestoorde bossen in groepen met ectomycorrhizavormende Caesalpiniaceae en aan de stambasis van *Uapaca*-soorten, terwijl één soort uitsluitend in verbinding met *Gnetum* werd aangetroffen. De meest soortenrijke groepen van de ectomycorrhizaschimmels behoorden tot de Amanitaceae, Russulaceae, Boletaceae en Cantharellaceae, terwijl slechts enkele soorten uit de Cortinariaceae, Sclerodermataceae, Gomphaceae, Clavulinaceae en Hymenochaetaceae werden waargenomen (Hoofdstuk 3). De soortensamenstelling op de verschillende onderzoekslocaties was sterk overeenkomstig, hetgeen erop wijst dat het onderzoeksgebied van het Tropenbos Cameroon Programme (TCP)

homogeen is. Dezelfde ectomycorrhizaschimmels (en dezelfde boomsoorten met ectomycorrhiza) komen ook voor in Korup National Park, Kameroen, een gebied met een geheel andere bodemgesteldheid en klimaatscondities dan het gebied van het TCP.
Verschillen in inoculumpotentiaal van arbusculaire-mycorrhizaschimmels (Hoofdstuk 4) en ectomycorrhizaschimmels (Hoofdstuk 5) in vegetatietypen, die behoren tot verschillende verstoringsstadia als gevolg van zwerflandbouw en selectieve houtkap, werden beschreven. Sporen van arbusculaire-mycorrhizaschimmels vormden de voornaamste bijdrage tot het inoculumpotentiaal van bodems in het TCP-gebied, zoals bleek uit een significant positieve correlatie tussen sporenaantallen en kolonisatie van proefplanten. Blijkbaar is het mycorrhizanetwerk van arbusculaire-mycorrhizaschimmels van minder belang voor het inoculumpotentiaal. Sporenaantallen en percentage wortelkolonisatie waren weinig verschillend tussen de onderzoekslocaties, hetgeen er eveneens op wijst dat het TCP-gebied homogeen is, ondanks verschillen in bodemstruktuur (kleigehalte), zuurgraad en beschikbaarheid van fosfaat. Sporen van arbusculaire-mycorrhizaschimmels waren relatief talrijk in de oudere bossen (in vergelijking met bossen in Zuid- en Midden-Amerika), maar in clusters van ectomycorrhizabomen werd slechts zeer weinig inoculum van arbusculaire-mycorrhizaschimmels gevonden. Het inoculumpotentiaal van arbusculaire-mycorrhizaschimmels was veel hoger in velden waar landbouw bedreven wordt en in braakland. Gedurende de secundaire successie, wanneer braakland overgaat in jong en tenslotte in oud bos, neemt het aantal sporen weer af. Het inoculumpotentiaal was (zeer) aanzienlijk verlaagd door plaatsen die door de bosbouwpraktijken waren beïnvloed (sleeppaden, houtverzamelplaatsen) in vergelijking met het ongestoorde bos, en dit negatieve effect van bosbouwpraktijken kon tenminste tien jaar voortbestaan. Een dergelijk laag inoculumpotentiaal riep de vraag op of de vestiging en groei van zaailingen van bomen negatief beïnvloed werd door afwezigheid van of een tekort aan inoculum en of de groei van deze zaailingen verbeterd kon worden door het toevoegen van mycorrhiza-inoculum.
Het inoculumpotentiaal van ectomycorrhizaschimmels was daarentegen meer afhankelijk van het intacte mycorrhizanetwerk. Het bepalen van het inoculumpotentiaal van ectomycorrhizaschimmels leidde tot verschillende uitkomsten bij twee verschillende boomsoorten met ectomycorrhiza, hetgeen erop lijkt te wijzen dat gastheerspecificiteit van ectomycorrhizaschimmels een belangrijke rol speelt. Wanneer *Tetraberlinia bifoliolata* (een soort die karakteristiek is voor clusters van ectomycorrhizabomen) gebruikt werd als proefplant, bleek ectomycorrhiza-inoculum voor te komen in braakland met *Gnetum* en in bossen van verschillende leeftijd, waarbij de hoeveelheid inoculum toenam met de leeftijd van het bos. Wanneer *Afzelia bipindensis* (een soort die vrijwel altijd solitair groeit tussen bomen met arbusculaire mycorrhiza) gebruikt werd als proefplant, bleek de grootste hoeveelheid inoculum voor te komen in landbouwveldjes en op braakland, en nam de hoeveelheid gedurende de successie naar oud bos weer af. Op plaatsen van bosbouwpraktijken (sleeppaden, kale en begroeide houtverzamelplaatsen) was geen ectomycorrhiza-inoculum aanwezig, hetgeen er op wijst dat de huidige bosbouwpraktijk geen garantie biedt voor het voortbestaan van de boslevensgemeenschap met ectomycorrhizabomen. De groei van zaailingen op bodems van deze landgebruiksvormen toonde een goede correlatie met de hoeveelheid inoculum van ectomycorrhizaschimmels. Een sleutelrol voor planten met dubbele

mycorrhiza, zoals *Afzelia bipindensis*, voor bosherstel kon niet worden aangetoond (Hoofdstuk 5). De gegevens van Hoofdstuk 4 en 5 geven geen aanwijzingen dat bomen met arbusculaire mycorrhiza en ectomycorrhiza een voorkeur hebben voor verschillende bodems. Het lijkt er derhalve op dat het gezamenlijk voortbestaan van beide bosgemeenschappen met verschillende mycorrhizatypen afhankelijk is van verschillen in hun regeneratienis.
In Hoofdstuk 6 werd het belang van het ectomycorrhizanetwerk voor de ectomycorrhiza-vorming en het functioneren van de plant bestudeerd. Zaailingen van de ectomycorrhiza-vormende boom *Paraberlinia bifoliolata* (Caesalpiniaceae), die in contact stonden met het mycorrhizanetwerk van volwassen bomen, bleken beter te overleven en vaker mycorrhiza te vormen dan zaailingen die geïsoleerd waren van het wortelstelsel van een volwassen boom. Het gebied met het hoge ectomycorrhiza-inoculumpotentiaal via het netwerk viel globaal samen met dat van de kroonprojectie van de volwassen ectomycorrhizaboom. De mate van ectomycorrhizavorming werd ook beïnvloed door de mate van gastheerspecificiteit van het mycorrhiza-inoculum, hetgeen in overeenstemming is met de resultaten die in Hoofdstuk 5 werden beschreven. De uitkomsten van de Hoofdstukken 5 en 6 houden in dat het beheer van de bosopstanden met ectomycorrhiza extra aandacht vraagt vanwege de grote betekenis van het ectomycorrhizanetwerk en de beperkte verspreidingsmogelijkheden van zowel ecto-mycorrhizaschimmels als ectomycorrhizabomen, die grote zaden vormen.
In Hoofdstuk 7 werd de mycorrhizakolonisatie en de groei van drie economisch belangrijke boomsoorten (*Terminalia superba*, *Distemonanthus benthamianus*, *Entandrophragma utile*) bestudeerd in bodems onder verschillende vegetatietypen die met verschillende verstorings-stadia corresponderen. Zowel de betekenis van het in die bodem aanwezige inoculum van arbusculaire-mycorrhizaschimmels, als het effect van toedienen van inoculum verzameld in een grasvegetatie, werden onderzocht. Zowel de mycorrhizakolonisatie als de groei van de zaailing waren afhankelijk van de boomsoort, inoculumpotentiaal van die bodem, en toe-voegen van grasinoculum. Zowel mycorrhizakolonisatie als de groei van zaailingen waren laag in grond met een lage inoculumpotentiaal zoals sleeppaden en houtverzamelplaatsen, en in oudere bossen. In zulke gevallen was het positieve effect van toevoegen van inoculum groot. In bodems van landbouwveldjes en braakland was het inoculumpotentiaal hoog; daardoor was de kolonisatie hoog en de plantengroei goed en het effect van toevoegen van inoculum gering en soms zelfs iets negatief. Bij *Terminalia superba* nam na toevoegen van inoculum de kolonisatie toe, maar de groei van de plant juist niet, terwijl bij *Distemonanthus benthamianus* het tegenovergestelde patroon werd waargenomen. De reactie van *Entandro-phragma utile* was intermediair. De resultaten van dit hoofdstuk bevestigen dat het inoculum-potentiaal van arbusculaire-mycorrhizaschimmels een goede voorspeller is van mycorrhiza-kolonisatie en groei van zaailingen. De resultaten tonen eveneens aan dat op plaatsen waar mycorrhiza-inoculum schaars is, het toevoegen van inoculum kan leiden tot meer resultaat bij de regeneratie van economisch belangrijke boomsoorten. Hoewel het ontbreken van voldoende mycorrhiza-inoculum op sommige plaatsen betekent dat het toevoegen van inoculum in potentie succesvol kan zijn, moet niet vergeten worden dat gastheervoorkeur van bepaalde soorten arbusculaire-mycorrhizaschimmels voor sommige boomsoorten eveneens een belangrijke factor kan zijn. Hoewel in zijn algemeenheid de zegswijze geldt dat elk willekeurig mycorrhiza-inoculum beter is dan geen mycorrhiza-inoculum voor herbebossing

op sterk verstoorde en gedegradeerde bodems, blijft het mogelijk dat succesvolle regeneratie van sommige economisch belangrijke boomsoorten beter verloopt als er gastheer-specifiek inoculum van hoge kwaliteit wordt gebruikt. In Hoofdstuk 8 worden de mycorrhizakolonisatie en de groei van zaailingen van twee andere boomsoorten, *Pterocarpus soyauxii* en *Lophira alata*, bestudeerd in bodems van verschillende verstoringsstadia, zowel in aanwezigheid van het inheemse inoculum als na toevoegen van inoculum dat verzameld is in de rhizosfeer van volwassen bomen van dezelfde soort. Een poging werd gedaan om de effecten van de inoculumhoeveelheid en de kwaliteit van dat inoculum te scheiden. De resultaten in dit hoofdstuk gaven aan dat inoculumkwaliteit een belangrijke rol speelt bij het vaststellen van het effect van inoculumtoevoeging, speciaal voor de stikstofbindende, voor oudere bossen karakteristieke soort *Pterocarpus soyauxii*. Voor *Lophira alata*, de economisch meest belangrijke boomsoort van zuid Kameroen, was de betekenis van inoculumkwaliteit veel minder, behalve op bodems van plaatsen van bosbouwpraktijken.
In Hoofdstuk 9 worden de resultaten van de verschillende hoofdstukken geïntegreerd. Nadruk wordt gelegd op het verband tussen inoculumpotentiaal van mycorrhizaschimmels en de groei van zaailingen en op de manieren waarop de mens het bosecosysteem verstoort door zwerf-landbouw en selectieve kap en daarmee de mogelijkheden tot duurzaam bosbeheer beïnvloedt. Het hoofdstuk laat eveneens zien dat er grote verschillen zijn tussen de ruimtelijke verspreiding van het inoculum van arbusculaire-mycorrhiza- en ectomycorrhizaschimmels, en daarmee ook in de manier waarop verstoringen van het ecosysteem invloed hebben op het behoud van soortenrijkdom van boomsoorten met arbusculaire mycorrhiza en ectomycorrhiza. In het licht van de grote snelheid van ontbossing in de tropen is het niet waarschijnlijk dat duurzaam gebruik van het regenbos mogelijk is zonder de soortenrijkdom van bomen in gevaar te brengen. Selectieve verrijking van bossen met economisch belangrijke boomsoorten en plantage-bosbouw zijn mogelijk duurzaam, hoewel vermoedelijk niet met behoud van de volledige rijkdom aan boomsoorten. In het licht van de verschillen in regeneratienis tussen bomen met arbusculaire mycorrhiza en ectomycorrhiza, is het noodzakelijk dat duurzaam bosbeheer rekening houdt met de mycorrhiza-associaties van tropische bomen. Omdat de bosgemeenschappen met ectomycorrhiza kwetsbaarder zijn voor vormen van verstoring dan bossen met arbusculaire mycorrhiza, verdienen zij speciale bescherming. Vanwege de verschillen in specificiteit voor ectomycorrhizaschimmelsoorten van boomsoorten die gewoonlijk solitair groeien en die gewoonlijk geclusterd groeien, moeten verschillende boomsoorten en vegetatietypen zorgvuldig beheerd worden Voor *Afzelia* (en andere boom)soorten die gewoonlijk solitair groeien, dienen kleine bosrefugia rondom deze bomen gemaakt te worden, wanneer zulke bossen voor selectieve kap worden opengesteld. Zulke refugia kunnen eveneens bescherming bieden aan wilde zoogdieren en vogels, en dienen het zaadreservoir te vormen voor de kolonisatie van gedegradeerd bos met ectomycorrhiza. Bossen waarin ectomycorrhizabomen geclusterd voorkomen, dienen integraal beschermd te worden. De methoden van inventarisatie ten behoeve van duurzaam beheer van tropische bossen in Kameroen dienen veranderd te worden om tijdens de inventarisatiefase deze bos-gedeelten op te nemen. De eerste stap moet dan zijn om deze groepen met ectomycorrhizabomen te karteren, zodat aanwijzing en bescherming beter mogelijk is. Ectomycorrhizabossen verdienen eveneens een bijzondere beschermde status omdat

vruchtlichamen van sommige soorten ectomycorrhizapaddestoelen een waardevolle bijdrage kunnen leveren aan de eiwitconsumptie van volkeren die in en van het bos leven. *Gnetum*, een andere belangrijke voedingsplant, dient slechts in kleine hoeveelheden geoogst te worden. Doordat het nog niet mogelijk is om deze liaan te kweken, vermoedelijk doordat zijn noodzaak tot ectomycorrhizavorming miskend is, bestaat het gevaar voor de grote oogst en daardoor van zijn achteruitgang en uiteindelijk verdwijnen. De locale bevolking dient bewust gemaakt te worden van het belang van de mycorrhizasymbiose, door aan de bevolking uitleg te geven over de noodzaak om deze bossen te beschermen op eenzelfde manier als vruchtbomen en andere bomen met sociale betekenis tijdens het kappen van het bos voor de aanleg van landbouwvelden worden beschermd.