Dipterocarpaceae:
Shorea leprosula Miq. Cuttings, Mycorrhizae and Nutrients

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DIPTEROCARPACEAE:

Shorea leprosula Miq. CUTTINGS, MYCORRHIZAE AND NUTRIENTS

R. Mulyana Omon

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus van
Wageningen Universiteit prof. dr. ir. L. Speelman
in het openbaar te verdedigen op dinsdag 14 mei 2002
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PROPOSITIONS

STELLINGEN behorende bij het proefschrift:
DIPTEROCARPACEAE: *Shorea leprosula* Miq. cuttings, mycorrhizae and nutrients

1. The huge number of possible combinations of environmental factors and their variability over very short distances as are occurring in natural forests, makes the reproduction of just a few sets thereof under artificial, controlled conditions a poor reflection of the reality of forest conditions (this thesis - 2.2.1).

2. *Shorea leprosula* seedlings are not only shade-tolerant, but seen their photosynthetic profile they are shade-requiring (this thesis).

3 Of the physical and chemical soil properties affecting the growth of *Shorea leprosula* cuttings, the granular composition of the soil is the predominant factor, certainly linked to available oxygen and water (this thesis).

4. Mycorrhizal types 3 and 4 show different behaviour that could be due to species differences of the mycobiont (*Amanita sp*), but in view of the squash test showing that there is only one *Amanita* species, differences are due to other factors, such as phytobiont physiology or age and/or external stress (this thesis).

5 Inoculation with a mixture of mycorrhizal fungal species stimulates growth of *S. leprosula* cuttings more than each fungal species on its own, so the use of such mixtures is to be preferred in nursery practice (this thesis).

6. In forestry practice, the use of soil inoculum containing a sufficiently high inoculum potential of efficient indigenous mycorrhizal fungi should be seriously considered (this thesis).

7. In Indonesia the *Orangutans* are better foresters than the *Orang Hutan*.

8. Dipterocarpaceae species still belong to the important supports of the Indonesian economy.

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FOREWORD

Scientific results in this book were obtained in the framework of the international cooperation between the Ministry of Forestry of the Republic of Indonesia and the Tropenbos Foundation. From 1994 to 2000, I was Project Manager. One of the project activities is vegetative propagation and the establishment of Dipterocarps.

Since 1987, this project has produced several articles, among them about the vegetative propagation of Dipterocarps (Yasman and Smits, 1988), hedge orchards of Dipterocarps (Leppe and Smits, 1988) and mycorrhizal inoculation of dipterocarp seedlings (Smits et al., 1988; Smits, 1994; Yasman, 1995).

The above-mentioned activities were implemented in nursery techniques with dipterocarp species, especially with cuttings of dipterocarps. To obtain healthy cuttings, the soil substrate, compatible mycorrhizal fungi, fertilization and environmental factors were considered. It was discussed with the Tropenbos Team Leader, dr ir W.T.M. Smits who suggested consulting with Professor Dr. Ir. R.A.A. Oldeman. In 1996, Professor Dr. Ir. R.A.A. Oldeman visited Indonesia for the Program Sustainable Forest Management (Hutan Lestari). On that occasion I discussed the ideas with him and he agreed to formulate a proposal for a Doctoral programme at Wageningen Agricultural University (WAU).

In October 1996, I was accepted in the Ph.D Sandwich programme of Wageningen University. In agreement with my supervisor, I invited Dr Ir Supriyanto, Faculty of Forestry, Bogor Agricultural University, expert in mycorrhizae, and Dr Ir W.T.M. Smits, Director of the Gibbon Foundation as co-supervisors.

After the approval of the proposal, I was given the opportunity to have consultations with Professor Dr.Ir. R.A.A. Oldeman, with experts from ALTERRA or WAU, and to conduct literature studies at Wageningen for three months a year.

During the experimental set-up and monitoring, some constraints were also met, especially on equipment. Due to the funding assistance from the Tropenbos Foundation and equipment from WAU, these constraints were overcome successfully.

It is expected that the results of this study will be useful for the development of mycorrhizal research in tropical countries or other related areas, as well as for silviculture close to nature.

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I thank my supervisor Dr Peter Evers who advised me on the ecophysiological aspects of this research. Special thanks I address to my dear friend Dr Limonard for advice and critical evaluation of the mycorrhizal aspect of this research. I would also like to express my gratitude for their support to Ir Soeparno W. M.Sc, Dr Ir Boen M. Purnama M.Sc, Mr Martinus de Kam, Ir Bas van Helvoort, Ing. J W. Hildebrand, Dr Ir Irsyal Yasman, Joke Mahulete, Jelle Maas, Ir Massofian Noor, Edy Supardi, Agus Akhmadi, Arianto, Priyono and Andini Fadma.

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Special thanks to the staff of the Tropenbos Foundation office in Wageningen especially Ir Erik Lammerts van Bueren, Director of the Tropenbos Foundation, Dr Hans H. de Iongh, Head Programme of the Tropenbos Foundation at Wageningen, the Netherlands and Henk Lijftogt, Head Business of the Tropenbos Foundation at Wageningen, the Netherlands for their valuable assistance with my study at Wageningen University. I thank all staff involved in the MOF-Tropenbos Kalimantan Project who made this study possible.

Last but not least, I would like to thank my beloved wife Nurul Chotimah for her understanding, and my sons Eka Permana, R. Fajar Dwi Permadi and Aria Tri Peryoga and also my brothers and sisters for their wholehearted support.

CHAPTER 1
GENERAL INTRODUCTION

1.1 INTRODUCTION

Dipterocarps are among the most important tree species of the South East Asian tropical rain forests. These species are distributed over an extensive area, from Burma (Myanmar) to Thailand and the Philippines and passing from Malaysia to the Indonesian archipelago and Papua New Guinea. The Dipterocarpaceae constitute a large family of tropical trees that includes three subfamilies viz. the Monotoideae, the Pakaraimoideae, and the Dipterocarpoideae. The first subfamily, the Monotoideae, is found in Africa and consists of some 36 species in two genera. The second subfamily, the Pakaraimoideaea, consists of one monotypic genus, which was found till now only in the Republic of Guyana in South America (Maguire, 1977; Maguire and Ashton, 1977). In Columbia a new species of Dipterocarpoideae was found, namely *Pseudomonotes tropenbosii* (Londoño et al., 1995). The third subfamily, the Dipterocarpoideae, consists of about 470 species divided into 14 genera (Kostermans, 1992) 267 of which are found in Borneo, 155 species being endemic to this island (Ashton, 1989). Many of Dipterocarpoideae are canopy trees and can make up to 80 % of the trees in this upper layer of the forest. They also have become the most important commercial tropical hardwoods in the world. Thus it is important that the Dipterocarpoideae should be managed in a sustainable way because of their important ecological role, as well as for their economic importance.

The main silvicultural system that has to be implemented by law in Indonesia is the Indonesian Selective Felling System, which was officially formulated in 1972 and slightly modified in 1990. The mixed dipterocarp forests of Sumatra and Kalimantan are virtually all to be managed under this system.

The most important genus of the Dipterocarpaceae family is *Shorea*, of which there are about 100 commercial species (Haggarsson et al., 1994; Smits, 1994). One of these commercial species is *S. leprosula*, which can grow in many soil types and under a wide variety of site conditions. It is frequently found on well-drained soils in lowland and hill dipterocarp forests up to 700 m above sea level (Ardikoesoema & Noerkamal, 1955; Meijer & Wood, 1964; Symington, 1974; Ashton, 1982). This species is able to achieve a mean annual increment in diameter of more than 2 cm (Harbagung & Wahyono, 1987; Masono, 1985). *S. leprosula* grows in sites that are less hospitable than the sites in which the species evolved (Hatta et al., in prep.), and in such sites, the availability of nutrients and the development of mycorrhizae are often critical factors determining success or failure of plantation establishment.

Logging activities in production forests with heavy machinery have significant ecological impacts, especially on the microclimate, the soil density and the

hydrological conditions (Soerianegara, 1972). It has been shown that, after logging, many seedlings and saplings die because of soil heating (up to 45^{0}C). This heat level is lethal to the development of most mycorrhizae associated with the dipterocarps that have been studied (Noor & Smits, 1987). According to Mason and Ingleby (1997), the size and effectiveness of populations of ectomycorrhizal fungi can be severely affected by damage to vegetation and soil, whether the damage results from natural causes or from human intervention. These destructive processes include intense fire, soil erosion, topsoil disturbance, clearing/logging, tillage, long fallow periods, soil compaction, and biocide application. As a consequence of so many negative impacts upon the natural ectomycorrhizal inoculum potential of the areas to be planted with dipterocarps, the dipterocarp planting stock must often be provided with suitable fungi before planting in order to grow well. However, conditions in nurseries can be quite different from forest conditions and often certain early ectomycorrhizal fungi, sometimes called weed fungi (Mason and Ingleby, 1997), may dominate the mycorrhizal community on the nursery planting stock. Therefore, ectomycorrhizal fungi used for inoculation in the nursery may be unsuitable for use in the field. The exclusive use of the former fungi may lead to the failure of dipterocarp seedlings to establish themselves in the field (Smits, 1992).

The main problem that has been encountered in establishing plantations of dipterocarp species on a large scale is related to the poor and uncertain supply of dipterocarp planting stock. This is due to either irregular or mast flowering behaviour of many members of this family, in which fruiting only occurs at intervals of 2 to 10 years (Janzen, 1974; Chan & Appanah, 1980; Ashton, 1982; Yasman & Smits, 1988. Further complicating the problem is that flowering seems to be irregular among the species (Chan and Appanah, 1980; Ashton, 1982). Furthermore, seeds of this family cannot be stored for periods longer than a few weeks (Tang & Tamari, 1973; Tamari, 1976; Tompsett, 1987). Producing dipterocarp-planting stock through vegetative propagation, which is carried out in nurseries using cuttings (Yasman and Smits, 1988) has solved these problems. The cuttings used are obtained from hedge orchards (Leppe & Smits, 1988). Once rooted, they are inoculated with mycorrhizal fungi. This method has, among others, been used successfully in plantations of the state forest enterprise PT. INHUTANI I in Long Nah (East Kalimantan). Various dipterocarp species were planted (*S. leprosula, S. johorensis, S. pauciflora* and *Dryobalanops lanceolata*). They have all shown very good survival and growth (Bachtaruddin et al., 1994). The total area reported following this methode since 1985 now has reached 2 million hectares. The Wanariset propagation method was applied on a production scale by PT. INHUTANI I in Long Nah (East Kalimantan), where a nursery was capable of producing more than half a million cuttings per year (Bachtaruddin et al., 1994). In 1994, a total of 328 forest concession holders in Indonesia had already established hedge orchards to support the vegetative propagation of dipterocarps (Leppe and Smits, 1996).

Another problem is that wood increment in East Kalimantan is calculated at only 1 to 3 m^3/ha/year for commercial species of diameters over 50 cm. These poor growth rates are due to the effects of long dry seasons and low soil moisture content (Oldeman & Iriansyah, 1993). In addition, low soil fertility, especially a scarcity of phosphorus, is a contributing factor. The presence of myccorhizae plays an important role in the uptake of phosphorus from the soil. Growth of Dipterocarps can be improved by the use of fertilizers. Fertilizers have been proven to be important for several Dipterocarps (Sundralingan, 1983). It has been found that NP fertilizer generally improved the growth of potted *Dryobalanops aromatica* and *D. oblongifolia.* Supriyanto et al. (1993) reported that a combination of mycorrhizal fungi (*Scleroderma dictyosporum*) with 2 g/plant NP fertilizer produced the strongest effect on the growth of *Shorea mecistopteryx* seedlings in nurseries.

Several recent reports on dipterocarp mycorrhizae have shown a triangular interaction between the type of soil, the dipterocarp species, and the fungal species in a particular location (Smits, 1994; Yasman, 1995; Brundrett et al., 1996). However, it has yet to be determined how the degree of fungal (mycobiont) specificity and environmental conditions are related to the developmental stage of the tree (phytobiont). Last et al. (1984) reported that a succession of mycorrhizal fungi on various temperate-zone trees is related to tree age, but no information is available as yet on the importance of this phenomenon in dipterocarps. Those fungi appearing on seedlings or in young plantations are referred to as early-stage fungi, those that replace the early stage ones as late-stage fungi (Mason et al., 1982; 1983; Deacon, et al., 1983). According to Dighton and Mason (1985), the succession of mycorrhizal fungi can possibly be explained by changes in the carbohydrate supply from the host tree. It can also be understood by increases in net photosynthesis (Hintikka, 1988), by tree vitality (Termorshuizen and Schaffers, 1987) or by an altered distribution of photosynthates over root and shoot (Hintikka, 1988). Furthermore, the mycorrhizae might be affected by an increased internal recycling of nutrients with increasing age of the trees and their roots (Miller et al., 1979; Theodorou and Bowen, 1971, see Fig. 1-1).

Smith and Read (1997), Smits (1994), Omon (1994), and Hadi and Santoso (1988) claimed that the Dipterocarps are more specific than many other plant groups in their symbiotic associations. This is suspected to be further complicated by the ageing process of the phytobiont. How do water stress, light, and nutrient conditions of the phytobiont influence the root metabolism, and with it the succession and performance of the mycobiont? Species succession of mycorrhizal fungi in association with the roots of *S.leprosula* is assumed to be influenced by the physiological age of the plants and environmental stress conditions as mentioned above. The present study attempts to elucidate some of these possibilities.

Photosynthesis and transpiration are important physiological processes in plant growth. These processes are not only related to productivity, but also to adjustment

to the environment (Oldeman, 1990). Both processes are very important for any given aspect of plant growth. Information on photosynthesis in tropical forest trees is still limited in comparison with temperate forest trees. When transplanting, it is important to know the reaction of a plant to suitable fungi under the environmental stresses of new habitats or under new environmental conditions. The rate of photosynthesis is controlled by a large number of external and internal factors. External factors include light (intensity, period and colour), heat, carbon dioxide concentration, wind (velocity), water (supply), nutrients (supply). Internal factors include chlorophyll content, leaf structure and stomata aperture (Harley, 1989) and the organization and state of the sap-stream (Zimmermann, 1963; Oldeman, 1974, 1990).

Yasman (1995) reported that on dipterocarp roots in nature the following environmental factors influence the development of mycorrhizal fungi: light intensity, soil fertility (expressed as the availability of nitrogen and phosphorus), soil moisture, heat, aeration, and pH. Light intensity is an important environmental factor for the growth of seedlings and indirectly, for associated mycorrhizae.

Considering the above facts, this study focussed on ectomycorrhizal development related to age and stress. Indicators of juvenility were considered to be important in assessing the developmental state of a plant. The indicators used for assessing juvenility in dipterocarp seedlings are leaf size, size ratio (length to width, shape of leaf), presence of a drip point, presence of green glands in the leaf, leaf thickness and presence of a thick cuticula (Smits, personal communication). The main environmental stress factor taken into consideration was drought. To a lesser degree, differential use of light and nutrients by young plants was also studied as a factor influencing the succession of mycorrhizal fungi on Dipterocarpaceae.

1.2 DIPTEROCARPACEAE AND ECTOMYCORRHIZAE: A SHORT REVIEW

A mycorrhizal root is an association of a fungus with the living root of a green plant, most often beneficial to the plant. This association usually occurs during the whole life of the plant. Mycorrhizae possess characteristics that differentiate them morphologically from other plant infections. Usually, both the fungus and the plant benefit from mycorrhizal symbiosis: the fungus obtains carbohydrates from the plant and the host plant obtains water, mineral nutrients and N- synthates from the fungus.

There are several mycorrhizal types, which differ in morphology and physiology. Harley and Smith (1983) recognized seven mycorrhizal types, namely Vesicular-Arbuscular Mycorrhizae (VAM), Ectomycorrhizae (ECM), Ectendomycorrhizae, Arbutoid mycorrhizae, Monotropoid mycorrhizae, Ericoid mycorrhizae and Orchid mycorrhizae. Only two of them, the ectomycorrhizae (ECM) and vesicular-

arbuscular mycorrhizae (VAM) occur in Dipterocarpaceae (Alexander and Hogberg, 1986; Smits, 1992). Smits (1994) also discovered an eighth, new type of dipterocarp root-fungus association, which differs from the seven types of symbiosis mentioned above, and for which he coined the name Amphimycorrhizae. He claims this to be a mycorrhizal association as well.

An ectomycorrhizal root is characterized by the presence of three structural components: (a) a sheath or mantle of fungal tissue which encloses the root, (b) a labyrinthine inward growth of hyphae between the epidermal and cortical cells called the Hartig net, and (c) an outwardly growing system of hyphal elements which form essential connections both with the soil and with the fruiting bodies of the ectomycorrhizal fungi (cf. Smith and Read, 1997).

Most ectomycorrhizae are characterized by the presence of the above-mentioned Hartig net. In dipterocarp roots the Hartig net always grows between the radially elongated epidermal layers (Lee, 1988; Supriyanto et al., 1993, Omon, 1994, Smith and Read, 1997). In addition, a pseudoparenchymatic fungal mantle surrounds the fine roots (Smith and Read, 1997).

World-wide, about 1000 species of ectomycorrhizal fungi are known at present, and these can form ectomycorrhizae with only a small number of plant families such as Betulaceae, Fagaceae, Pinaceae, Leguminosae and Dipterocarpaceae (Jülich, 1988). On the other hand, the relatively small number of 70 to 80 species of vesicular-arbuscular mycorrhizal fungi can enter into symbiosis with a very large number of plant families. Contrarily to Jülich (1988), Molina et al.(1992) estimate that a total of 5000 to 6000 species of fungi form ectomycorrhizae or endomycorrhizae. All dipterocarp species have mycorrhizal associations, in two forms, i.e. VA- and ectomycorrhizae. Almost all dipterocarps form ectomycorrhizae with several fungi belonging to the Basidiomycetes. Ectomycorrhizae have been found on all Dipterocarpoidae.

The results of previous studies proved that several dipterocarp species are obligately ectomycorrhizal during their entire life cycle (Hadi and Santoso, 1988; Smits, 1994; Omon, 1994 and Yasman, 1995).

The significance of ectomycorrhizae for the trees can also include an increased resistance to pathogens besides better provision of water and nutrients. The increased provision of water and nutrients is caused by the fact that the absorption surface of the mycelium of mycorrhizal fungi is much larger than that of associated plant roots, whereas their thin hyphae can enter water-filled soil pores too small for roots (Oldeman, 2001).

Several mechanisms are involved in the protection afforded by mycorrhizal fungi against pathogens. They are supposed to be caused by (a) the fungal mantle, which

serves as a physical barrier to pathogens, (b) recycling of root exudation substances, which are lost to the soil in non-mycorrhizal roots, and (c) the production of antibiotics by mycorrhizal fungi.

In return, the mycorrhizal fungus obtains carbohydrates from the plant. One should best view the nature of mycorrhizae as a dynamic but stable balance, in which fungus and plant are able to withdraw nutrients from each other. The result is a mutualistic symbiosis. Ectomycorrhizae are also seen to play a role in minimizing nutrient losses through leaching (Read et al., 1989).

Yasman (1995) reported that the Dayak people of West Kalimantan (Indonesia) practiced a method of planting dipterocarp trees, in which they unknowingly used mycorrhizae, long before mycorrhizae on dipterocarp roots were first described by Van Roosendaal and Thorenaar (1924, p. 530). The latter authors observed mycorrhizae associated with the roots of *Hopea mengarawan* growing in Sumatra. They were subsequently also mentioned by de Voogd (1933, p 707) who observed them on roots of *Shorea platyclados*. The local people in Krui (Sumatra) also have planted *Shorea javanica* trees for several hundreds of years for the production of resin (Torquebiau, 1984). So the existing previous practical experience involving dipterocarp mycorrhizae, until recently was neither applied knowingly nor to large-scale dipterocarp forests in Indonesia.

1.3 LIGHT INTENSITY, NUTRIENTS, AND MYCORRHIZAE

The development of mycorrhizal associations is influenced by several environmental factors, namely light intensity, soil fertility (such as available nitrogen and phosphorus), soil heat, soil moisture, aeration, pH, etc. The present study focuses on the role of nutrients, light and soil heat and their influence upon the dipterocarp mycorrhizae. Indeed, besides being extremely important biologically these factors are also the only ones that can be silviculturally more or less controlled in the field.

Light and mycorrhizal formation have been so intimately associated that it is impossible to consider the problem of mycorrhizal morphogenesis without examining the direct and indirect effects of light on root development (Harley, 1989).

Light influences mycorrhizal development in several ways. There is the direct influence of light through carbohydrate production by host plants and the influence of radiation affecting other factors such as evapotranspiration and soil heat.

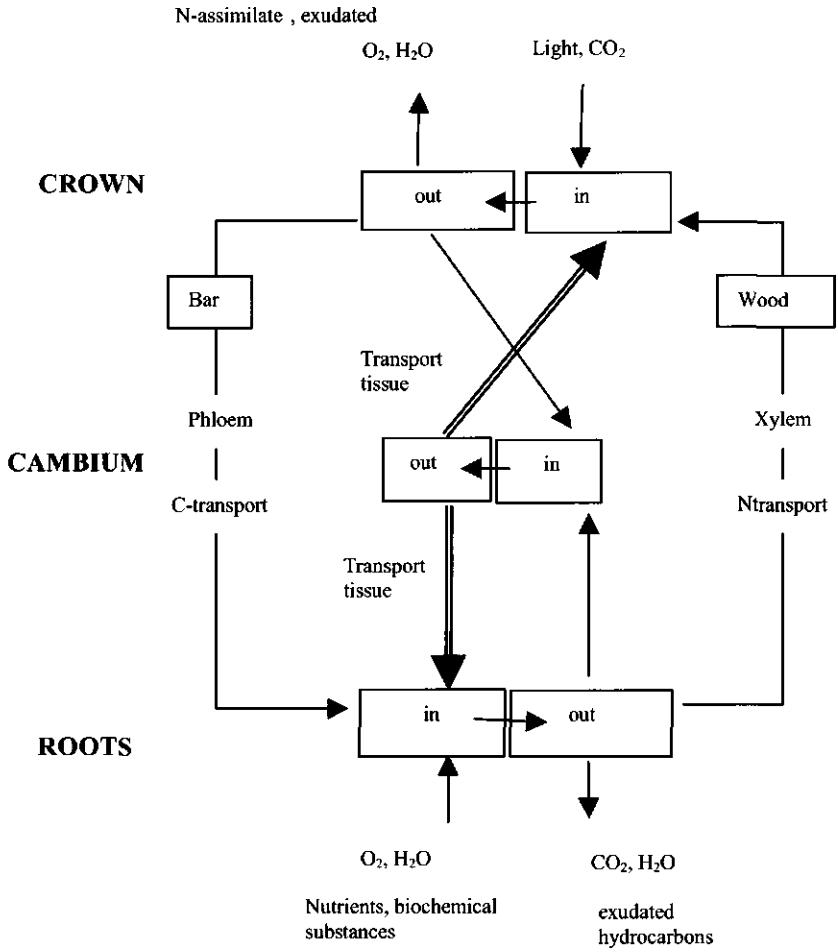


Figure 1-1 Diagram of the sap-stream (inspired by Oldeman, 1990)

High soil heat, due to increased solar irradiation, becomes limiting for growth of dipterocarps (Nicholson, 1960; Noor and Smits, 1987; Smits, 1994). The relationship between light and mycorrhizae in Dipterocarpaceae is furthermore complicated by the fact that different mycorrhizal associations may show different reactions to light intensity and / or soil heat (Yasman, 1995).

The diagram of sap-stream (Fig. 1-1), also see Oldeman (1990), shows the input-output relationships among roots, cambium and crown.

Photosynthesis receives inputs of light and carbon dioxide (CO_2) from the atmosphere and water plus organic nitrogen compounds from the roots by the sap-stream and the output is oxygen and sugars. Production rates of these processes depend on different leaf properties such as leaf area, chlorophyll content or dry matter content or specialization as shadow or sun leaf.

Rhizosynthesis inputs are water and mineral nutrients from the environment and sugar from the crown. Outputs contain N-syntheses made by the roots including vitamins and hormones that are distributed throughout the tree by the sap-stream.

In cambial production, the inputs arrive with the sap-stream in the form of water, sugar, nitrogen compounds and the outputs are xylem and phloem. Functionally, these provide transport capacity for sap, upwards in the xylem and downwards in the phloem.

The production systems are interrelated by feedback loops, in which the crown produces mainly sugars, the root system produces nitrogen compounds, and the cambium produces transport capacity in the form of vessels or tubes.

Fertilizer affects the input in the root system. Fertilizer trials have been conducted for several dipterocarp species. Tangau (1983) found that potted *Shorea bracteolata* and *Shorea parvifolia* seedlings had improved growth and increased nutrient uptake at higher fertilizer levels, particularly when water supply was abundant. Turner et al., (1993), however, found that potted *Shorea macroptera* seedlings did not respond to fertilizer application but he did not include possible absence of suitable mycorrhizae. Newton and Piggott (1991) working with oak and beach found that the application of fertilizer could reduce the number of mycorrhizal types and their relative abundance. In other words, fertilizer inputs might trigger feedback loops stimulating growth by the cambium and the crown, but they did not always do so.

Lee and Alexander (1994) working with *Hopea helferi* and *H. odorata* found a positive growth response to mycorrhizal infection but a variable response to nutrient treatments. The presence and abundance of mycorrhizae, as influenced by nutrients, affects the amount of ascending sap (Figure 1-1) and, therefore, influences the drought tolerance of the host plants. It therefore has implications for dipterocarp forest management. An understanding of the relationship between fertilizer application, growth, mineral uptake and ectomycorrhizal infection for rational forest management and for an efficient use of fertilizers, is therefore of paramount importance.

1.4 SIGNIFICANCE OF THE STUDY

It is now realized that the role of mycorrhizae in the establishment of plantations with dipterocarp species is much more important than was previously known. Research activities involving dipterocarp mycorrhizae, however, are still scattered and too scarce. Some studies only, the majority being due to research of Tropenbos Kalimantan Center (East Kalimantan) reported on the role and function of mycorrhizae in dipterocarp forests (Smits, 1982; Smits et al. 1987; Lee, 1988, Oldeman, 1990; Supriyanto et al., 1993; Omon, 1994; Smits, 1994; Yasman, 1995; Smith and Read, 1997). The present project is part of the ongoing effort at the Wanariset station. In this context, the objectives of the present investigations were:

1. To study the development of dipterocarp mycorrhizae
2. To understand the succession of their mycobiont species on vegetatively propagated *S. leprosula* plants.

Factors such as physiological ageing, environmental factors (e.g. deficiencies in or excesses of light and nutrients), and the presence or availability of suitable fungi are expected to influence the succession of ectomycorrhizal fungi, and are therefore important to study.

The investigation had three specific objectives:

* To study the effects of mycorrhizal fungal species, soil substrate characteristics, nutrients and some environmental factors on the growth of cuttings of *Shorea leprosula* Miq.
* To study the effects of interactions between the applications of fertilizers, different ectomycorrhizal fungi and different soil substrates on the growth of *S. leprosula* cuttings.
* To study the development of mycorrhizae in different soil substrates under known environmental conditions

A better understanding of all the above relations is important for the formulation of better silvicultural guidelines for dipterocarp forest. In view of the great ecological importance of dipterocarps in the lowlands of the Sunda land region, and their economic importance, this study is therefore highly relevant and of great significance both scientifically and practically.

The parallel aspects of water stress physiology in *Shorea leprosula* will be covered in a study by Aldrianto Priadjati, soon to be published.

1.5 HYPOTHESES

In this study two hypotheses were tested:

Environmental factors, particularly light and nutrients, are critical to the growth and development of small dipterocarp plants, but only so in combination with mycorrhizal fungi.

1. Different mycorrhizal associations result in different growth performances of *Shorea leprosula.*
2. The environmental conditions affect the interaction between soil mycorrhizae and *Shorea leprosula* plants.

CHAPTER 2
EFFECT OF ECTOMYCORRHIZAE, NPK FERTILIZER AND SOIL SUBSTRATE ON THE GROWTH OF *Shorea leprosula* Miq. CUTTINGS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS IN THE GREENHOUSE

2.1 INTRODUCTION

Shorea leprosula (red meranti) is one of the most important Dipterocarp species in South East Asia. This species was selected for present study because it is a widely occurring, important commercial species and is considered as one of the fastest growing dipterocarp species (Mayer and Wood, 1964; Harbagung and Wahyono, 1987; Masano, 1985; Zipperlen and M.C. Press, 1996). Since this species can be propagated successfully by cuttings, there is no problem in the provision of planting stock materials (Omon and Soeseno, 1984; Yasman and Smits, 1988). The success of the rooted cuttings depends, however, on the presence of ectomycorrhizae, and proper plant handling in the nursery.

Ectomycorrhizal fungi (ECM) can provide a wide range of amino acids and more complex organic nitrogen compounds. Supriyanto (1999) reported that in *S. leprosula*, the concentration of nitrogen in the leaves was significantly increased by inoculation with *Scleroderma columnare, Laccaria laccata* or *Amanita umbonata.* Most of the ectomycorrhizal fungi readily convert NH_4, NO_3 and some simple organic N compounds, although there are differences at both the interspecific and intraspecific levels (Smith and Read, 1997).

In this study only N, P and K are used as nutrient inputs from fertilizer. Some specific results are known of the role of these nutrients in mycorrhizal growth and development. It was shown that rock phosphate was as effective as super phosphate for the formation of ECM. Rock phosphate applied at an amount of 25 ppm proved to be adequate for the improvement of ECM formation as compared to the controls (Hadi and Nuhamara, 1995). Extension of hyphae beyond the root depletion zone causes solubilization, and an uptake of P from originally unavailable organic-P forms (Pedersen and Sylvia, 1996).

In the majority of investigations involving nutrient applications, K was found to be present at lower concentrations in the tissues of mycorrhizal plants than in those of non-mycorrhizal plants. Accumulation of K is strongly influenced by the form of available N (NO_3 or NH_4) as well as by other cations, particularly Na^+. It is also influenced by the synthesis and storage of phosphate, and carefully designed experiments to investigate the influence of the mycorrhizal colonization on K nutrients need to take all potentially confounding factors into account (Smith and

Read, 1997). In the sap-stream, K^+ ions are vital for phloem transport (Zimmermann, 1983; Zimmermann and Brown, 1977).

In Chapter 1 it was already shown that in general mycorrhizae play a beneficial role in nutrient uptake by the phytobiont. In the case of the dipterocarps growing in normal, nutrient-poor ultisols, this role is even more important. Most of the soils in East Kalimantan belong to the red-yellow podsolic soils (FAO Soil Taxonomy) or Ultisols (USA Soil Taxonomy) which are generally known to have a very poor nutrient content, especially in phosphorus (Oldeman and Iriansyah, 1993). Therefore, the presence of mycorrhizae can be expected to be even more important in the uptake of phosphorus from the soil by dipterocarps.

In nature, nutrients as well as the type and species of ectomycorrhizal fungus can both influence the growth of the plants. Under extremely low nutrient availability the presence of carbohydrate consuming fungi in the mycorrhizal association may lead to facultative parasitism causing growth depressions (Yasman, 1995). The same is true for many plants grown under ideal nutrient supply conditions where the mycorrhizal presence does not contribute much to the growth of the plant but only takes away carbohydrates (Harley and Smith 1983). In limited light intensity, the carbohydrate production by the phytobiont decreases, whereas the carbohydrate consumption by ectomycorrhizae is still required. Such conditions affect the net amount of photosynthates available to the phytobiont and the mycobiont. For Dipterocarpaceae, mycorrhizae are obligate, so they always influence plant growth. Mycorrhizal performance is then expected not to be relevant (Smits, 1994). In between those extremes of nutrient supply, mycorrhizal fungi provide the largest benefit for the associated trees. There are different responses of the trees to different combinations of the nutrient supply and the presence of mycorrhizal fungi (Supriyanto, 1999).

The soil does not only have a great influence upon the plant and mycorrhizae by its nutrient status, but also by its physical characteristics. Water retention capacity is, of course, important but in this study water availability was not a limiting factor. Rainfall, the principal supply of water for natural forests, cannot be controlled in practice. Soil drainage and aeration are two related factors very important for plant growth and mycorrhizal developments, which can be somewhat manipulated. Periodical inundation, influencing the breathing of the soil, micro-life and plant roots can have serious impacts upon the survival and performance of mycorrhizae. In the field almost all dipterocarps are by nature confined to locations with relatively good drainage. In the experiment described here, inundation was not a limiting factor. This leaves us with the factor of aeration. Smits (1994) found significant differences between mycorrhizal fungi in sandy and clayey soils. He also found that the presence of mycorrhizal roots was directly related to soil depth. Almost all dipterocarp ectomycorrhizae were confined to the top 10 cm of the soil in natural

forests, confirming the thinness of the living forest soil layer in general (Oldeman, 1990).

The hyphae growing in the soil function as "bridges" allowing the absorption of certain otherwise unattainable nutrients needed for plant growth and mycorrhizal development. In general, the availability of nitrogen and phosphorus are known to affect the development of ECM on different tree species. Fertilizer trials have been conducted with several dipterocarp species, both in pots as well as under field conditions. For practical reasons, the present study made use of the range of fertilizer quantities already known to achieve significant growth increases in other *Shorea* species. The optimum dosage to support the optimum growth of seedling still needs further study (Supriyanto, 1999).

In practice, fertilization is also commonly used in nursery techniques. Nursery fertilization can increase plant production efficiently and rarely has adverse effects. Properly applied fertilizer stimulates seedling growth after outplanting (Fischer and Mexal, 1983). Response to nursery fertilization is influenced by a number of factors including soil and plant nutritional status, bed density, length of growing season and soil organic matter (Fischer and Mixal, 1983). Application of fertilizer after establishment of abundant ectomycorrhizae on root systems in cuttings of dipterocarp species has no negative effects (Smits, 1992).

Omon (1999) reported that inoculation with *Amanita* sp in combination with the application of a dosage of 100 mg NPK fertilizer resulted in a significantly higher percentage of of *S. leprosula* stem cuttings carrying mycorrhizae.

The interaction of several environmental factors with plant growth and the performance of the mycorrhizal association further cloud the role of nutrients and mycorrhizal fungi as independent factors. This interaction can involve various factors, among them culture medium (soil substrate), light intensity, soil heat and humidity (Hadi and Nuhamara, 1995).

Soil heat can have a dramatic impact upon the presence and performance of mycorrhizae. Smits (1994) describes several experiments showing that at soil temperature above 32 to 35°C several mycorrhizal root types disappeared completely. In open terrain, solar radiation can lead to very hot topsoil (Noor and Smits, 1987). Soil temperature can amount to more than 50° C at the soil surface and still be above 35° C at 10 cm depth, the deepest level at which most ectomycorrhizal types still have enough aeration to function normally.

Yasman (1995) also reported that an increase in the usual soil temperature had a negative impact upon the development of ectomycorrhizae. To find out the optimum growth of *S. leprosula* cuttings, the effect of ectomycorrhizae, NPK fertilizer, soil

substrate and their combination in different controlled and semi-controlled conditions, including light, were studied.

The aims of the experiments described in the present chapter address the following questions in the above framework:

- What are the effects of mycorrhizal fungal species, soil characteristics, fertilizers and some climatic factors on the growth of *Shorea leprosula* Miq. cuttings?
- What are the effects of interactions between the application of fertilizers, different ectomycorrhizal fungi and different soil substrates on the growth of *S. leprosula* cuttings?

2.2 MATERIALS AND METHODS

2.2.1 Introduction

Tropical forests consist of extremely complicated ecosystems. They are generally growing in extremely heterogeneous sites, in which conditions may vary from one relatively small location to the other. These variations express themselves in such factors as local topography, soil type, drainage, light intensity, etc, which are interrelated.

Hence it is intrinsically impossible to find experimental conditions which are representative of the tropical forest in general, or even for a forest of a certain area (e.g. East Kalimantan, Indonesia). Van Rompaey, for Ivory Coast, correctly states (1993, "Stellingen"): "There exist neither homogeneous sample plots in tropical rain forest, nor replications and the sampling of independent variables within one and the same ecosystem is a *contradictio in terminis*".

The experiments reported here address a particular situation at the very detailed scale of one cutting in a small environment. In fact it is the environment of pots with soil substrates growing under a number of greenhouse conditions, which can only represent a small fraction of those occurring in the forest. Even this small fraction shows heterogeneity again, at its own more detailed scale, because a greenhouse is a partly open, heterogeneous environment. The only thing possible is therefore to compare similar (but not identical) experimental samples with and without a certain treatment or combinations of treatments. In this way there can be no really "zero" control experiment. Unless one creates very artificial phytotron-like conditions, which are very costly and moreover far removed from those occurring in the forest, zero conditions are almost impossible to achieve in mycorrhizal work in particular.

Under non-hermetic greenhouse conditions, the use of sterilized soil creates a chance for certain opportunistic fungi (and other organisms) to colonize it. This is

also the case with certain opportunistic mycorrhizal fungi, notably *Thelophora terrestris*. This fungus is well known for its capacity to infect seedlings in nurseries all over the world (Mason and Ingleby, 1997).

It is, however, at the same time a weak parasite, often smothering tree seedlings. Its mycorrhizal benefit is doubtful and not considered to be very clear. In our experimental setup, we lacked the elaborate and costly installations such as those built by Marx (1973) in his work with Southern pine species in the USA. We had to accept the possible occurrence of fungi such as *T. terrestris*. Therefore, the effect of inoculations could not be measured as a difference between plants with and without mycorrhizae, but it had to be treated as the difference between inoculated and non-inoculated plants, accepting the "noise of nature" in the resulting numerical data.

One should moreover be aware of the fact that in the nursery one is presented with the same situation. Here the young seedlings or cuttings are usually potted in sterilized soil. The conditions in the nursery are, however, far from sterile. Hence the conditions of the presently described greenhouse experiments were very much a representation of nursery conditions.

2.2.2 Location and time of the experiment

The experiment was carried out in a greenhouse at the Wanariset research station, located some 38 kilometers Northeast of Balikpapan in East Kalimantan (Fig. 2-1). The experiment started in April 1999 when the cuttings were planted, and ended in February 2000 by harvesting the cuttings to obtain numerical data on the mycorrhizae and the plant material itself. Soil analyses, root investigations and mycorrhizal assessment were done in the Silviculture Laboratory, SEAMEO (South East Asian Ministers of Education Organization), BIOTROP (Southeast Asian Regional Center for Tropical Biology) at Bogor.

2.2.3 Preparation and experimental design

In March 1998, seeds of *S. leprosula* were collected from the Darmaga Experimental Forest of the Forest and Nature Conservation Research and Development Center at Bogor. The trees had been planted in 1958 from seeds brought from Sumatra (Ardikoesuma and Noerkamal, 1955). The choice of the seed material for these experiments from outside Kalimantan was made because of the fires raging through Kalimantan from mid-1997 until mid-1998.

The seeds of *Shorea leprosula* were made to germinate in clean washed river sand in the greenhouse. One week after sowing, most of the seeds had germinated and produced the typical first pair of green leaves, while the cotyledons were still attached. When these first leaves were fully developed, the seedlings were transplanted to the potting medium. The pots were made of concrete, 22 cm high, tapering from 10 cm diameter at the base to 15 cm diameter at the top. A mixture of topsoil and white sand at a ration 3:1 was used as the potting medium. These

seedlings were grown for six months in the greenhouse at the Wanariset research station before the first cuttings were taken. After the first harvest of the orthotropic shoots, more cuttings could be produced every two or three months from the newly appearing sylleptic shoots. In this way the collection of seedlings developed into a small-scale hedge orchard (Leppe and Smits, 1988). The cuttings were rooted according to the method developed by Yasman and Smits (1988).

The cuttings taken from the seedlings were some 5 to 7 cm long and consisted of two or three internodes while the stem diameter in general was between 3 and 5 mm (Omon and Soeseno, 1984; Yasman and Smits, 1988). To prevent wilting, the leaves were pruned according to the methodology described by Yasman and Smits (1988). To induce root formation the cuttings were treated with Rootone F at a dosage of approximately 5 mg per cutting (Omon, 1994). Rootone F contains on a weight basis 0.057 % IBA, 0.113 % of three chemical derivatives of NAA (0.067 % 1-Napthalene Acetamide, 0.033 % 2-Methyl-1-Napthalene Acetic Acid, 0.013 % 3-Methyl-1-Napthalene Acetamide) and 4 % of the fungicide thiram (Tetra Methyl Thiuram Dissulfate), suspended in talcum (Manurung, 1987).

After leaf pruning and application of the rooting stimulant, the cuttings were planted in vermiculite under glass cover to maintain high air humidity. Two months later most of them had formed roots, which is in agreement with other reports (Omon, 1994; Tolkamp and Priadjati, 1996). The rooted cuttings were transplanted to plastic pots containing 300 gram of soil substrate. At that time all traces of fungicide from Rootone F were washed out before transplanting.

Three different soil substrates (clay, sandy loam and sandy clay) were used. These soils were collected from the PT. KEM (Kalimantan Equatorial Mining Company) rehabilitation area in the Wanariset research forest area, the site locally known as Kilometer 42 to the left of the main road from Balikpapan to Samarinda. The soils were chosen on the basis of available soil analyses. These soil types were selected after their phosphorus content and drainage values. Three soils were used, namely clay (content 66.98 ppm. P available or a high P content), sandy loam (content of 39.86 ppm. available P, or a moderate P content) and sandy clay (content of 2.87 ppm. P available, or a low P content). They all represent soil types on which *S. leprosula* can be found growing naturally (Iriansyah et al., 1998). These soil types are quite common in the province of East-Kalimantan. For each of the three soil types used some three samples were analysed before the start of the experiment after autoclaving. At the time of harvesting of the plants at the end of the experiments, one soil sample per treatment was taken and analysed in detail. This amounted to 120 samples in total. The BIOTROP laboratory at Bogor carried out the analyses of these samples. Soil sample analysis followed standardized methods. The BIOTROP laboratory has its standard samples regularly checked through the international network of soil laboratories and has a good and consistent record for the data

obtained. The soil substrates had all three been previously sterilized by autoclaving at 121°C during two hours.

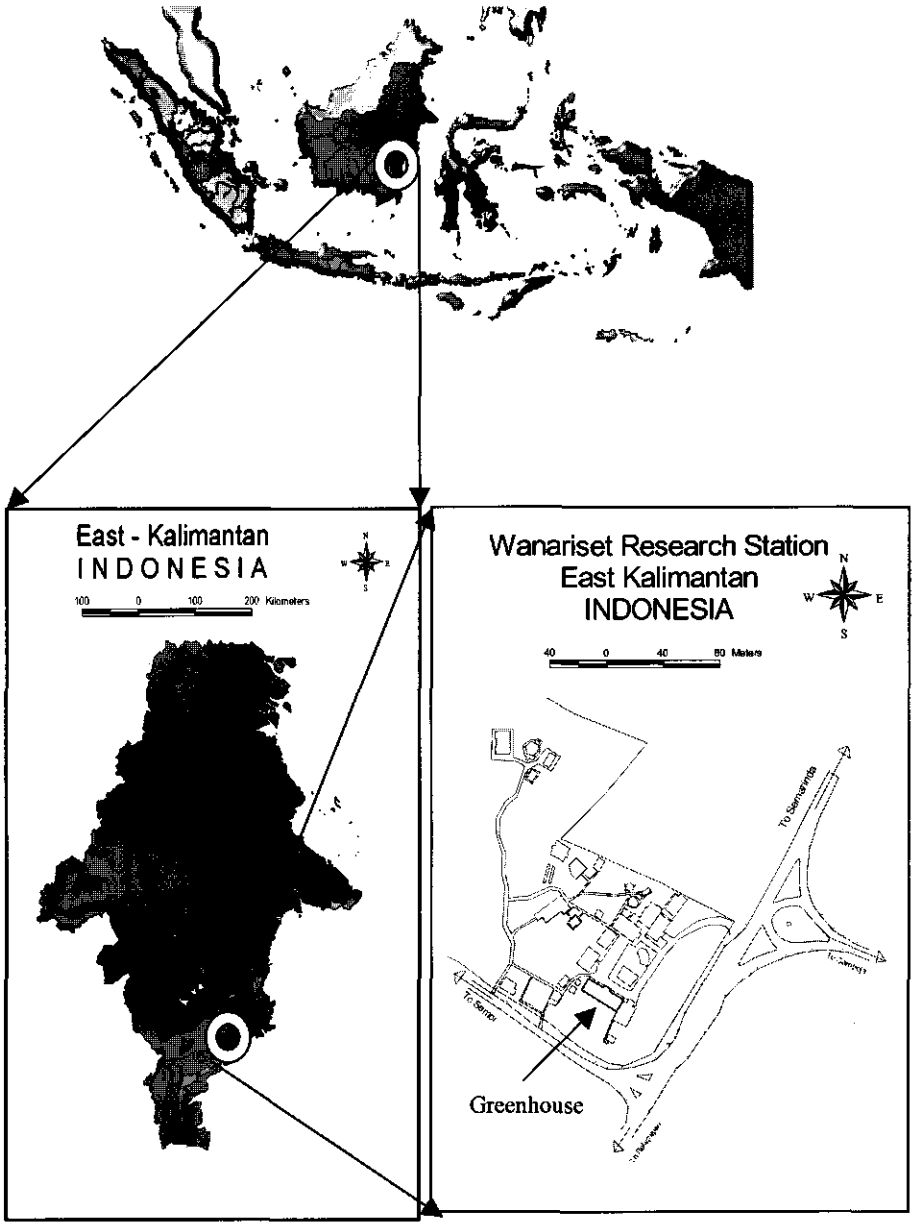


Figure 1-2 Map of the Wanariset Forestry Research Station, Samboja, East Kalimantan Indonesia

The experiments were carried out in the Wanariset greenhouse. The pots were placed in nursery beds of 1 by 6 meters. Two sets of environmental conditions were used, which are indicated henceforth as either "semi-controlled" or "controlled conditions". The plants growing under the semi-controlled conditions were grown under a simple plastic cover that surrounded the plants (see Fig. 2-2 at the left side).

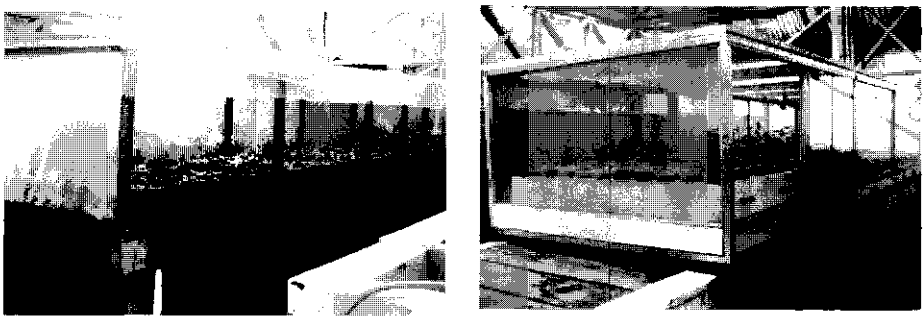


Figure 2-2 Transplanted *Shorea leprosula* cuttings placed in 1 m x 6 m compartments and covered by either polyethylene sheets ("semi-controlled conditions" left), or under glass ("controlled conditions" right). Note that the coverings were opened only for taking the picture and for measurement.

The plants grown under controlled conditions were placed in glass boxes in the greenhouse (Fig. 2-2 at the right side). This construction used an automatic water circulation system, activated by a thermostat whenever the inside air temperature reached 30° C. Fig. 2-3 shows the set-up of the water circulation system, which utilized a small reservoir under the concrete tables on which the plants were placed (A). A small aquarium pump (B) was used to lift the water up to the rim of the glass roof. From there, it flowed through a narrow gutter on the higher front part of the glass roof to the glass edge at the lower back of the roof part in a closed film of water, maximizing the cooling effect achieved underneath the glass, through evaporation of the water.

Besides situating the experiment in a greenhouse with only a minimal movement of air, and placing the inoculated cuttings under plastic or glass enclosures to reduce the risk of unwanted ectomycorrhizal inoculum coming into contact with the plants, the watering was done with a misting head. The misting head produced a fine spray of droplets to prevent splashing drops that could throw soil particles from one plastic container to the other. Watering was done once a day, except during unusually warm days when more misting was applied to prevent wilting of the plants. The water originated from a local deep well, considered to be free of ectomycorrhizal inoculum.

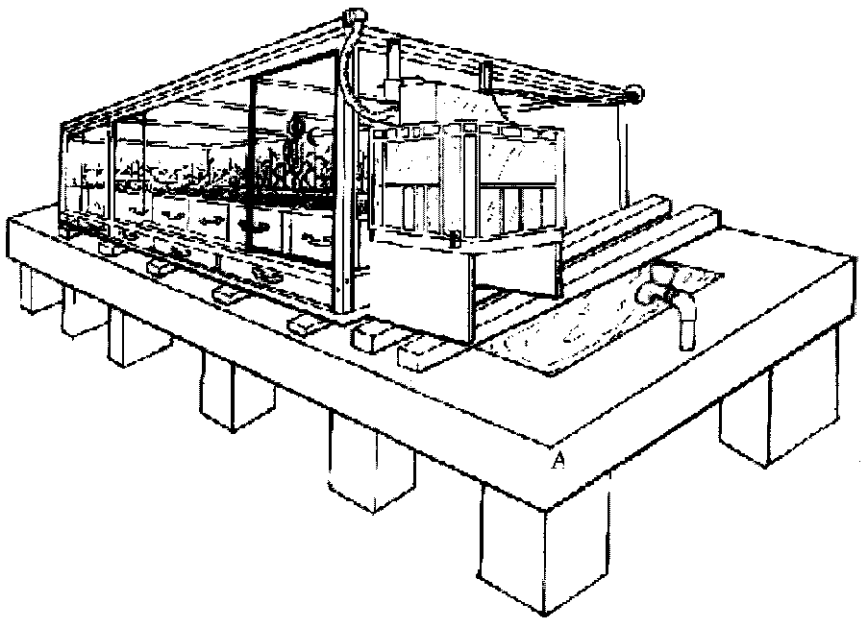


Figure 2-3 In "controlled" conditions the water was circulated through an automatic system to keep inside air temperature between 27° and 30° C. A = concrete table, B = small aquarium pump, C = thermostat.

A combined temperature and humidity measuring device was hung in each of the enclosures (see hanging copper circular device on the right in Fig. 2-3C) to measure the inside temperature and air humidity. Temperatures in the substrates were measured with a special thermometer for soil temperatures, 5 cm below the surface.

The photosynthetically active radiation was measured using a PAR-measuring device (LI-COR Type LI-250). All temperature, humidity and light measurements were taken per block. The light measurements were taken above the leaves of the cuttings in the enclosures. The layout of the blocks I to V in the two plant beds in the greenhouse is shown in Fig. 2-4. The semi-controlled setup is located further to the West and therefore received direct sunlight about 3 minutes later than the controlled setup because of the presence of a building towards the East.

The four inocula used in this experiment contained material of either one of the following ectomycorrhizal fungi: *Amanita* sp., *Russula* sp., *Scleroderma columnare*, or a cocktail (mixture) of these three fungi (Fig. 2-5). Fruiting bodies of *Amanita sp* and *Russula sp* were collected along the trail ("rintis") locally known as the "Rintis Wartono Kadri", located in a primary dipterocarp lowland forest four kilometers West of the Wanariset research station. The two species had previously been shown to improve the growth of *S. leprosula* (Smits, 1994). Fruiting bodies (sporocarps) of

Scleroderma columnare were obtained from the Laboratory of Silviculture at SEAMEO, BIOTROP, in Bogor. They were collected from the nearby *S. leprosula* plantation in Haurbentes.

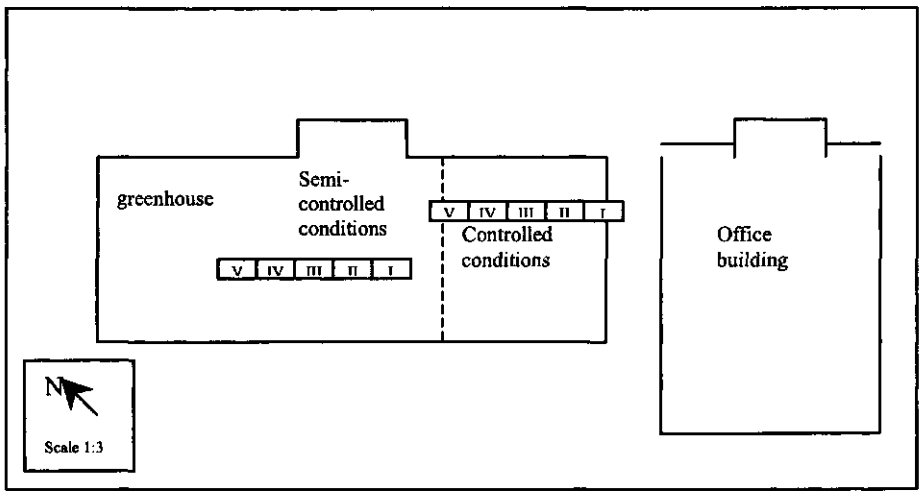


Figure 2-4 Layout of controlled and semi-controlled conditions in the Wanariset experimental greenhouse at the Station (Research station). I to V are similar treatments in two blocks.

The fruiting bodies of the three ectomycorrhizal fungi were cut into pieces and further treated for a short time in a blender according to the method described by Mason and Ingleby (1997). Then the fungal material was mixed with water to form a suspension. To assess the number of spores in 1 ml of suspension, microscope counts were made. Each of the plants was inoculated by 1 ml of this watery suspension, containing in the order of 10.000 spores. The treatment without mycorrhizal inoculation was not given a 1 ml clean water drop since one milliliter of water was insignificant compared with the daily watering needs of the plants.

One week after the inoculation, the fertilizer treatment, consisting of the addition of NPK (15:15:15), was applied in concentrations of 0, 50, 100 and 200 mg per plastic container each of which contained 300 grams of soil.

The experiment was laid out as a factorial set-up in a completely randomized design with three factors (3 x 4 x 5), in 5 similar treatments (not identical replications, Fig. 2-4) both under controlled and semi-controlled conditions. An outline of the experimental design is presented in Table 2-1.

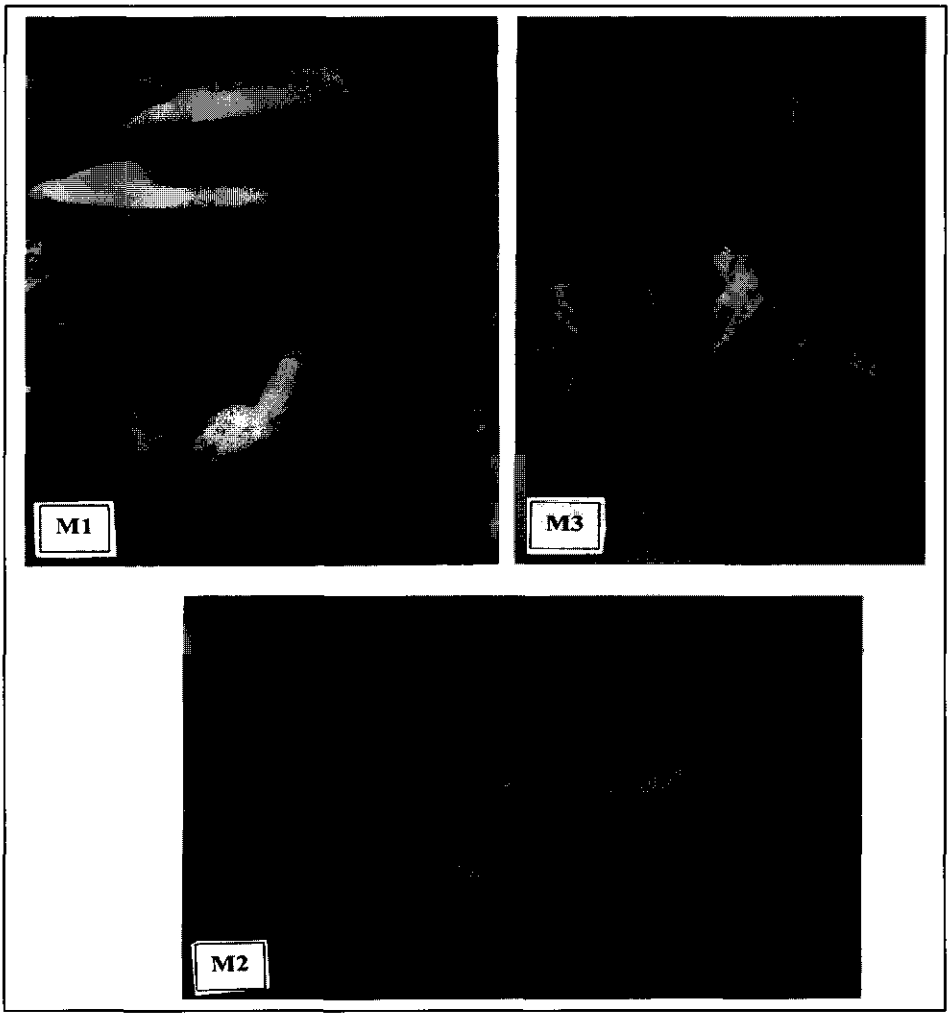


Figure 2-5 Fruiting bodies of *Amanita sp* (M_1), *Russula sp* (M_2) and *Scleroderma columnare* (M_3)

Table 2-1 Summary of the experimental design for testing the effects of ectomycorrhizal fungi, NPK fertilizer and soil substrates on the growth of *S. leprosula* cuttings under both controlled and semi-controlled conditions.

Type of treatment	Code	Number of treatments	Descriptions
Soil substrate	S	3	Clay, sandy loam and sandy clay
NPK fertilization	F	4	0, 50, 100 and 200 mg per cutting
Ectomycorrhizal inoculation	M	5	Untreated, *Amanita sp., Russula sp, Scleroderma columnare*, and cocktail *)
Similar treatment		5	Layout see Fig. 2.4
Total of cuttings		300	Two conditions means twice 300 in total

Note: *) Cocktail refers to a mixture of the three mentioned ectomycorrhizal fungi.

2.2.4 Data collection and analysis

During the experiment, measurements were taken and observations made regularly. The parameters measured were plant height, stem diameter and number of newly produced leaves per cutting per month. The first measurements testing the effects of treatments were taken one month after transplantation, time enough for all plants to have produced at least one new leaf. Measurements of height and diameter, and counting of the number of leaves were repeated every month.

Soil temperature was measured once a week at 9.00 hrs, 12.00 hrs, and 16.00 hrs, using a thermometer reading at 5 cm below the ground surface for each plant. These measurements are important in view of the above-mentioned effect of soil heat upon ectomycorrhizal growth and functioning (Smits, 1994).

Light intensity (Phosynthetically Active Radiation), humidity and air temperature were measured per block at 9.00 hrs, 12.00 hrs, and 16.00 hrs once a week for both controlled and semi-controlled conditions.

After 10 months, the experiment was stopped and the plants were harvested. The experiment was limited to 10 months because after that length of time plants growing in the nursery are normally started to be hardened to field conditions by gradually removing the plastic cover. Usually, they are then planted out into the field at age 18 months.

All parts above the ground as well as all underground parts were harvested. The plant parts were oven-dried at 80° C for 48 hours for further assessments. The soil was carefully removed from the roots and one soil sample per treatment (2 conditions x 3 soil types x 5 mycorrhizal treatments x 4 fertilizer levels, amounting to a total of 120 samples) was analyzed in the BIOTROP laboratory (see 2.2.2).

The leaf length and leaf width of all the leaves was measured. The leaf area was calculated based upon the formula of Sturges (1926) as applied by Omon (1994) to

calculate the form factor of the leaf. The frequency distribution of width and length of leaves of *S. leprosula* is presented in Appendix 1.

The plant parts were weighed, then oven-dried and their dry weight was assessed, after drying at 80^0 C for 48 hours in the oven. The ratios of fresh weight/dry weight were determined.

After drying, one sample per treatment was analyzed in the BIOTROP laboratory for nutrient content, totaling 120 samples. The nitrogen, phosphorus, potassium, calcium and magnesium contents were determined in percentages of dry weight. The iron content was measured in parts per million (ppm).

The fresh roots of one sample per treatment, totaling 120 samples were immediately stored in FAA (Formaldehyde Acetic Acid) to prevent any deterioration taking place. Then these preserved roots were taken to the Silviculture Laboratory, SEAMEO BIOTROP, at Bogor. After arrival in Bogor the number of infected and non-infected roots and the number of root tips were counted.

Histological analyses of mycorrhizal roots were performed according to the SASS method of 1958 (ex Berlyn and Miksche, 1976). First, the mycorrhizal roots were dehydrated (sequential application of ethanol at 20, 40, 60, 80% for 3 x 5 minutes and finally 3 x 10 minutes in ethanol 96%), then mounted in paraffin, and cut by microtome. After the microtome slicing the samples were stained with safranin to enhance the visibility of the fungal material.

Growth data (height and diameter of stem, number of leaves, leaf area, total fresh weight, total dry weight, number of root tips and percentage of mycorrhizal roots) of stem cuttings were treated in an analysis of variance (ANOVA). This was done to test and compare among treatments the effect of each factor of inoculation, fertilization and soil substrate as well as the interaction between those factors in determining the growth of *Shorea leprosula* cuttings at 10 months after inoculation and fertilization. Data were analysed using the GLM-ANOVA procedure of the SAS version 6.12. Significant F values found by ANOVA were further examined by pair-wise comparisons of means (Duncan's Multiple Range Test in the SAS software).

2.3 RESULTS

The results of the experiment are presented in three consecutive groups, namely the effect on plant growth, mycorrhizal development and nutrient uptake of each treatment or interacting set of treatments. To find out the significant level of each parameter measured, the mean values were tested by statistical analysis. The levels of significance for the measured effects of ectomycorrhizae, NPK fertilization, and soil substrates on the growth of *Shorea leprosula* cuttings, at 10 months after inoculation and fertilization for both the controlled and the semi-controlled condition are presented in Appendix 2.

Appendix 2 shows that single treatment factors (environmental conditions, soil substrates, fertilization and mycorrhizae) influenced plant growth and mycorrhizal development, with a significance level mostly exceeding 95 %. The combination of two treatments gave a lower level of significance, mostly none at all, except in two cases. The interaction between environmental conditions and soil substrate or between fertilizer, plant growth and mycorrhizal development, scored both more than 90 % significance. The combination of three or four factors did not show any significant effect. The importance of the significance level depends on the type of experiment. Laboratory and greenhouse experiments require a level of a significance to be more than 90 %, while a field experiment needs a minimal 80 % (Gomez and Gomez, 1984).

2.3.1 Effects of environmental conditions

2.3.1.1 Effect on plant growth

In Table 2-2 and Fig. 2-6 a comparison is made between the mean values of plant height, stem diameter, number of leaves per plant, leaf surface area per leaf per plant, fresh weight and dry weight. It appears from Duncan's Multiple Range Test that for all these factors, the controlled conditions show considerably more significant results than the semi-controlled conditions. Also the number of surviving plants at the end of the experiment is higher. There were 284 out of 300 cuttings surviving under controlled conditions vs. 255 under semi-controlled conditions.

Overall, the growth of cuttings in controlled conditions was stronger than in semi-controlled conditions. Height and diameter growth, number of leaves, leaf area, total dry weight under controlled conditions increased by 40 %, 50 %, 59 %, 71 %, 21 % and 11 %, respectively (Table 2-2, Fig. 2-6).

Table 2-3 shows that the measurements of some physical environmental factors such as atmospheric humidity and heat were lower under controlled conditions than under semi-controlled conditions.

Table 2-2 Effects of controlled and semi-controlled conditions on some growth parameters in *Shorea leprosula* cuttings, 10 months after treatment. E_1 = controlled conditions, E_2= semi- controlled conditions, ΔH= average height growth, ΔD = average diameter growth, N_l = number of leaves, A_l = leaf area, W_{tf} = total fresh weight, W_{td} = total dry weight, N_{rt} = number of root tip, ECM % = percentage of mycorrhizal roots.

		Mean response						
Treatment	N	ΔH (cm)	ΔD (mm)	N_l	A_l (cm^2)	W_{tf} (g)	W_{td} (g)	ECM %
Controlled (E_1)	284	13.3 a	0.3 a	11.6 a	8.2 a	3.4 a	1.0 a	69.1 a
Semi-Controlled (E_2)	255	9.5 b	0.2 b	7.3 b	4.8 b	2.8 b	0.9 b	60.5 b
Increment E_1 vs E_2 (%)		40	50	59	71	21	11	14

Values followed by the same letter (a or b) in the same column are not significantly different at the 5% level as tested with Duncan's Multiple Range Test.

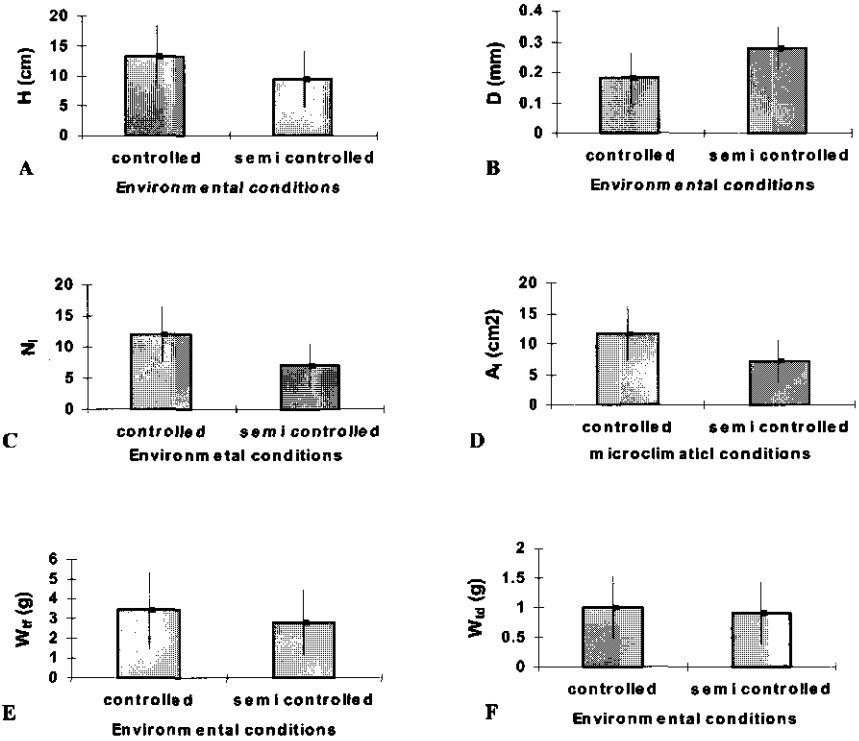


Figure 2-6 Environmental conditions and averages of some growth variables: (A) height growth; (B) diameter growth; (C) number of leaves; (D) leaf area; (E) total fresh weight and (F) total dry weight at harvest 10 month after inoculation and fertilization. The length of the vertical line at the top of the bars indicates the interval of incertitude at the 5 %-level of significance (Duncan' s test).

The average air temperature under controlled conditions was two degrees Celsius lower than under semi-controlled conditions (30^0C vs. 32^0C). Soil temperature was the same for both conditions (28^0C). Under controlled conditions, atmospheric humidity was much higher than under semi-controlled conditions (91% vs. 68%). A significant difference between environmental factors was found in atmospheric humidity only.

Table 2-3 Average soil temperature, humidity and air temperature measured under controlled and semi-controlled conditions during 10 months of observation.

	Controlled conditions			**Semi-controlled conditions**		
Series	Soil temp. (^{0}C)	Humidity (%)	Air temperature (^{0}C)	Soil temp. (^{0}C)	Humidity (%)	Air temperature (^{0}C)
I	28	91	31	29	72	32
II	28	90	29	29	71	33
III	27	89	30	28	67	31
IV	28	93	29	27	68	31
V	29	92	31	27	64	31
Average	28	91	30	28	68	32

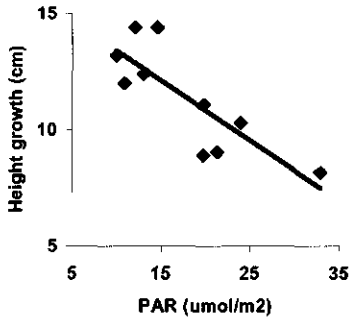
Light intensity (PAR) under controlled conditions was lower than under semi-controlled conditions, 12 and 24 μmol.m^{-2}, respectively. This difference is due to the high PAR values under semi-controlled conditions at 09.00 h and spectacularly so at 12.00 h, while the PAR value in both conditions at 16.00 h looks similar. In Table 2-4 the average PAR values at three different times of the day are given for both the controlled and semi-controlled conditions.

Table 2-4 Average PAR (μmol.m^{-2}) intensity measured under controlled and semi-controlled conditions during 10 months of observation.

	Controlled conditions				**Semi –controlled conditions**			
Series	09.00	12.00	16.00	Average	09.00	12.00	16.00	Average
I	9.56	10.92	9.50	10.00	14.90	45.64	11.00	24.00
II	10.60	11.96	10.00	10.90	14.57	34.36	10.00	19.64
III	11.13	14.29	11.00	12.14	18.58	34.15	11.00	21.24
IV	11.78	21.32	11.00	14.70	17.16	71.04	11.00	33.07
V	12.98	14.44	12.00	13.14	16.77	33.00	9.30	19.69
Average				12				24

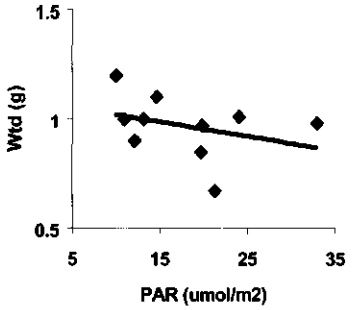
The growth of the cuttings, as shown in Table 2-2 was affected by the light intensity. They showed higher growth rates under light intensities between 10.0 to 14.7 μmol.m^{-2} (controlled conditions) than under 19.6 to 33.1 μmol.m^{-2} (semi-controlled conditions). In this case, light intensity acted as a growth factor, with an optimum dosage.

The photosynthetically active radiation (PAR) under controlled conditions was 12 μmol.m^{-2} of full daylight and in semi-controlled conditions was 24 μmol.m^{-2} of full daylight. The relationship between light intensity and height growth is presented in Fig. 2-7. The height growth of *Shorea leprosula* cuttings was significantly correlated to light intensity. At a higher light intensity the growth of cuttings tends to become lower than at a lower light intensity, with a correlation coefficient r = - 0.82.



Y = - 0.26x + 16.00 (r = 0.82; P = 0.01)

Figure 2-7 Relationship between light intensity (PAR) and heig ht growth of *S. leprosula* cuttings. P = significance (probability).



Y = - 0.001x + 1.09 (r = - 0.33 ; P = 0.35)

Figure 2-8 Relationship between the light intensity (PAR) and total dry weight of *S. leprosula* cuttings. P = significance (probability)

The total dry weight values in Table 2-2 and Fig.2-8 show that the biomass of the cuttings was not significantly correlated to light intensity (r = - 0.33, P = 0.35). The dry weight under controlled conditions increased 11 % more than under semi-controlled conditions. It looks as if the cuttings planted in controlled conditions

contained more water than those planted in semi-controlled conditions. Since the humidity under controlled conditions was higher than under semi-controlled conditions (91 % vs. 68 %), this is certainly possible.

2.3.1.2 Effect on mycorrhizal development

The mycorrhizal development can be expressed by the percentage of mycorrhizal roots, which is a parameter of the mycorrhizal ability to colonize the root system. Table 2-2 shows that the percentage of mycorrhizal roots (ECM %) under controlled conditions was higher than under semi-controlled conditions (69 % vs 61 %), an increase by 14 %. Factors affecting the mycorrhizal development could be light intensity, soil heat, humidity, soil pH, and /or oxygen supply.

Table 2-5 shows the mycorrhizal development in the roots to be slightly influenced by light intensity, soil heat measured by temperature, atmospheric humidity and temperature, but their correlation is very weak (r <0.5). Among the environmental factors tested, atmospheric humidity and soil heat were very important factors in the mycorrhizal development during the period of research.

Table 2-5 Correlation between light intensity, atmospheric humidity and temperature, soil temperature and percentage mycorrhizal roots (ECM %).

Correlation	Equation	r	P
Light intensity vs ECM %	Y = - 0.08 x + 67.57	0.22	0.54
Soil temperature vs ECM %	Y = 0.36 x + 56.06	0.11	0.81
Air humidity vs ECM %	Y = - 0.07 x +71.41	0.31	0.38
Air temperature vs ECM %	Y = - 0.29 x + 75.07	0.19	0.61

Note: r = correlation coefficient; P = significant (probability)

2.3.1.3 Effect on nutrient uptake

Nutrients absorbed by the mycobiont are used directly for fungal growth and development, and are indirectly transmitted for growth of the phytobiont. The effect of these nutrients was analysed using the biomass of the cuttings as a parameter. The relationship between nutrient uptake and biomass of cuttings is presented in Table 2-6, Fig. 2-9 and Fig. 2-10.

Table 2-6 Relationship between the nutrient uptake and the biomass (W_{td}) of the cuttings

No	Variable	Controlled conditions			Semi-controlled conditions		
		Equation	r	P	Equation	r	P
1	N	$Y = - 9.6x^2 + 248.5x - 152.4$	0.91	0.01	$Y = 7.5 x^2 + 72.9x + 323.1$	0.85	0.01
2	P	$Y = - 201.5 x^2 + 983.7x + 66.1$	0.83	0.01	$Y = - 44.8 x^2 + 345.2 x + 489.2$	0.57	0.01
3	K	$Y = - 9.9 x^2 + 249.0x - 75.6$	0.81	0.01	$Y = - 2.70 x^2 + 145.6 x + 235.2$	0.86	0.01
4	Ca	$Y = - 2.0x^2 + 97.8x + 302.1$	0.72	0.01	$Y = 2.6 x^2 + 24.7 x + 632.6$	0.59	0.01
5	Mg	$Y = - 185.7 x^2 + 1009.9x + 123.0$	0.92	0.01	$Y = - 269.7 x^2 + 1095.9x + 110.1$	0.67	0.01
6	Fe	$Y = - 2E+07 x^2 + 218176 x + 570.9$	0.62	0.01	$Y = - 1.8x^2 + 80.2x + 605.4$	0.58	0.01

Note: r = correlation coefficient ; P = significance (probability)

Table 2-6 shows a very strong correlation between nutrient uptake and biomass of the cuttings in controlled conditions ($r>0.80$; $P = 0.01$), except for Ca and Fe with $r = 0.72$, and $r = 62$. The biomass of the cuttings hence was affected strongly by the nutrients N, P, K and Mg. The nutrient uptake of Ca and Fe affected the biomass of cuttings moderately. Meanwhile, the nutrient uptake in semi-controlled conditions was somewhat correlated ($0.5 \leq r \leq 0.8$), except for N and K ($r = 0.85$ and $r = 0.86$). Both environmental conditions hence had a similar effect upon the uptake of N and K, controlled conditions a little stronger.

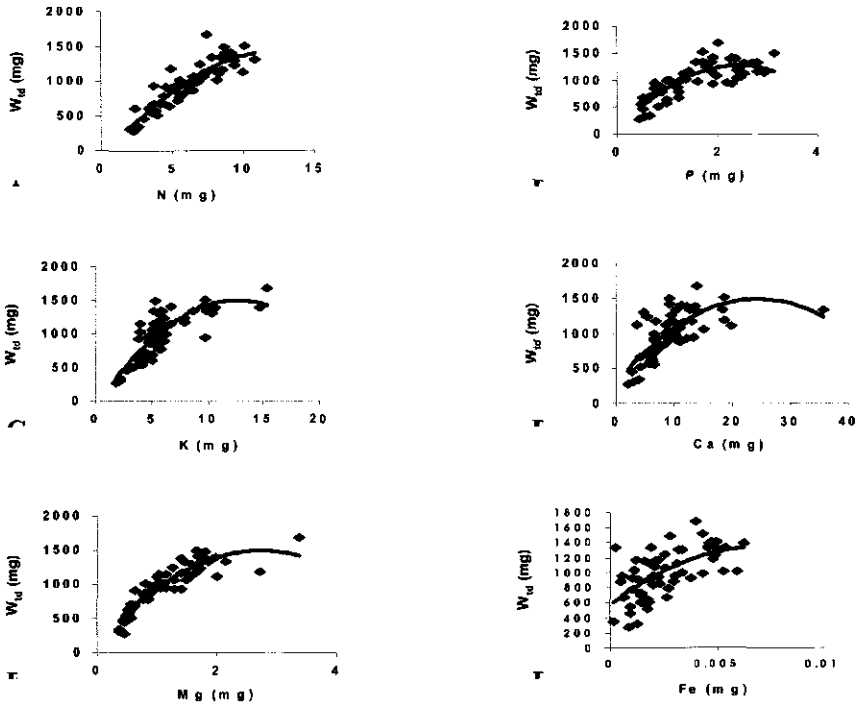


Figure 2-9 Correlation between dry weight (W_{td}) and (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake under controlled conditions.

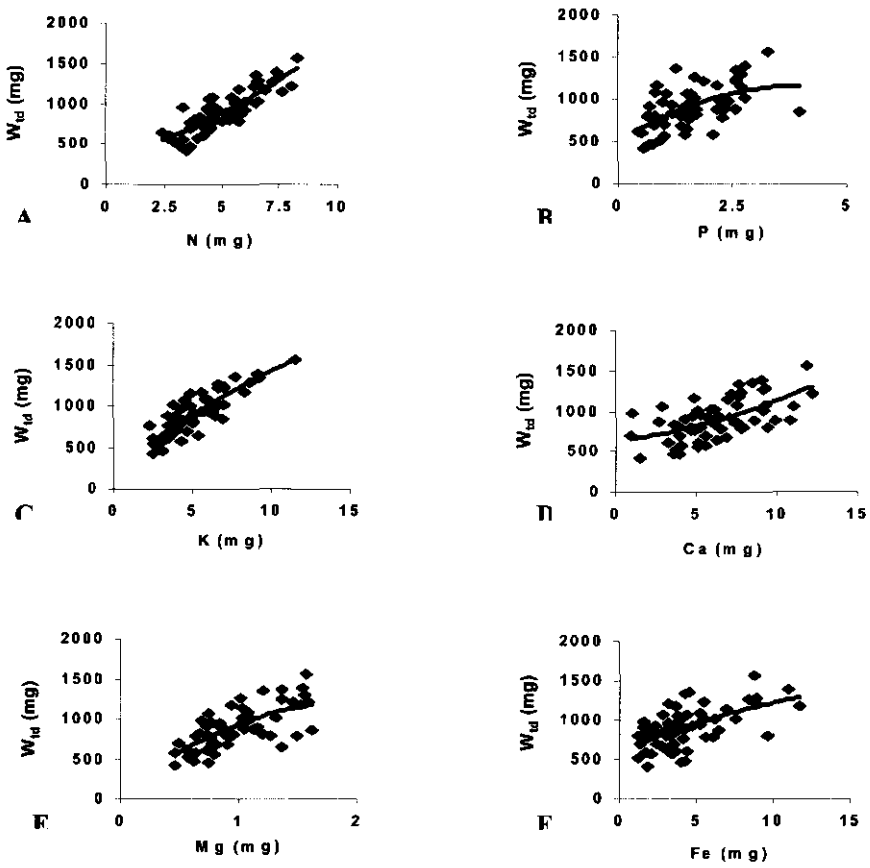


Figure 2-10 Correlation between dry weight (W_{td}) and (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake under semi-controlled conditions.

Nitrogen uptake under controlled and semi-controlled conditions is accompanied by a raise in biomass of the cuttings, particularly leaf biomass. Table 2-6 shows that nutrient uptake significantly affected the biomass increment of *S. leprosula* cuttings.

It can be confirmed that environmental factors influence the growth of cuttings, the mycorrhizal development and the nutrient uptake. Under semi-controlled conditions these effects are clear. For the 10 months that the experiment lasted the mycorrhizal development and the nutrient uptake effects tend to increase and according to the shape of the curves, they were not yet at their maximum in month 10.

2.3.2 Effects of soil substrates

2.3.2.1 Effect on plant growth

Of course, the substrate used in the nursery should meet the physical and chemical properties required for supporting the growth of cuttings. Some physical properties of the soil substrates used in the experiment are presented in Table 2-7. Regarding these substrate properties, the sand fraction in sandy clay (70 %) is higher than in sandy loam (58 %) and lowest in clay (35 %). Porosity and aeration in sandy clay are expected to be higher than in sandy loam and in clay. More intense aeration generally promotes both mycorrhizal development and plant growth.

Table 2-7 Summary of physical properties of the soil substrates in experiment 1.

	Fraction Distribution		
Soil substrate	Sand (%)	Silt (%)	Clay (%)
Clay	35	28	37
Sandy loam	58	19	23
Sandy clay	70	15	15

Table 2-8 and Fig. 2-11 and 2-12 show that the growth of *Shorea leprosula* cuttings planted in sandy loam and sandy clay was not significantly different as concerns height growth, diameter growth, number of leaves, leaf area, total fresh weight, and total dry weight (Duncan test at a 5 % level of significance). According to Table 2-7 and Table 2-8, the clay consistently was the poorest medium, reflected by the growth of *S. leprosula* plants and their percentage of roots with mycorrhizae. The height increment in sandy loam and sandy clay was 27 % and 28 % higher than in cuttings grown in clay, in parallel to the decreasing sand fraction (Table 2-7). More sand enhances aeration in sandy loam and sandy clay, as compared to clay substrate.

Table 2-8 Effects of soil substrate on various growth parameters of *Shorea leprosula* cuttings 10 months after treatment. ΔH = average height growth, ΔD = average diameter growth, N_l = number of leaves, A_l = leaf area, W_{tf} = total fresh weight, W_{td} = total dry weight, N_{rt} = number of mycorrhizal root tips, ECM % = percentage of mycorrhizal roots.

	Mean response								
Treatments	N	ΔH (cm)	ΔD (mm)	N_l	A_l (cm^2)	W_{tf} (g)	W_{td} (g)	N_{rt}	ECM (%)
Clay (S1)	178	9.7 a	0.2 a	9.0 a	4.5 a	2.4 a	0.8 a	394 a	62 a
Sandy loam (S2)	174	12.3 b	0.3 b	9.7 ab	7.8 b	3.6 b	1.1 b	443 a	67 b
Sandy clay (S3)	187	12.4 b	0.3 b	10.0 b	7.5 b	3.5 b	1.0 b	564 b	67 b
Increment S2 vs. S1 (%)		27	50	8	73	50	38	12	8
Increment S3 vs. S1 (%)		28	50	11	67	46	25	43	8

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

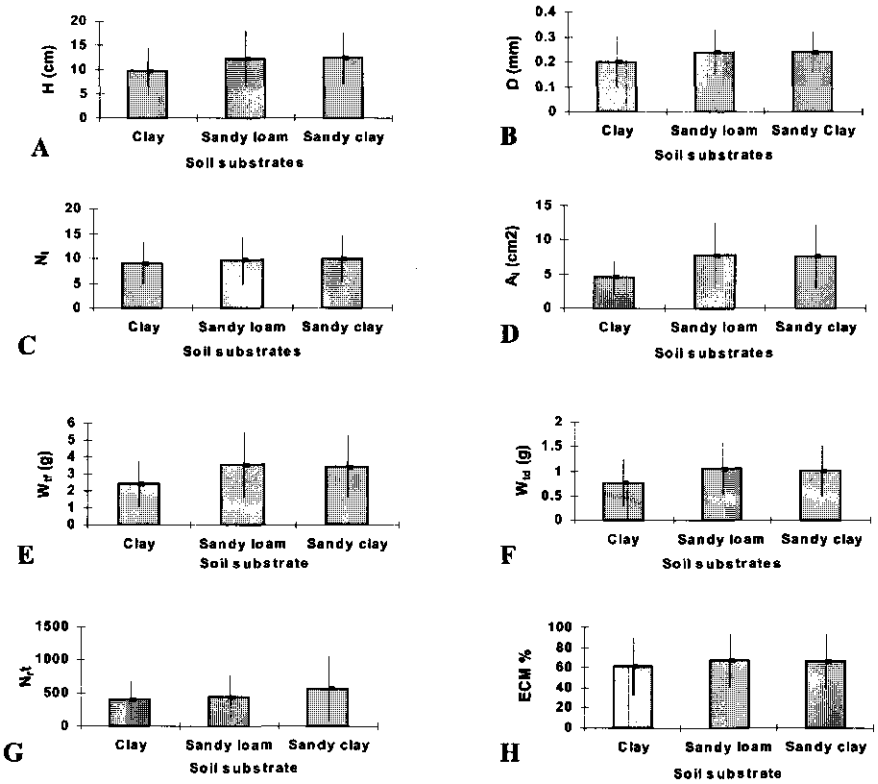


Figure 2-11 Soil substrate and average values of some growth variabl es. (A) height growth; (B) diameter growth; (C) number of leaves; (D) leaf area; (E) total fresh weight; (F) total dry weight; (G) number of root tips and (H) percentage of mycorrhizal roots at harvest 10 months after inoculation and fertilization. The length of the vertical line at the top of the bars indicates the interval of incertitude at 5 %-level of significance (Duncan test).

The total dry weight of the cuttings represents the structural matter, formed in interaction between ecological and physiological processes. Fresh weight includes water, important in sap-stream dynamics. The increment of total dry weight in sandy loam and sandy clay substrates was 38 % and 25 % higher, respectively, than dry weight in clay. The other important increment was in leaf area. The increment of leaf area represents the increment of the photosynthetic surface involved in feeding biomass production of the cuttings. Leaf area increment in sandy loam and sandy clay substrate was 73 % and 67 % higher than with the in clay. The differences in relation with the sand fraction of the soil substrate (see the results of soil analyses in Appendix 3 and 4) are closely correlated with this observation.

2.3.2.2 *Effect on mycorrhizal development*

The development of mycorrhizal hyphae in the substrate is influenced by oxygen supply (Yasman and Smits, 1988). A higher share of sand in the soil is expected to make the soil more porous and so to enhance oxygen supply. Table 2-8 shows that the percentage of mycorrhizal roots (ECM %) in sandy clay and sandy loam was indeed a little higher than in clay substrate, 67%, 67% and 62%, respectively. However, these differences were not statistically significant.

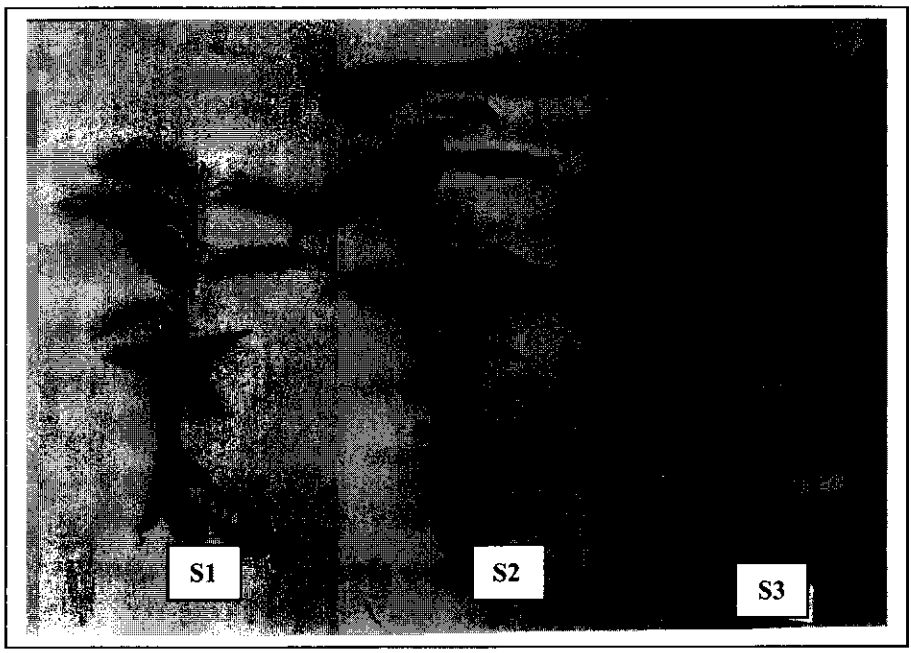


Figure 2-12 Effect of soil substrate on growth of *Shorea leprosula* cuttings after 10 months. S1 = Clay, S2 = Sandy loam and S3 = Sandy clay

2.3.2.3 *Effect on nutrient uptake*

The nutrient availability of each substrate is different. The plants absorb nutrient, translocated to meristematic tissues and either consumed or finally stored, either in structural forms or in reserves. Nutrient uptake by cuttings of *Shorea leprosula* in soil substrates of clay, sandy loam and sandy clay is presented in Fig. 2-13 for N, P, K, Ca, Fe and Mg.

The figure shows that the nutrient uptake in sandy loam and sandy clay was higher than in clay for all elements considered. The growth pattern as shown in Table 2-8 by height and diameter increment combined, and the dry weight of cuttings in sandy loam and sandy clay is related to the increase in nutrient uptake in those substrates.

Regarding these results, in terms of application, sandy loam and sandy clay can both be used to optimize the planting stock production of *Shorea leprosula* cuttings in the nursery (also see 2.5).

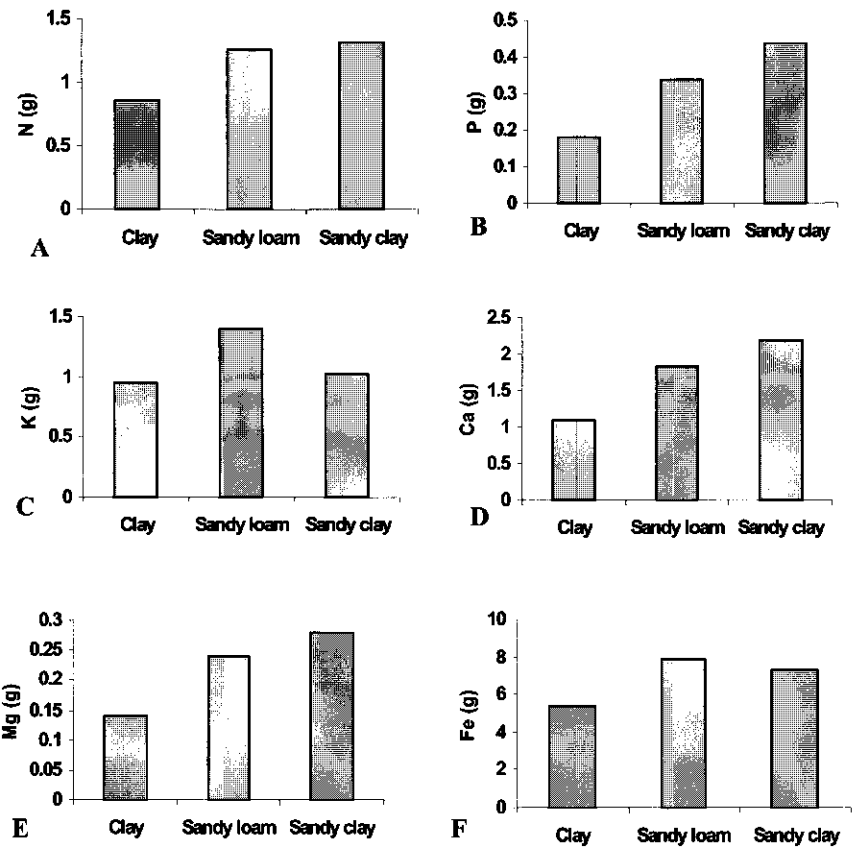


Figure 2-13 Nutrient uptake (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake of cuttings in different soil substrates

2.3.3 Effects of NPK fertilization

2.3.3.1 Effect on plant growth

Statistically, fertilization by NPK fertilizer at a dosage of 0, 50, 100, and 200 mg/cutting did not significantly affect the growth of cuttings, mycorrhizal development and nutrient uptake. Appendix 2 shows that fertilization most probably had some effect only on height and diameter growth and the percentage of

mycorrhizal roots, the probabilities being 63 %, 78 % and 83.0 %, respectively. The number of leaves, leaf area, total fresh weight and total dry weight of *S. leprosula* cuttings with different dosages of NPK fertilizer are presented in Fig. 2-14 and 2-15.

The number of leaves tends to decrease and leaf area, total fresh weight and total dry weight tend to increase after the application of NPK>50 ppm./cutting. None of these tendencies is statistically significant.

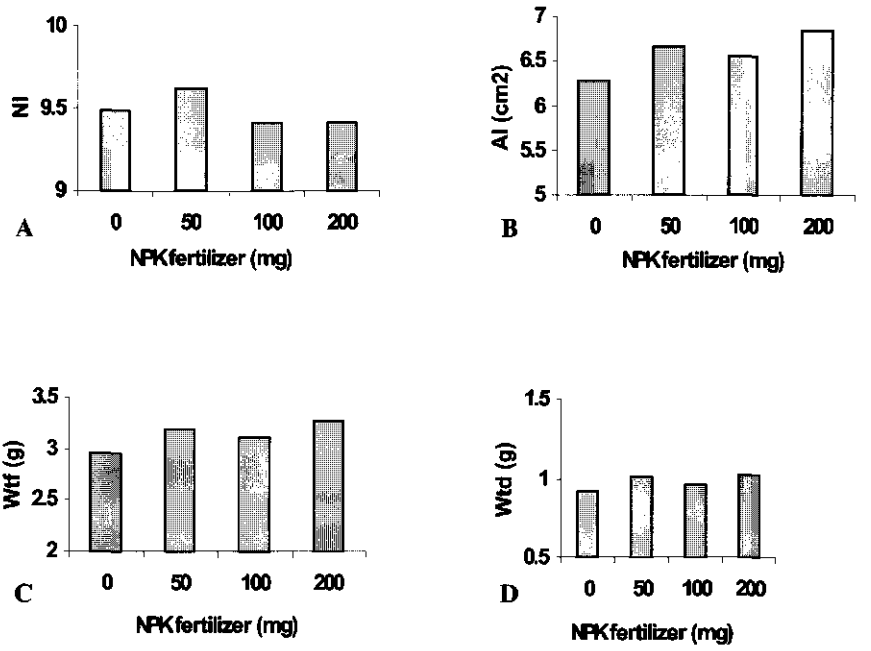


Figure 2-14 NPK fertilizer and (A) number of leaves; (B) leaf area; (C) total fresh weight and (D) total dry weight.

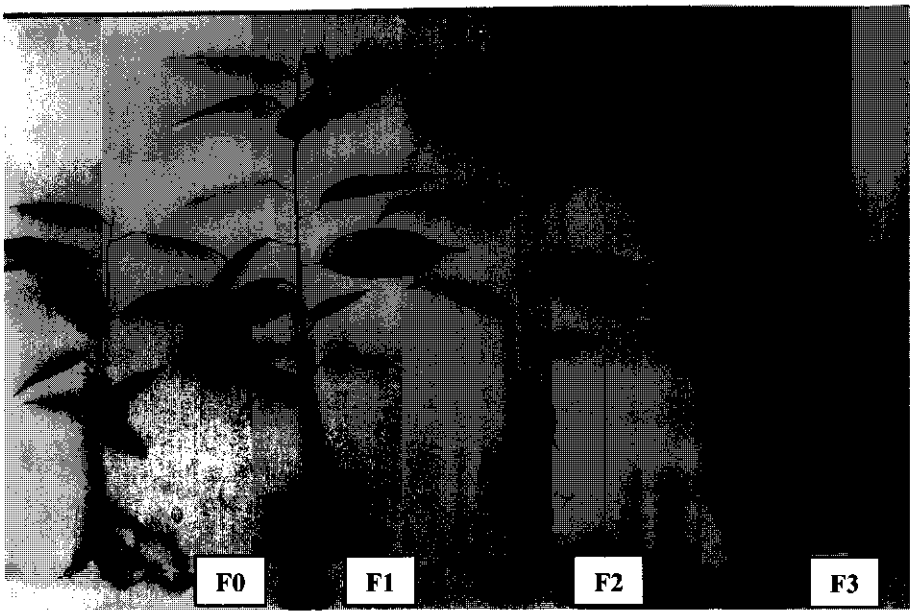


Figure 2-15 NPK fertilizer and growth of *Shorea leprosula* cuttings after different dosages of NPK fertilizer/cutting. F_0 = no fertilizer, F_1 = 50 mg NPK, F_2 = 100 mg NPK F_3 = 200 mg NPK

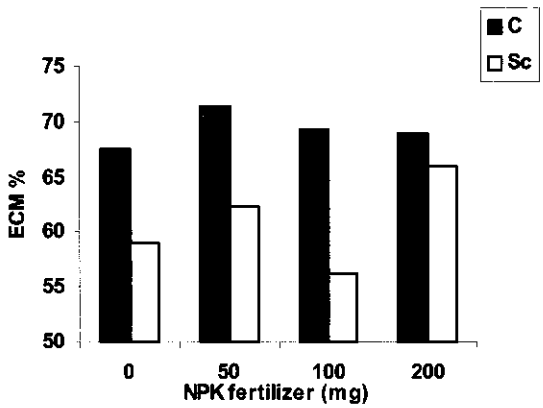


Figure 2-16 Effect of NPK fertilization on percentage of mycorrhizal roots (ECM %). C= controlled conditions, Sc = semi-controlled conditions

2.3.3.2 *Effect on mycorrhizal development.*

From Fig. 2-16 it is unclear whether or not NPK fertilization under semi-controlled conditions had any effect on mycorrhizal development, but under controlled conditions there is an "optimal" NPK dosage of 50 mg/cutting. This shows that the effect of NPK fertilizer as a mycorrhizal facilitator under controlled condition was more effective and less variable than under semi-controlled conditions, where it was inconsistent.

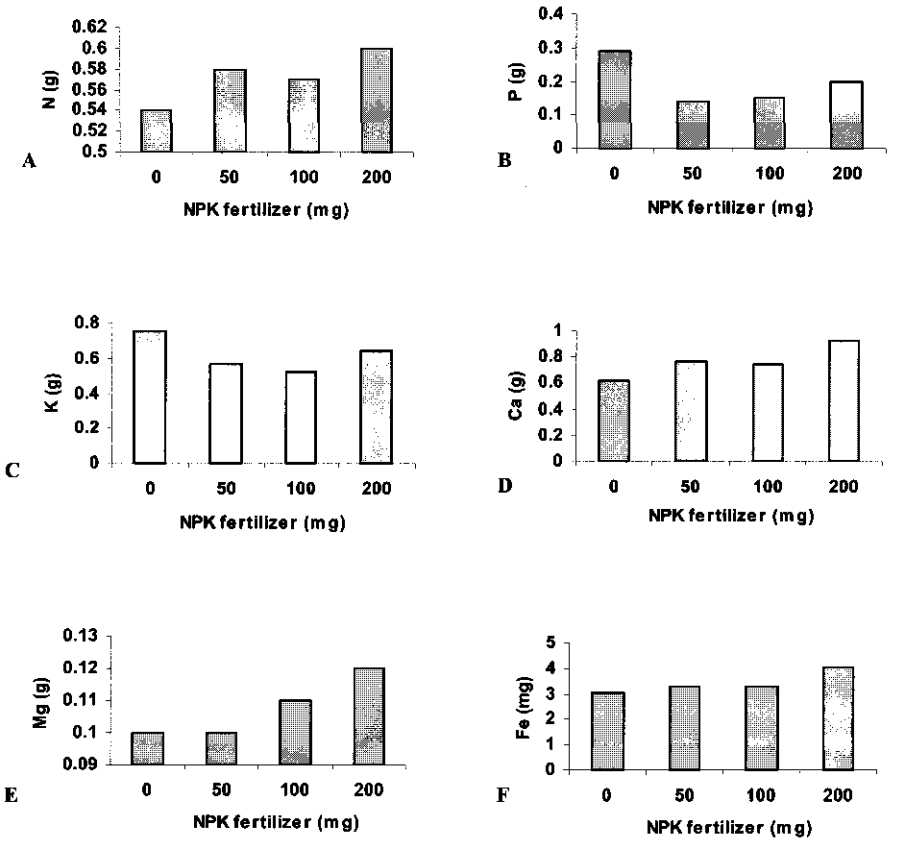


Figure 2-17 Nutrient uptake (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe after different dosages of NPK fertilizer

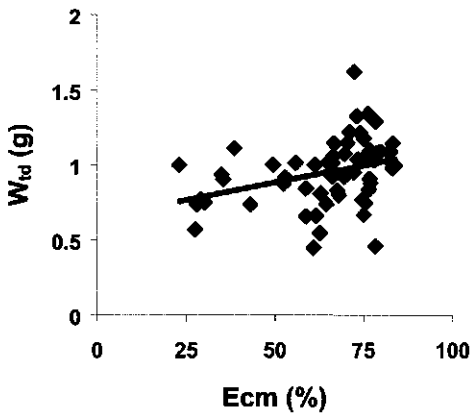
2.3.3.3 Effect on nutrient uptake

NPK fertilization affects nutrient uptake (Fig. 2-17). The uptake of N, Ca, Mg and Fe tended to increase when NPK fertilizer was added at 200 mg/cutting, but the nutrient uptake of P and K tends to decrease when NPK was added at 50 mg/cutting. Note in Fig. 2-14B, that leaf area is shown to increase with the NPK.

2.3.4 Effects of mycorrhizal inoculation

2.3.4. 1 Effect on plant growth

Mycorrhizal inoculation affected the growth of *Shorea leprosula* cuttings as well as their number of leaves, total fresh weight, total dry weight and the percentage of mycorrhizal roots. However, there was little variation between the various mycorrhizal inoculations with regard to the effect upon the growth of the plants. The percentage of mycorrhizal roots is set against dry weight in Fig. 2-18.



$Y = 0.005x + 0.65$ ($r = 0.35$; $P = 0.01$)

Figure 2-18 Relationship between total dry weight (biomass) and percentage of mycorrhizal roots (ECM %). P = significance (probability).

Fig. 2-18 shows that mycorrhizal inoculation significantly affected the growth of *S. leprosula* cuttings. The correlation was weak (r = 0.35), perhaps because of the observation period of 10 months only (cf. 2.3.1.3).

Table 2-9 shows that *Shorea leprosula* cuttings inoculated with *Scleroderma columnare, Amanita* sp, *Russula* sp and a mixture of these fungi have a significantly different number of leaves, total fresh weight and total dry weight (Duncan test at 5 % level of significance). The growth of cuttings without inoculation was

significantly lower than in cuttings with inoculation. The largest response was found in the growth of cuttings inoculated with *Scleroderma columnare*: 11 leaves /cutting (N_l), 3 g/cutting (W_{tf}) and 1 g/cutting (W_{td}). Only for total dry weight, inoculation with the cocktail gave a higher response than inoculation with *S. columnare* alone.

In general, the leaf numbers, total fresh weight and total dry weight of inoculated cuttings was higher than in plants without inoculation. The increment of the number of leaves, total fresh weight and total dry weight ranged from 8 to 19 %, from 7 to 21 % and from 11 to 22 %, respectively.

Table 2-9 Mycorrhizal inoculation and the number of leaves, total fresh weight and total dry weight in *Shorea leprosula* cuttings, 10 months after treatment. N_l = number of leaves, W_{tf} = total fresh weight, and W_{td} = total dry weight.

		Mean response		
Mycorrhizal treatment	N	N_l	W_{tf} (gr)	W_{td} (gr)
No treatment (M0)	107	8.8 b	2.8 b	0.9 b
Amanita sp (M1)	109	9.5 ab	3.1 ab	1.0 ab
Russula sp (M2)	102	9.5 ab	3.0 ab	1.0 ab
Scleroderma columnare (M3)	108	10.5 a	3.4 a	1.1 a
Cocktail of fungi (M4)	107	9.8 ab	3.4 a	1.1 a
Increment M1 vs. M0 (%)		8	11	11
Increment M2 vs. M0 (%)		8	7	11
Increment M3 vs. M0 (%)		19	21	22.
Increment M4 vs. M0 (%)		11	21	22

Values followed by the same letter (a, b, ab) in the same column are not significantly different at 5% level if tested with Duncan's Multiple Range Test.

Table 2-10 Mycorrhizal inoculation and percentage of mycorrhizal roots (ECM %) of *Shorea leprosula* cuttings 10 months after treatment.

Mycorrhizal treatment	ECM %
No treatment (M0)	37 b
Amanita sp (M1)	72. a
Russula sp (M2)	72 a
Scleroderma columnare (M3)	75 a
Cocktail of fungi (M4)	76 a
Increment M1 vs. M0 (%)	95
Increment M2 vs. M0 (%)	95
Increment M3 vs. M0 (%)	103
Increment M4 vs. M0 (%)	105

Values followed by the same letter (a ,b) in the same column are not significantly different at 5% level if tested with Duncan's Multiple Range Test.

2.3.4.2 Effect on mycorrhizal development

The degree of compatibility of mycorrhizal fungi with their host plant species is also important (Supriyanto, 1999). Each mycorrhizal fungus plays a specific role in promoting the growth of the host plant. The ability of each mycorrhizal fungus to colonize the host plant is also different. Table 2-10 shows that *Amanita sp, Russula*

sp, Scleroderma columnare and their mixture were able to colonize the roots of *S. leprosula,* with percentages of mycorrhizal roots of 72 %, 72 %, 75% and 76% respectively. These values were quite similar. An unknown mycorrhizal fungus also colonized the untreated *S. leprosula* spontaneously, causing an infection of 37% of the roots.

Nevertheless, the percentage of mycorrhizal roots inoculated by known fungi approximately doubled (between 95 % and 105 %) the proportion of roots colonized by unknown fungi in the cases without treatment. Moreover, the mixed inoculum of different mycorrhizal fungi (cocktail) clearly colonized the roots of the host plant more intensively than the single-species inocula.

Histological analysis (Fig. 2-19 and Fig. 2-20) shows that inoculated mycorrhizal fungi were able to form a mantle and a Hartig net. The mycorrhizal roots (Fig. 2-19 and Fig. 2-20) formed Radially Elongated Epidermis Cells (REEC) both in controlled and semi-controlled conditions.

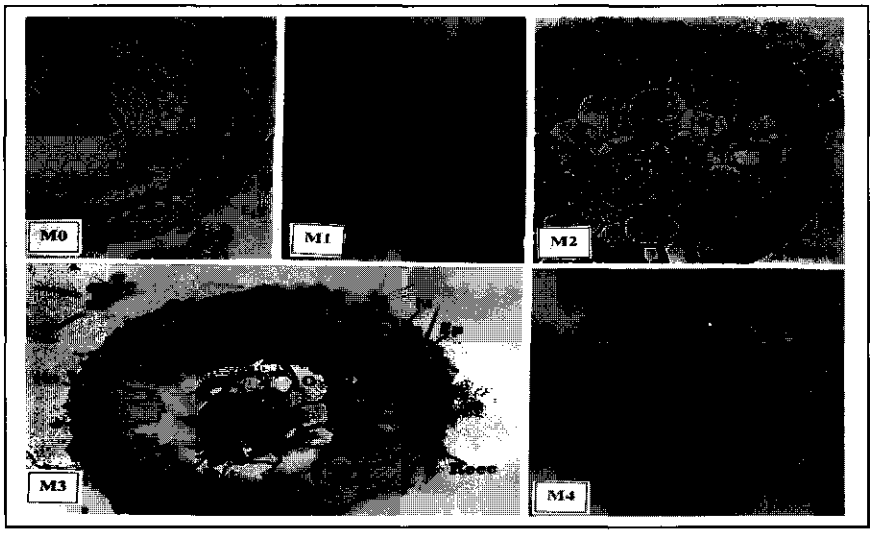


Figure: 2-19 Tissue analysis of mycorrhizal and non-mycorrhizal roots in controlled conditions .M0 = without treatment, M1= inoculated with *Amanita* sp, M2 = inoculated with *Russula* sp, M3= inoculated with *Scleroderma columnare* and M4 = inoculated with a cocktail of those fungi; M= mantle, Hn = Hartig net, Ep= Epidermis, C = Cortex, Reec= Radially elongated epidermis cells.

Figure 2-20 Tissue analysis of mycorrhizal and non-mycorrhizal roots in semi-controlled conditions. M0 = without treatment, M1=Inoculated with *Amanita* sp, M2= Inoculated with *Russula* sp, M3= Inoculated with *Scleroderma columnare* and M4= inoculated with a cocktail of these fungi; M= mantle, Hn = Hartig net, Ep= Epidermis, C= Cortex, Reec = Radially elongated epidermis cell.

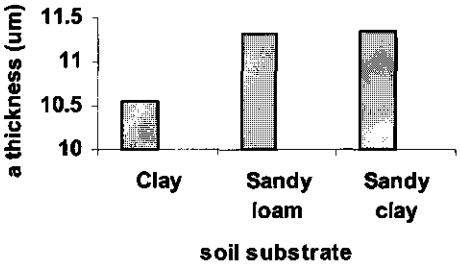


Figure 2-21 Mycorrhizal inoculation and mantle thickness in different soil substrates. Note that the difference is "either sand or no sand."

Fig. 2-21 shows that the mantle thickness was affected by the kind of soil substrate. The mantle thickness in substrates containing a sand fraction was thicker than in a substrate without sand fraction.

Fig. 2-22 shows that the mantle thickness ranged from 6 to 9 μm and was affected by the type of mycorrhizal fungi.

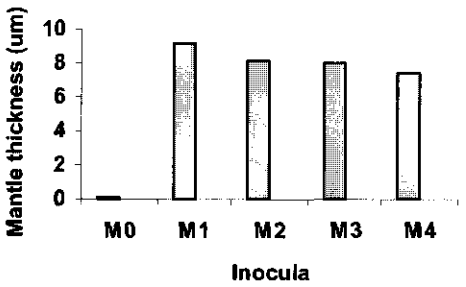


Figure 2-22 Mycorrhizal inoculation and mantle thickness after inoculation with different species of mycorrhizal fungi. M0 = without treatment, M1= *Amanita sp*, M2=*Russula* sp, M3= *Scleroderma columnare*, M4= cocktail of these fungi.

2.3.4.3 Effect on nutrient uptake

The uptake N, P, K, Ca, Mg and Fe by cuttings inoculated with different species of mycorrhizal fungi is shown in Fig. 2-23. In general, the nutrient uptake in inoculated cuttings was better than in cuttings without treatment.

The highest N uptake was found in the cuttings inoculated by a cocktail of mycorrhizal fungi consisting of *Amanita* sp, *Russula* sp and *Scleroderma columnare* (M4). Fig. 2-23 shows a similar ability of those mycorrhizal fungi to promote the nutrient uptake of P, K, Ca and Fe (Fig. 2-23 B, C, D and F). Mg uptake by the *S. leprosula* cuttings inoculated with *Amanita* sp was the highest among all other cuttings inoculated with mycorrhizal fungi (M1 to M4).

2.3.5 Interaction among environmental conditions, fertilization and soil substrate

2.3.5.1 Interaction between environmental conditions and fertilization

A. Effect on plant growth

The effects of interaction between environmental conditions and fertilization on the growth *of S. leprosula* cuttings are presented in Table 2-11. The number of leaves was higher under controlled conditions than under semi-controlled conditions. It ranged from 11 to 12 leaves per cutting, while under semi-controlled conditions there were 7 to 8 leaves per cutting. The number of leaves under controlled conditions increased by 54 % as compared to semi-controlled conditions without

fertilizer. The increment in leaf number under controlled conditions was somewhat lower, in between 41 % to 54 %.

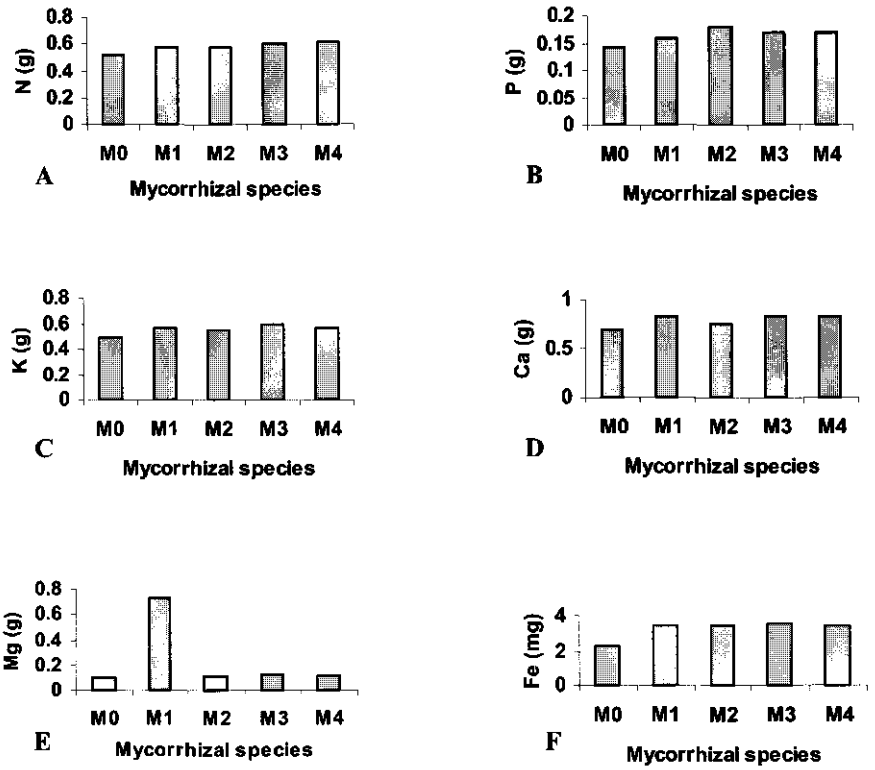


Figure 2-23 Mycorrhizal inoculation and (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake by inoculated cuttings of *Shorea leprosula*. M0 = without treatment, M1=*Amanita* sp, M2=*Russula* sp, M3= *Scleroderma columnare* , M4= mixture of M1, M2, and M3.

The leaf area of *S. leprosula* cuttings under controlled conditions and fertilized with NPK at 0, 50, 100 and 200 mg/cutting increased by 42 %, 62 %, 73 % and 65 % respectively. The total biomass, expressed either as fresh or dry weight, under controlled conditions was higher than under semi-controlled conditions. The highest increment was obtained in the cuttings fertilized with 100 mg per cutting under controlled conditions, i.e. 31 % in fresh weight and 22 % in dry weight.

Table 2-11 Interaction between environmental conditions and NPK fertilizer affecting growth parameters of *Shorea leprosula* cuttings 10 months after treatment. Number of leaves (N_l), leaf area (A_l), total fresh weight (W_{tf}) and total dry weight (W_{td}).

	Mean response				
	N	N_l	A_l	W_{tf}	W_{td}
Interaction treatments			(cm2)	(gr)	(gr)
Controlled + 0 mg NPK fertilizer (E1F0)	68	11.1 a	7.4 b	3.0 bc	0.9 ab
Controlled + 50 mg NPK fertilizer (E1F1)	71	11.6 a	8.4 ab	3.6 ab	1.1 a
Controlled + 100 mg NPK fertilizer (E1F2)	68	12.2 a	9.0 a	3.8 a	1.1 a
Controlled + 200 mg NPK fertilizer (E1F3)	73	11.7 a	8.6 ab	3.5 ab	1.1 a
Semi-controlled + 0 mg NPK fertilizer (E2F0)	66	7.9 b	5.2 c	2.9 bc	0.9 ab
Semi-controlled + 50 mg NPK fertilizer (E2F1)	63	7.6 b	4.9 c	2.8 c	0.9 ab
Semi-controlled + 100 mg NPK fertilizer (E2F2)	64	6.6 b	4.2 c	2.4 c	0.8 b
Semi-controlled + 200 mg NPK fertilizer (E2F3)	60	7.1 b	5.1 c	3.0 bc	1.0 ab
Increment E1F0 vs E2F0 (%)		41	42	3	0
Increment E1F1 vs E2F0 (%)		47	62	24	22
Increment E1F2 vs E2F0 (%)		54	73	31	22
Increment E1F3 vs E2F0 (%)		49	65	21	22
Increment E2F1 vs E2F0 (%)		- 3	-6	-3	0
Increment E2F2 vs E2 F0 (%)		- 16	-19	-17	- 11
Increment E2F3 vs E2 F0(%)		- 10	-2	3	11

Values followed by the same letter (a ,b) in the same column are not significantly different at 5% level if tested with Duncan's Multiple Range Test.

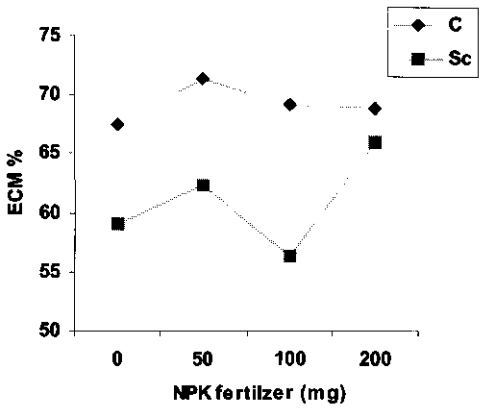


Figure 2-24 Percentage of mycorrhizal roots (ECM %) after growing at a different dosage of NPK fertilizer. C = controlled conditions, Sc = Semi-controlled conditions.

B. Effect on mycorrhizal development.

The effect of environmental conditions and NPK fertilization on mycorrhizae is presented in Fig. 2-24. The number of mycorrhizal roots under controlled conditions increased when the cuttings were fertilized with 50 mg NPK per cutting, the response tending to become similar to no treatment when NPK is added in a dosage

of 100 and 200 mg NPK per cutting. Under semi-controlled conditions the response is irregular.

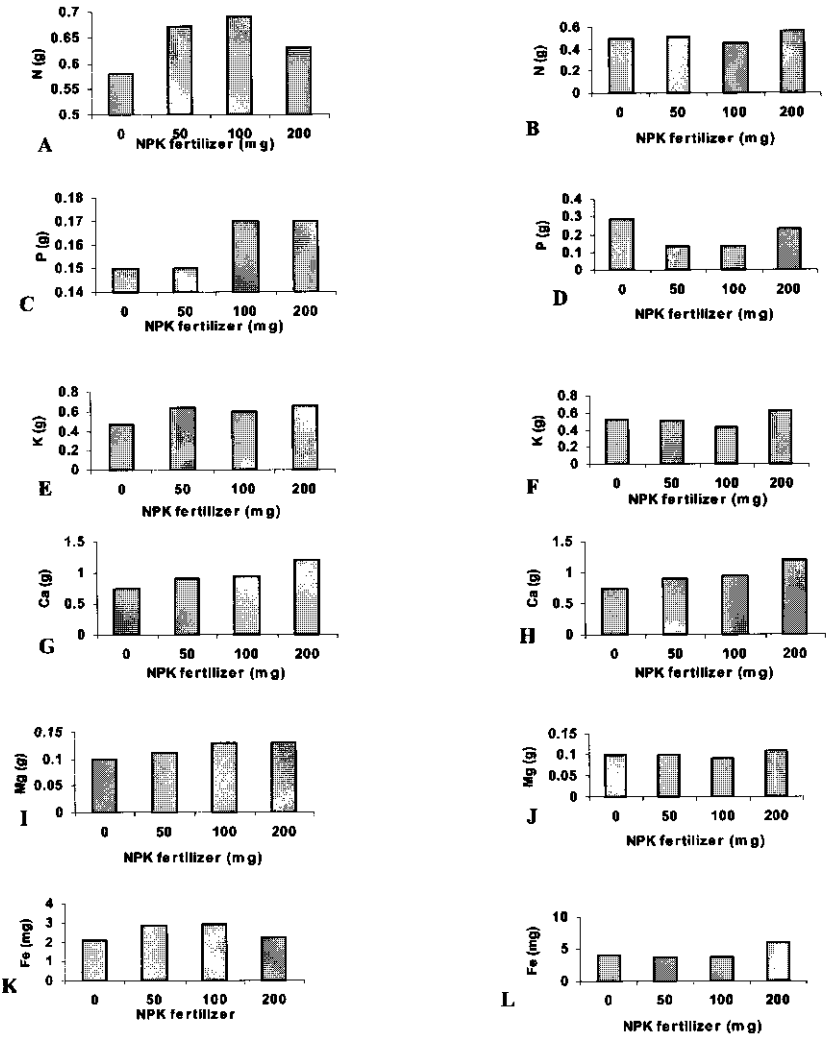


Figure. 2-25 NPK fertilization and uptake of (A,B) N-uptake; (C,D) P-uptake; (E,F) K-uptake; (G,H) Ca-uptake; (I,J) Mg-uptake and (K,L) Fe-uptake under controlled and semi-controlled conditions.

C. Effect on the nutrient uptake

The effect of fertilization on nutrient uptake was not significantly different for the dosages tested (Duncant test at 5 % level of significance). Nitrogen uptake under controlled conditions, at a dosage of 50 to 100 mg NPK per cutting enhanced the growth of *S. leprosula* cuttings, but under semi-controlled conditions this effect became irregular and statistically non-significant (see Fig. 2-25)

Phosphorus uptake under controlled conditions clearly increases at a dosage of 100 to 200 mg NPK/cutting. The nutrient uptake data for K, Ca, Mg and Fe under controlled as well as under semi-controlled conditions show similar results, see Fig. 2-25. Although none of these differences are significant in their own right, this consistence of all tendencies strengthens the overall image.

2.3.5.2 Interaction between environmental conditions and soil substrate.

A. Effect on plant growth

Table 2-12 and Fig. 2-26 show the interaction between environmental conditions and soil substrates. Height growth of *S. leprosula* cuttings planted in sandy clay and sandy loam under controlled conditions increased by 68 % and 56 % respectively, compared with the cuttings planted under semi-controlled conditions in a clay substrate. On the other hand, the diameter of those cuttings did practically not increase.

Table 2-12 Interaction between environmental conditions and soil substrate according to various growth parameters of *Shorea leprosula* cuttings 10 months after treatment. Average height growth (ΔH), average diameter growth (ΔD), leaf area (A_l), total fresh weight (W_{tf}), total dry weight (W_{td}).

		Mean response				
	N	ΔH	ΔD	A_l	W_{tf}	W_{td}
Interaction of treatments		(cm.)	(mm)	(cm^2)	(g)	(g)
Controlled + Clay (E1S1)	84	10.2 b	0.1 c	4.1 c	2.3 c	0.7 c
Controlled + Sandy loam (E1S2)	79	14.2 a	0.2 b	9.8 a	4.0 a	1.2 a
Controlled + Sandy clay (E1S3)	92	15.3 a	0.2 b	10.2 a	4.1 a	1.3 a
Semi-controlled + Clay (E2S1)	92	9.1 b	0.3 a	5.0 bc	2.5 bc	0.9 b
Semi-controlled +Sandy loam (E2S2)	94	10.1 b	0.3 a	5.5 b	3.0 b	1.0 b
Semi-controlled +Sandy clay (E2S3)	94	9.6 b	0.3 a	5.0 bc	2.8 b	0.9 b
Increment E1S1 vs. E2S1 (%)		12	- 67	- 18	- 8	- 22
Increment E1S2 vs. E2S1 (%)		56	- 33	96	60	33
Increment E1S3 vs. E2S1 (%)		68	- 33	104	64	44
Increment E2S2 vs. E2S1 (%)		11	NI	10	20	11
Increment E2S3 vs. E2S1 (%)		5	NI	NI	12	NI

Values followed by the same letter (a, b, ab, c, bc) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test; NI= No increment

In general, cuttings planted in sandy clay and under controlled conditions showed higher growth rates than in clay under semi-controlled conditions. Under controlled conditions a rise by 68 %, 104 %, 64 % and 44 % was observed for height growth, number of leaves, fresh and dry weight, respectively.

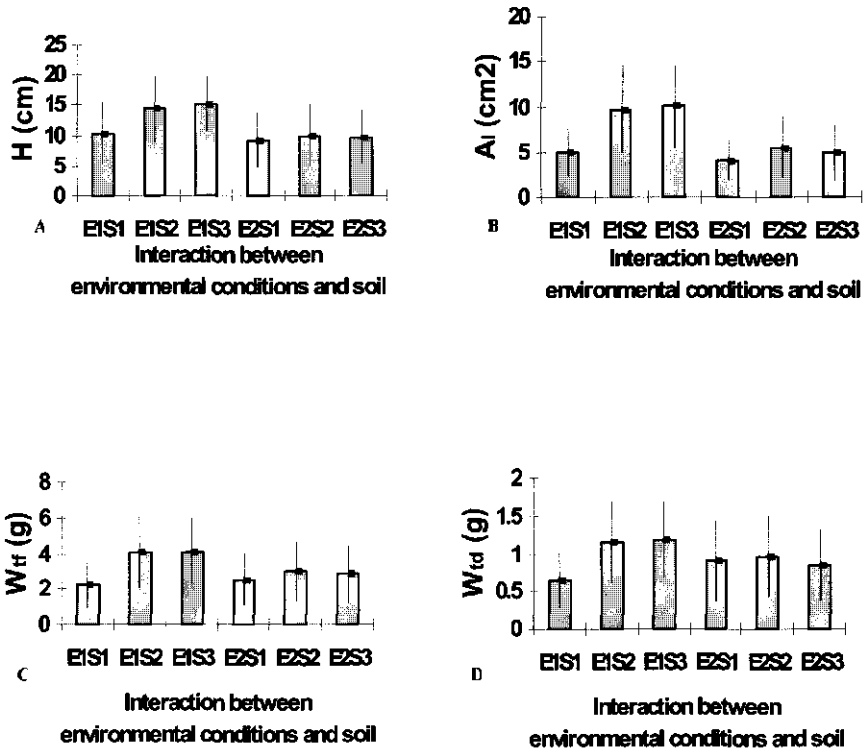


Figure 2-26 Interaction between environmental conditions and soil substrate expressed by (A) height growth; (B) leaf area; (C) total fresh weight and (D) total dry weight at harvest, 10 months after treatments. E1 = controlled conditions; E2 = semi-controlled conditions; S1 = clay; S2 = sandy loam; S3 = sandy clay.

B. Effect on the mycorrhizal development.

The effect of interaction between environmental conditions and soil substrates is presented in Table 2-13. Table 2-8 showed mycorrhizal development in sandy loam and sandy clay to be higher than in clay. But when the environmental conditions are controlled, with concomitant high humidity, mycorrhizal developments change drastically. Table 2-13 shows that the highest mycorrhizal development was obtained in cuttings planted in a clay substrate in controlled conditions (72 %). When conditions were semi-controlled, mycorrhizal development in clay substrate was much lower (53 %). Statistically, the mycorrhizal development was not significantly different in sandy loam and sandy clay, whatever the environmental conditions. The difference once more is "with sand vs. without sand".

C. Effects on nutrient uptake

Fig. 2-27 shows that N, P, K, Mg and Fe uptake under controlled conditions was higher than under semi-controlled conditions. This is a parallel tendency with the higher percentage of mycorrhizal roots under controlled conditions (Table 2-13).

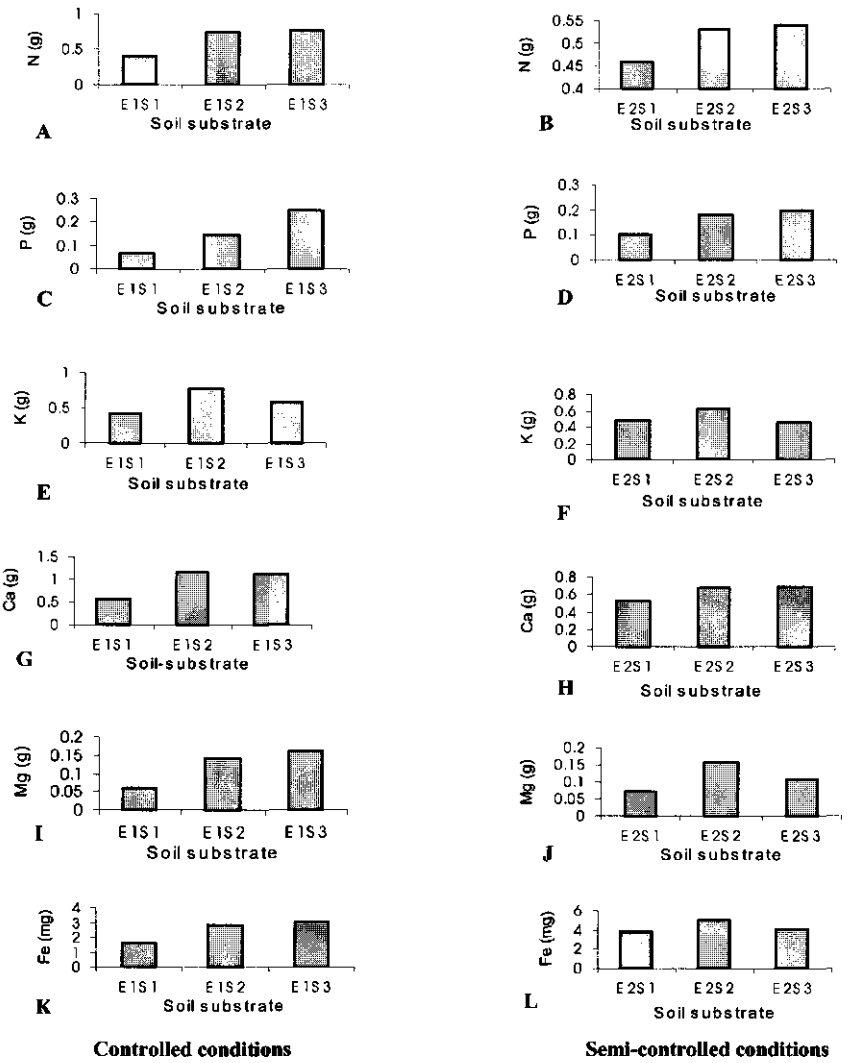


Figure 2-27 Nutrient uptake (A, B) N-uptake; (C,D) P-uptake; (E,F) K-uptake; (G,H) Ca-uptake; (I,J) Mg-uptake and (K,L) Fe-uptake under controlled and semi-controlled conditions on different soil substrates. S1 = Clay, S2=Sandy loam, S3=Sandy clay, E1= controlled conditions, E2=semi-controlled conditions.

Table 2-13 Interaction between environmental conditions and soil substrate expressed by percentage mycorrhizal roots (ECM %) in *Shorea leprosula* cuttings during 10 months of observation.

Interaction treatments	ECM %
Controlled + Clay (E1S1)	72 a
Controlled + Sandy loam (E1S2)	69 a
Controlled + Sandy clay (E1S3)	67 a
Semi-controlled + Clay (E2S1)	53 b
Semi-controlled +Sandy loam (E2S2)	67 a
Semi-controlled +Sandy clay (E2S3)	69 a
Increment E1S1 vs. E2S1 (%)	36
Increment E1S2 vs. E2S1 (%)	30
Increment E1S3 vs. E2S1 (%)	26
Increment E2S2 vs. E2S1 (%)	26
Increment E2S3 vs. E2S1 (%)	30

Values followed by the same letter (a, b, ab, c, bc) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test

2.4 DISCUSSION

Mycorrhizal fungi, soil substrate, fertilization and environmental conditions affect the growth of *Shorea leprosula* cuttings independently or in interaction. Interaction of several treatments may produce a synergetic effect on the growth of cuttings, but it also may produce an adverse effect. Such kinds of arguments are discussed below.

2.4.1 The growth of *Shorea leprosula* cuttings

On the whole, the growth of *S. leprosula* cuttings under controlled conditions was strongest (see Table 2-2; Fig. 2-6). The increment of the parameters (height, diameter, number of leaves, leaf area, total fresh and dry weight) under controlled conditions was from 11 % to 71 % higher than under semi-controlled conditions. These differences are of course related to different environmental factors. Air temperature was higher under semi-controlled conditions, whereas relative humidity was higher under controlled conditions. We may assume that the cooling system as a tool of control played an important role in the higher performance of the plants, although the later reception of morning sunlight may have also played a role. Atmospheric humidity is an important factor for the survival of cuttings after being transplanted in pots (Omon and Soeseno 1984; Yasman and Smits 1988; Omon 1994).

Soil temperatures for both conditions are presented in Table 2-3 (2.3.1.1). This experiment shows that even slight differences in soil heat have a great impact. This result confirms Smits, conclusions (1994) on sensitivity of dipterocarp ectomycorrhizae to soil heat and the large effect of soil heat upon the functioning of ectomycorrhizae. In a pot experiment employing *Shorea leavis* Smits et al. (1987) reported that at five centimeter depth in the pot the soil temperature varied between

32° and 35° C. In the present experiment, however, soil temperature was 28° C on the average, so it could not affect ectomycorrhizae.

When the air was more humid and cooler, less wilting of the leaves occurred of the plants under controlled conditions. Photosynthesis is so kept at higher levels during longer periods, resulting in a better overall growth. This fits in with the balance of light and atmospheric humidity as a regulator of tree growth and forest dynamics; light is metabolically balanced by humidity (Oldeman, 1974).

Another factor influencing the growth of *Shorea leprosula* cuttings was light intensity. In this experiment, the light intensity under controlled conditions was 12 $\mu mol.m^{-2}$ of full daylight and under semi-controlled conditions it was 24$\mu mol.m^{-2}$ of full daylight. Comparing light intensity in both controlled and semi-controlled conditions, we found that under controlled conditions light intensity was lower than under semi-controlled conditions (Table 2-4). The effect on height growth of *Shorea leprosula* cuttings was significant and negative ($r = -0.84$; see Fig. 2-7). At the higher value of PAR (24 $\mu mol.m^{-2}$), height growth of *Shorea leprosula* became slower. Clearly, the growth of *Shorea leprosula* was affected by light intensity. When light intensity is doubled, height growth of cuttings is retarded. In practice, *Shorea leprosula* cuttings must be shaded to keep them under a light intensity of 12 $\mu mol.m^{-2}$ of full daylight.

In the forest the highest canopy layer receives full light. The average light intensity gradually decreases to the lower part of the canopy and this happens so swiftly that it can reach less than one percent as an average near the ground. On the one hand the difference in light intensity with different height in the forest causes different reactions of the plant leaves (Yasman, 1995). On the other hand, plants growing under these different light conditions also adapt physiologically, for instance by reducing transpiration rates, by increasing the efficiency of their photosynthetic processes under low light (Fitter and Hay, 1981), or by having low light compensation points (Bazzaz, 1989).

Total dry weight increment of cuttings under controlled conditions increased by 11 % as compared to that under semi-controlled conditions. It was not affected very much by light intensity ($r = -0.33$). It is possible that the cuttings growing under controlled condition contained more water as a consequence of high humidity and concomitant low transpiration. The higher dry weight may also mean that there are more wood vessels that were filled with more water in the living plant.

The culture medium was another important factor influencing the growth of the cuttings. The analysis of physical properties of media used shows that the sand portion of the soil substrates differed (see Table 2-7).

Cuttings *of S. leprosula* performed better in sandy loam and sandy clay (Table 2-8). Their height growth was 27 and 28% higher, and their dry weight 38 and 25 %, respectively. The strong correlation between plant growth and aeration of the sandy loam and the sandy clay soil samples supports the notion that these differences are due to a better aeration of the two sandier media. This is in accordance with the results obtained by Oldeman and Iriansyah (1993). The dependence on adequate oxygen supply is also an important feature for mycorrhizal development. Mycorrhizal beech seedlings attain their maximum production rates when supplied with an oxygen concentration close to that of the air, cease producing when oxygen concentration drops below 3 % (Harley, 1989).

Cuttings grown in sandy clay and sandy loam under controlled conditions performed better than those grown in clay under semi-controlled conditions. Height growth was raised by 68% and 56 % and total dry weight by 44 % and 33 % respectively (Table. 2-12).

There was hardly any noticeable effect of NPK-fertilization. It had neither effect on height and diameter growth of the cuttings, nor on their biomass. Perhaps the nutrient dosage applied is still too small.

To enhance the growth of *Shorea leprosula* cuttings, mycorrhizal fungi of the species *Amanita* sp, *Russula* sp, *Scleroderma columnare* and their cocktail were inoculated to transplanted cuttings. Those mycorrhizal fungi can be found under natural Dipterocarp forest. One might think that none of these fungi enhance the growth of cuttings, because the results showed that neither height nor diameter growth was affected by inoculation with mycorrhizal fungi. However, there is significant increase of the number of leaves (8 % to 19 %), total fresh weight (7 % to 21 %) and total dry weight (11 % to 22 %). Refer to Table 2-9.

The cocktail of mycorrhizal fungi was the most performing inoculum as compared to single species inoculum. Each fungus may well have a specific functional role in promoting plant growth. *Amanita* sp. for instance, is an Mg-booster, and Mg is essential for building chlorophyll (Fig. 2-23E).

Interaction between climatic conditions and fertilization affected many growth parameters (Table 2-11). The highest growth increment as a result of fertilization under controlled conditions was obtained in *Shorea leprosula* cuttings fertilized with NPK 100 mg/cutting. The number of leaves increased by 54 %, the total leaf area by 73 %, the total fresh weight by 31 %, and the total dry weight by 22 %.

In the case of single factor treatment of climatic conditions, the dry weight obtained was higher in controlled condition (increase by 11 %). In fertilization treatments, the dry weight obtained was even higher (additional increase to a total of 22 %).

In the interaction between climatic condition and fertilization, the total dry weight increment obtained was higher in controlled conditions and fertilized with 100 mg NPK/cutting (increase 22%) - see Table 2-2, Fig. 2-14, and Table 2-11. Fertilization is more effective in controlled conditions, fertilized with 100 mg NPK/cutting. Fertilization in controlled conditions was more effective than in semi-controlled conditions because less fertilizer was applied to obtain a response. Perhaps, under semi-controlled conditions some nutrients precipitate or evaporate.

It was already pointed out that soil substrate and environmental conditions together affected the growth of cuttings. The interaction between climatic conditions and soil substrate significantly affected the height and diameter growth, leaf area, total fresh weight and total dry weight of the cuttings (Table 2-12). The highest increment of height growth under controlled conditions was 40 %, and 28 % in sandy clay, while the height growth increment due to interaction of both factors was 68 % (Table 2-2; Table 2-8 and Table 2-12). Therefore, in practice, the production of *Shorea leprosula* cuttings benefits from culture under controlled conditions and the use of sandy clay substrate. The same is also true for other growth variables such as diameter growth, number of leaves, leaf area, and total fresh weight of the cuttings.

2.4.2 Mycorrhizal development

The mycorrhizal fungi *Amanita* sp., *Russula* sp., *Scleroderma columnare* and their cocktail were inoculated to transplanted cuttings of *S. leprosula*. The effect of climatic conditions on mycorrhizal development is described in the next pages.

The percentage of mycorrhizal roots (ECM %) under controlled condition was higher than under semi-controlled conditions (69 % and 61 %, see Table 2-2). Yasman and Smits (1988), Yasman, (1995), Noor and Smits (1987) reported that mycorrhizal development is influenced by soil heat, light intensity, pH, humidity, moisture content and aeration or oxygen supply. These authors also produce evidence that increasing light intensity reduces the percentage ofmycorrhizal roots.

The percentage of mycorrhizal roots in the experiment was 37 % (without inoculation), 72 % (*Amanita* sp.), 72 % (*Russula* sp.), 75 % (*S. columnare*) and 76 % (cocktail of fungi), see Table 2-10.

The results indicate a spontaneous mycorrhizal infection level of 37 %. This infection was due to contaminating fungi from the greenhouse environment. It is usually caused by fungi with a low or even negative level of benefit to the plants. The inoculation with *Amanita* sp., *Russula* sp., *Scleroderma columnare* and their cocktail did increase the percentage of mycorrhizal roots significantly. They were able to form a mantle and a Hartig-net structure and increased the total dry weight of the cuttings by 11 % (*Amanita* sp), 11 % (*Russula* sp), 22 % (*Scleroderma columnare*) and 22 % (their cocktail), compared to the untreated ones. The

"spontaneous" fungi thus were able to colonize the roots of *S. leprosula*, but their effect on total dry weight is less than that of the inoculated mycorrhizal fungi.

All treatments involving inoculation showed a significantly higher result than the untreated. This indicates the importance of proper mycorrhizal fungus for a successful growth of *S. leprosula*. Each mycorrhizal fungus may play a specific role in promoting the growth of the plant. The ability of each mycorrhizal fungus to colonize the host plant is also species-dependent (Smits, 1994; Smith and Read, 1997). The results indicated that there was no significant difference betweeninocula as judged by the percentage of mycorrhizal roots produced.

Histological analysis from data on roots grown under both conditions showed that the mycorrhizae formed after inoculation with mycorrhizal fungi possessed a mantle and a Hartig-net (see Figure 2-19 and Figure 2-20). The Hartig-net for Dipterocarp species always was found in Radially Elongated Epidermis Cells (Lee, 1988, Omon, 1994, Smith and Read, 1997, Supriyanto, 1999). A mycorrhizal fungal species that is able to form a mantle and Hartig-net structure in several plant species has a high mycorrhizal compatibility (Supriyanto, 1999). So the results of inoculation with *Amanita* sp, *Russula* sp, and *S. columnare* also indicated a high compatibility with *S. leprosula* seedling roots.

The mantle thickness substrate was affected by the sand fraction in the soil (Figures 2-21) and by the species of mycorrhizal fungi (Fig.2-22). Maybe this is due to ECM fungi in sandy loam and sandy clay being able to penetrate easier than in clay substrate with its narrow pores.

NPK fertilization did not significantly affect the percentage of mycorrhizal roots. Even an application of NPK with 200 mg/cutting did not depress the mycorrhizal development (Figure 2-16). Therefore the claim that fertilizers and mycorrhizae are incompatible (Newton & Piggott, 1991) is not true in the present case.

The percentages of mycorrhizal roots found in clay, sandy loam and sandy clay were 62 %, 67 % and 67 % respectively (Table 2-8). The pH levels of these soil substrates were 4.8, 5.0 and 5.3 respectively. Hence, mycorrhizal development in those substrates was not affected by soil pH. Harley (1989) stated that the optimum pH for mycelial growth of mycorrhizal fungi is always on the acid side of neutrality. Each species has its own pH preference, for example *Amanita* sp prefers pH 3.5 to 4.5, while *Rhizopogon* sp. prefers pH 5.5 to 6.0 (Harley, 1989). According to Anggangan et al. (1996) an increase in soil pH levels does not affect ectomycorrhizal formation, but it reduces the effectiveness of different fungi in promoting plant growth.

The combination of climatic conditions and soil substrate affected mycorrhizal development. In Table 2-13 the highest percentage of mycorrhizal roots in sandy clay was found under semi-controlled conditions (69 %) as compared with root

growth found in clay under controlled conditions (30 %). The mycorrhizal fungi used, therefore, were able to colonize the roots of the cuttings, but their most important asset is their ability to increase the growth performance of cuttings.

2.4.3 Nutrient uptake

Uptake of essential nutrients is linked with, and depends upon, metabolic rates. Hence, factors affecting metabolism are important, such as availability of respiratory and other substrates, oxygen supply, heat, concentration and forms of nutrients and metabolic poisons or inhibitors. Where some nutrient, during or after absorption, is converted to other forms, or built into organic compounds, the supply of respiratory substrate is necessary not only for energy expended in uptake but also for the synthesis itself (Harley, 1989).

Physiologically, the growth of *Shorea leprosula* cuttings is of course affected by nutrient uptake. Nutrient uptake is affected by many factors, among them the climatic conditions, the presence of beneficial microorganisms such asmycorrhizal fungi, and nutrient availability in the substrate.

The nutrient uptake of N, P, K, Mg and Fe under controlled condition was higher than under semi-controlled conditions. The highest nutrient uptake under controlled conditions was correlated with the development ofmycorrhizae in the root system. A high correlation between nutrient uptake and total dry weight of *S. leprosula* cuttings can also be seen. Therefore, nutrients uptake was more effective under controlled conditions.

The biomass of *Shorea leprosula* cuttings under controlled conditions was strongly affected by the nutrient uptake of N, P, K and Mg ($r \geq 0.80$), except for Ca ($r = 0.72$) and Fe ($r = 0.61$). Meanwhile, the relation between biomass and nutrient uptake under semi-controlled conditions is moderate ($0.5 \leq r \leq 0.8$) except for N ($r = 0.85$) and K ($r = 0.86$), see Table 2-6. The nutrient uptake of N, P, K, Ca, Mg and Fe (Figure 2-13) tends to increase when the percentage of sand fraction in the substrate increases.

In this experiment the nutrient uptake of N, P, K, Ca, Mg and Fe was influenced by environmental conditions and soil substrate, but neither mycorrhizae, nor NPK fertilizer had any effect on total mineral nutrient uptake. In general, the nutrient uptake of inoculated cuttings was higher than in cuttings without artificial inoculation (Figure 2-27). Hadi and Santoso (1988) reported that species of *Russula* sp, *Scleroderma* sp and *Boletus* inoculated on *Hopea odorata*, *Vatica sumatrana*, *Shorea stenoptera*, *S. pinanga* and *S. compressa* were able to enhance nutrient uptake and accumulation in plant tissues.

Interaction between NPK fertilization and environmental conditions influenced P and Ca uptake in *S. leprosula* cuttings (Figure 2- 25). The highest P and K uptake

occurred under controlled conditions and in sandy clay. The highest P and Ca uptake occurred when the cuttings were planted in sandy loam with 200-mg/cutting NPK fertilizer. The differences in nutrient uptake between controlled and semi-controlled conditions were certainly due to the differences in environmental factors.

2.5 CONCLUSIONS

Light intensity affected the height growth of *S. leprosula* cuttings, showing an optimum at 12 $\mu mol.m^{-2}$ under controlled conditions.

Growth of *Shorea leprosula* cuttings was stronger under controlled conditions with a fertilizer dosage of 100 mg/cutting than under semi-controlled conditions and other fertilizer dosages.

Mycorrhizal inoculation with a mixture of fungal species stimulated the growth of *S. leprosula* cuttings more than inoculation with a single species.

The nutrient uptake of N, P, K, Ca, Mg and Fe by *S. leprosula* was influenced by the development of mycorrhizae, and was higher in sandy loam or sandy clay than in clay.

Sandy loam and sandy clay are better than clay as culture media for dipterocarp nurseries, especially for *S. leprosula* cuttings.

CHAPTER 3
MYCORRHIZAL DEVELOPMENT IN ROOTS OF CUTTINGS OF *Shorea leprosula* Miq. IN DIFFERENT SOIL SUBSTRATES IN PERFORONS

3.1 INTRODUCTION

Shorea leprosula can grow in a wide variety of soil types and sites (Ardikoesoema and Noerkamal, 1955). The annual diameter increment of adult *S. leprosula* can exceed 2 cm (Harbagung & Wahyono, 1987; Masano, 1985). This species also is an indicator for soil fertility in tropical forests (Shariff, 1989). Its mycorrhizae play an important role in the uptake of phosphorus from the soil. The purpose of the present experiment is to study this mutualistic symbiosis by the use of perforons.

Perforons are simple constructions used for the observation of living roots *in situ* without causing root damage and with the least soil disturbance (Figs 3-1 to 3-4). Nevertheless, its use may cause some root aberrations (Van Sonderen, 1986 cited by Oldeman, 1990 and by Smits, 1994). The first perforons were described and tested by Tweel (1979). The method is technically simple and relatively inexpensive. It makes direct access to the roots possible for the assessment of root lengths and number of root tips (Bosch 1984). The method is also suitable for studying root architecture without destroying the root system and allows the bulk of the roots to grow in the soil medium under study (Bosch, 1984). Smits, (1994) used this method to study mycorrhizal development in *Anisoptera marginata* seedlings. The construction and the use of perforons are further described in the "materials and methods" section 3.2.2.

One of the advantages of the use of perforons for mycorrhizal studies is the possibility of inoculation *in situ*. The inoculum can be applied directly in places where mycorrhizal formation can take place (Smits, 1994). Another advantage of the use of perforons for mycorrhizal studies is the possibility to know the age of the observed ectomycorrhizae and to follow the changes in root morphology and the ageing process. In case root pieces are needed for more detailed studies, samples can be taken with a biopsy forceps for further microscopic examination, without breaking up the system. The accessibility of individual roots to direct inoculation, inspection and sampling compares favourably with other methods. Aboveground developments and phenomena can be directly related to underground infection. Finally, mycorrhizal types can be seen *in vivo*.

In the host plant, the root systems usually consist of both mycorrhizal roots and uncolonized roots. The total system also includes hyphae ramifying in the soil, connected with the root tissue or even penetrating the root cells. The mycorrhizal

zones lie between the root cap and the mature root parts. The green phytobionts produce carbon compounds by photosynthesis. These compounds are used both by the host plant and the fungus. Mycorrhizal fungi absorb nutrients and water from the soil, parts of which are translocated to the host as input into the sap-stream (Fig. 1-1).

There is a variation in the efficiency of fungi, which can form mycorrhizae. A strain may be more effective or efficient in some environmental conditions in promoting the growth of a green plant, and less effective or efficient in others. Soil substrate, nutrients, soil pH, soil moisture content, soil heat, light intensity and the resulting "hospitality" of the environment for both symbiotic partners affect the development of mycorrhiza in the root system (Oldeman, 1983, 1990; Bowen, 1994; Yasman, 1995). Further detailed studies on the structure and extent of hyphal connections with soil and plant should be made. Newton (1992) claimed that factors decisive for a successful establishment of mycorrhizal associations are the soil type, the source of inoculum (spores or mycelium), and root branching or the growth rate at the moment of encounter with a particular fungus.

In the nursery, the substrate for growing the seedlings is taken from the forest nearest to the nursery. This is certain to contain indigenous mycorrhizal fungi. However, their effectiveness in promoting the growth of cuttings and their characteristics should be studied in more detail. Previous reconnoitering shows that soil obtained from different sources (clay, sandy loam, and sandy clay) contains different indigenous mycorrhizal fungi, and so favours the formation of different types of mycorrhizae.

The effect of clay, sandy loam and sandy clay on the growth of cuttings, mycorrhizal development and nutrient uptake is discussed below. *Shorea leprosula* cuttings were inoculated with *Amanita* sp, *Russula* sp, *Scleroderma columnare,* and a cocktail of these mycorrhizal fungi. Heat treatment of the soil substrate by autoclaving also allowed to drawing some conclusions about the importance of indigenous mycorrhizal fungi already present in the soil substrate.

Summarizing, the aim of the experiments described in this chapter was to study the development of mycorrhizae in different soil substrates under known environmental conditions.

3.2 MATERIALS AND METHODS

3.2.1 Location and time of the experiment

The experiment was laid out in a greenhouse at the Wanariset Research Station of the Forest Research Institute Samarinda (FRIS), located 38 km northeast of Balikpapan in Kalimantan Timur. It was started in December 1998, when cuttings of

S. leprosula were planted in perforons and it ended in January 2000 by harvesting the plants. Between start and harvest of the experiment various observations on the aboveground parts of the plants, as well as of their roots and mycorrhizae via the perforations, were made and recorded. At harvest, various parameters of the young trees and their mycorrhizae were recorded. Histological analysis of the roots was subsequently carried out in the Silviculture Laboratory, SEAMEO, BIOTROP in Bogor.

3.2.2 Preparation and experimental design

The plantlets of *S. leprosula* that were used at the start of the experiments originated from cuttings, rooted and grown for two months on vermiculite. Art and manner of obtaining these plantlets were described in section 2.2.2. At the start of the present experiment these plants were transplanted to the same three substrates as in experiment I, viz. clay, sandy loam and sandy clay (cf. 2.2.2). Half of each of these substrates was heated by means of autoclaving at 121^0 C during two hours to prevent possible contamination by unknown fungi (cf. 2.2.1); the other half was left untreated.

In the following figures (after Smits, 1994) the perforon technology is presented to demonstrate the method used in more detail. The system as shown in Fig. 3-1 shows some of the types of perforons that are possible (Smits, 1994).

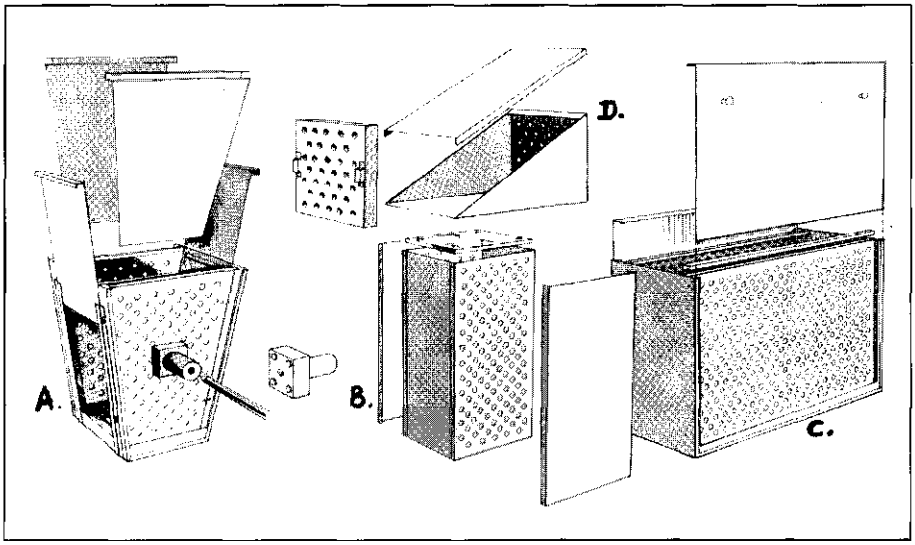


Figure 3-1 Four types of "perforons" suitable for use with the horizontally perforated soil system (from Smits, 1994)

As described by Smits (1994, pp 131 to 134) "All parts of the perforons in contact with soil are made of stainless steel, while the coverings are made of aluminium. Type A represents a type that is especially suitable for use with artificially composed soil media. Because of the tapering towards the base, newly filled soil will not settle as fast as would be the case in types B and C. The tapering towards the base provides an upward pressure of the soil clod. Therefore the perforations in the soil clod will stay longer in front of the perforations in the stainless steel plates and remain more rounded. The sides and the bottom of type A are made of glass, through which the root growth can also be observed in a more traditional way. The equipment used to make the perforations is made of PVC and can be used for all types of boxes. Type B is especially used to store natural soil profiles. Type C is of a type suitable for studying interactions between roots of different plants, providing space for a row of plants. Type D can be placed in natural terrain. Here the perforations are made at an angle down into the soil. The upper lid lies horizontally over the type D box after digging of its hole. Special equipment is used to manipulate the intrascope for this type of boxes.

A soil clod is horizontally perforated before or after planting. New roots that cross the perforations can be observed by means of an intrascope (a kind of endoscope) with an external light source that illuminates the object of study with halogen light transported through glass fibres (see Figure 3-2 and 3-3). The light that reaches the root is therefore "cold".

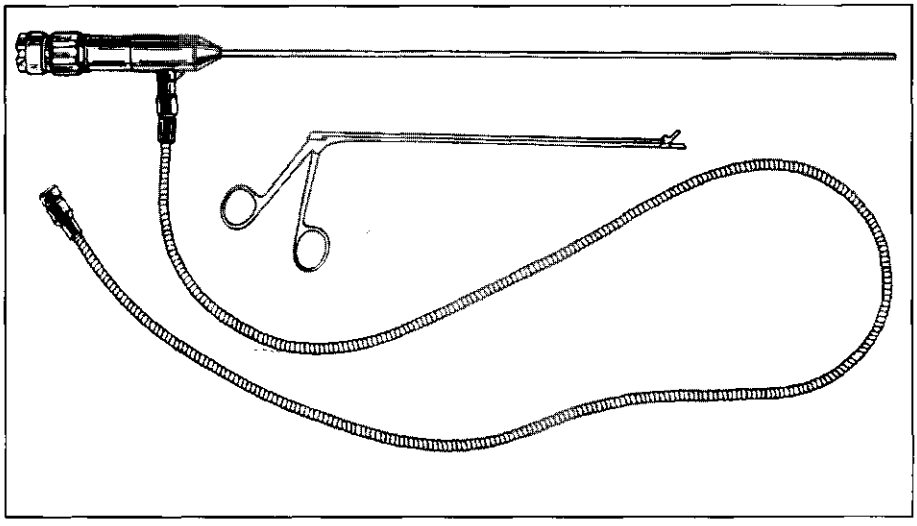


Figure 3-2 An intrascope (non-flexible endoscope) with flexible optical glass fibre connector to guide the light beam and medical biopsy forceps. The length of the thin tube that is to enter the perforations and the length of the biopsy forceps should fit the depth of the perforations (from Smits, 1994).

Only during observations the removable plates at the front and the back of the perforons are set aside. Their function is to prevent light from entering the perforations and thus inducing changes in root growth direction. They also function to keep air humidity within the perforations high so as to prevent desiccation of the roots growing through the perforations. Small root samples can be taken out by means of a long biopsy forceps without the need of breaking up the whole soil clod as in traditional methods of removing all roots from a pot (see Figure 3-2)".

Connecting a camera to the intrascope, one can make time lapse recordings of the infection process. A set-up as used in the greenhouse at Wanariset Research Station is shown in Figure 3-3. It is possible to get very close to the root so as to be able to see some hyphal characteristics.

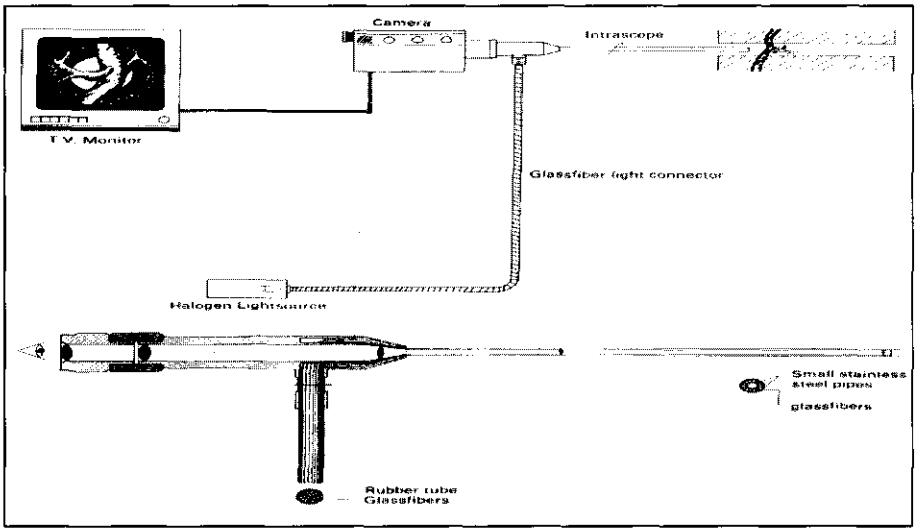


Figure 3-3 Simple way of recording the root growth in the perforations on photo or movie camera. The light that has passed the glass fibre light connector is "cold"(from Smits, 1994)

For studying the development of mycorrhizae in different soil substrates, one such perforon system was used in the present project (Fig. 3-4). It consists of root-boxes (perforons), an Intrascope and boring equipment (Bosch, 1985; Smits, 1994), as described above.

In the experiment the perforon used was the tapering type A. The perforons (root-boxes) used were made of stainless steel and had a rectangular bottom of 10 x 7.5 cm and a 12 x 10 cm top. The height was 32 cm. The front and back wall of the box were perforated with 14 holes of 12 mm in diameter and 4 cm apart from each other

(see Fig. 3-1). At the sides of the perforated front and back wall there were double grooves, in which transparent plates in the back and covering stainless steel plates in the front could be lowered down and lifted up. These were normally lowered to seal the soil perforations off from the greenhouse atmosphere and light, but could be removed for making the necessary observations.

The Intrascope system used for facilitating observations within the perforations consisted of a Volpi 2200 Intrascope together with a Volpi 6000 light source and a glass fiber light conductor. The boring equipment used for drilling the perforations in the soil at the start of the experiment, consisted of a positioned drill and 14 individual support tubes with a diameter of 12 mm.

Figure 3-4 Cutting of *S. leprosula* in a perforon.

Each of the root-boxes (perforons) was planted with one rooted cutting of *S. leprosula*. The experiment was carried out in the same greenhouse and on the same 6 x 1 m nursery beds as used for experiment 1 (chapter 2, Fig. 2-2). They were covered with plastic sheets to keep humidity and air temperature at the same level (Fig. 3-5; cf. semi-controlled conditions in experiment I).

Inoculation with ectomycorrhizal fungi was made after some roots of each of the cuttings were penetrating the holes in the perforated soil substrate in the perforons, four months after transplantation. The three fungi used in this experiment were the same as those used in Experiment I, viz. *Amanita sp, Russula sp* and *Scleroderma columnare.* The inoculum was prepared in the same way, and was again composed of 1 ml of a watery suspension containing 10.000 spores (cf. 2.2.3.). The spore suspension was applied to roots growing through the perforations. Plants with non-inoculated roots constituted the "no treatment" group. To facilitate observations of roots inside perforons the intrascope was used, as described above.

Figure 3-5 *S. leprosula* cuttings in perforons were placed in 1 m x 6 m compartments under a roof of polyethylene sheets.

Table 3-1 Summary of the experimental design for testing the effect of different soil substrates on the development of mycorrhizae on the roots of *S. leprosula* cuttings in perforons.

Treatment	Code	Number of treatments	Description
Soil substrate	S	3	Clay, sandy loam and sandy clay
Autoclaving of soil substrate	St	2	Sterilized and unsterilized
Ectomycorrhizae inoculated	M	5	*Amanita* sp, *Russula* sp, *Scleroderma columnare*, cocktail of fungi *) and without inoculation.
Similar blocks		5	
Total cuttings		150	

*) cocktail of fungi (mixture of *Amanita sp, Russula sp* and *Scleroderma columnare*)

The experiment was carried out as a factorial set-up in a completely randomized design with three factors (3 x 2 x 5), in 5 similar blocks. A summary of the design is presented in Table 3-1.

3.2.3 Data collection and analysis

The first measurements and observations were recorded three months after transplantation, to give the cuttings ample time to grow roots into the perforations. The parameters for plant growth measured were height, diameter growth and number of leaves of each plant. During these three months no cutting died. The soil temperatures were measured weekly for each perforon. Light intensities, atmospheric humidity and air temperature were measured per similar block weekly at 9.00 hrs, 12.00 hrs and 16.00 hrs.

After inoculation the number and types of mycorrhizae formed were recorded every month. The different mycorrhizal types were described according to the method of Mason and Ingleby (1997). Features of associated hyphae were, for instance ornamentation, branching pattern, presence and configuration of plant connection, mantle surface and inner mantle. Mantle structures are broadly categorized according to basic features of whether (a) the mantle is loosely structured or compact, or (b) whether the mantle cells retain an elongated hyphal shape or become more isodiametric.

During the execution of the experiment, the mycorrhizal type colonizing the root system of *S. leprosula* cutting was recorded. These mycorrhizal roots were classified into four types. Each mycorrhizal type was then dissected, squashed and identified following the method described by Mason and Ingleby (1997).

At the end of the experiment when the plants were harvested, the following data were recorded for each plant: height and diameter growth, number of leaves, leaf area, total fresh weight, total dry weight, dry shoot-root ratio and number of root tip infection by mycorrhizal fungi. Histological analysis of mycorrhizal roots was performed by use of the SASS method 1958 (Berlyn and Miksche, 1976) in the Silviculture Laboratory, SEAMEO, BIOTROP in Bogor. Mineral contents (N, P, K, Ca, Mg and Fe) of leaves, stems and roots of cuttings were determined at the Natural Products Laboratory, SEAMEO, BIOTROP in Bogor. Mineral contents of the soil substrates (N, P, K, Ca, Mg and Al) were determined at the beginning and at the end of the experiment in the same laboratory.

The data obtained for the various parameters of growth for the experimental plants were subjected to an analysis of variance (ANOVA) to test the significance of the effect of each of the factors, i.e. of soil substrate, of autoclaving of soil substrate, and of inoculum. The GLM-ANOVA procedure of the SAS version 6.12 was used. Significant F values obtained by ANOVA tests were further examined by pair-wise comparison of means using Duncan's Multiple Range Tests in the SAS software.

3.3 RESULTS

The results of the experiment are presented for three main factors and their interaction effects, namely: soil substrate (Table 3-3), soil sterilization (3.2.2.) and mycorrhizal inoculation. The effect of each factor on plant growth, mycorrhizal development and nutrient uptake is presented consecutively. To determine the significance level of the parameters measured, the mean value was tested by statistical analysis (Appendix 6). The results of the analyses of variance (ANOVA) show that soil substrate, and sterilization influenced the growth of *S. leprosula* cuttings. This is especially the case with the parameters of height growth, the number of leaves, total fresh weight and total dry weight. The number of mycorrhizal root tips and the percentage of mycorrhizal roots were significantly affected by the soil substrate in which the cuttings were grown. The soil substrates and their sterilization had a highly significant effect on diameter growth and number of leaves. Inoculation with mycorrhizal fungi affected the percentage of mycorrhizal roots (ECM %). Interactions between soil substrates and sterilization influenced the growth parameters of height and diameter growth, number of leaves, leaf area, total fresh weight and total dry weight.

3.3.1 Effects of Soil Substrate

A. Plant growth

In Table 3-2, Fig. 3-6 and Fig. 3-7, the mean value is shown of the plant growth parameters height, diameter, number of leaves, leaf area, total fresh weight and total dry weight. Table 3-2 shows that the growth of cuttings planted in clay was significantly lower than in sandy loam and sandy clay in regard to height and diameter growth, number of leaves, leaf area, total fresh weight and total dry weight. This was demonstrated by Duncan's Multiple Range Test.

Table 3-2 Effects of soil substrate on some growth parameters of *Shorea leprosula* cuttings. Average height growth (ΔH), average diameter growth, (ΔD), number of leaves (N_l), leaf area (A_l), total fresh weight (W_{tf}), total dry weight (W_{td}).

		Mean response					
Soil Treatment	N	ΔH (cm)	ΔD (mm)	N_l	A_l (cm^2)	W_{tf} (g)	W_{td} (g)
Clay (S1)	46	10.5 a	0.1 a	6.1 a	5.2a	4.1 a	1.0 a
Sandy loam (S2)	47	25.3 b	0.4 b	11.6 b	21.3 b	13.9 b	4.5 b
Sandy clay (S3)	48	24.1 b	0.4 b	11.7 b	19.9 b	14.0 b	4.3 b
Increment S2 vs S1(%)		141	300	90	310	239	350
Increment S3 vs S1 (%)		130	300	92	283	241	330

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level as tested with Duncan's Multiple Range Test.

Figure 3-6 Effect of soil substrate on growth of *Shorea leprosula* cuttings. S1= Clay, S2 = Sandy loam, S3 = Sandy clay

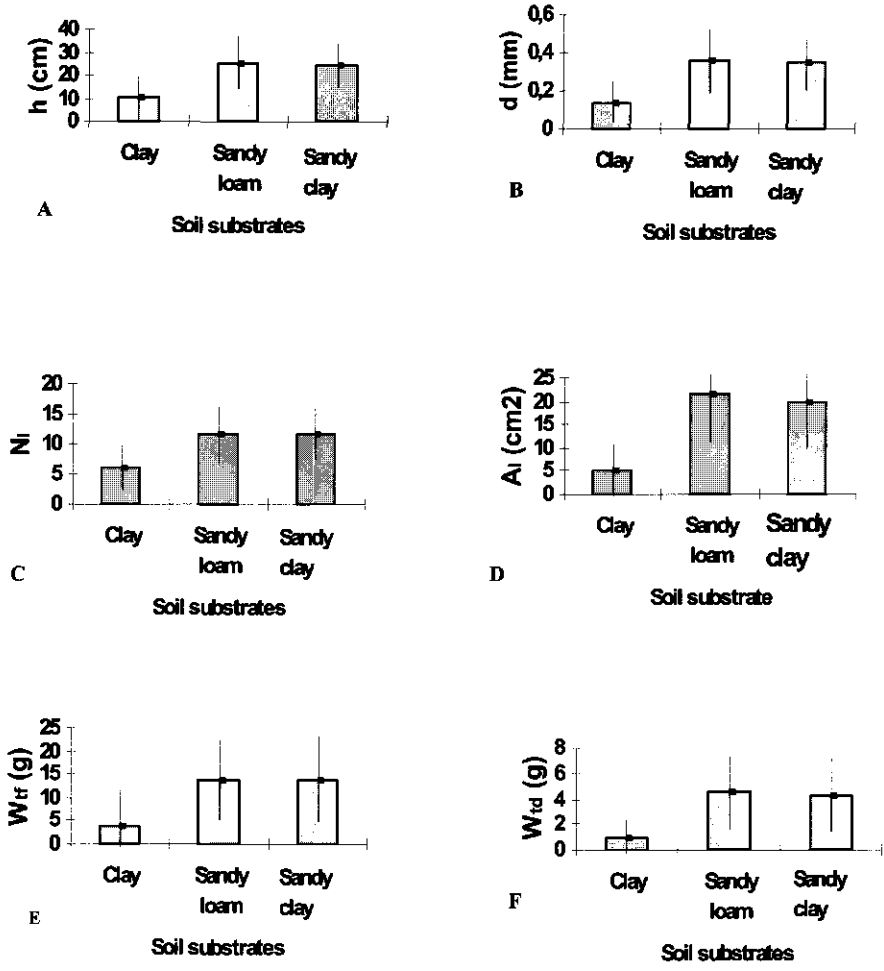


Figure 3-7 Effect of soil substrate on (A) height growth; (B) diameter growth; (C) number of leaves; (D) leaf area; (E) total fresh weight; (F) total dry weight at harvest 10 months after inoculation. The length of the vertical line at the top of the bars indicates the amount due to chance at 5 %-level of significance with Duncan's Multiple Range Test.

The differences in plant growth per soil substrate were caused by the granulometric and chemical soil properties required for supporting the growth of cuttings. The granulometric properties of the soil substrates used in the experiment are presented in Table 3-3. The sand fraction was higher in sandy clay than in sandy loam and lowest in clay. Porosity in sandy clay, hence, was higher than in sandy loam and in

clay. Good, clear aeration of soil substrates was expected to promote mycorrhizal development and plant growth.

Height and diameter growth of cuttings in sandy loam and sandy clay were higher than in clay (Table 3-2). The increment of total dry weight in sandy loam and sandy clay was 350 % and 330 % of that in clay, respectively. Leaf area increment in sandy loam was 310 % and in sandy clay 283 % of that in clay. Differences in aeration were correlated closely with this observation. The physical and chemical properties of each substrate are presented in Appendix 7.

Table 3-3 Summary of physical properties of the soil substr ate in the perforons

	Fraction distribution		
Soil substrates	Sand (%)	Silt (%)	Clay (%)
Clay (S_1)	43	24	33
Sandy loam (S_2)	61	20	19
Sandy clay (S_3)	71	16	13

B. Mycorrhizal development

In this experiment the number of mycorrhizal root tips and the percentage of mycorrhizal roots are considered parameters for mycorrhizal development. Aeration, pH, light intensity, and soil heat affect the development of mycorrhizae in a soil. In general, the number of mycorrhizal root tips increases after mycorrhizal colonization.

Table 3-4 Effects of soil substrate on the number of mycorrhizal root tips per perforon and percentage of mycorrhizal roots (ECM %) in *Shorea leprosula* cuttings 10 months after inoculation. N_{rt} = number of mycorrhizal root tips and ECM % = percentage of mycorrhizal roots

	Mean response	
Soil type	N_{rt}	ECM %
Clay (S1)	584 a	55 a
Sandy loam (S2)	1645 b	60 b
Sandy clay (S3)	2022 b	69 b
Increment S2 vs S1 (%)	182	9
Increment S3 vs S1 (%)	246	25

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level as tested with Duncan's Multiple Range Test.

Fig. 3-8 and Fig. 3-9 show that the mycorrhizal root type in one and the same phytobiont (*S. leprosula*) varied from a simple type (type 1) to a branching type (type 4). This response was caused by the mycorrhizal fungus species (Mason and Ingleby, 1997). It is important to describe any unknown mycorrhizal fungus colonizing the host plant, especially when using unsterilized substrate. The

description of each mycorrhizal type is therefore summarized in Table 3-5. Types were classified according to colour, mantle thickness, hyphal type, surface mantle type and inner mantle type. The type of mycorrhizal colonization in *S. leprosula* cuttings is presented in Fig. 3-8. Based on the descriptions in Table 3-5 and Fig. 3-10, type 2 represents mycorrhizal fungi of *Thelephora sp*, while type 3 and 4 represent *Amanita* sp1 and *Amanita* sp2 (Ingleby et al., 1990) Type 1 is difficult to identify as to the species of fungus involved. In the following, Type 1, 2, 3 and 4 will be used as designations of the above types.

Table 3-5 Macro- and micro- description of ectomycorrhizal types in the perforon experiment in different soil substrates and inoculation with different ectomycorrhizal fungi.

Designation	Type 1	Type 2	Type 3	Type 4
Colour	Brown-black	Brown	Silver-White	Silver-White
Mantle thickness	0.01- 0.02 mm	0.01-0.03 mm	0.01-0.04 mm	0.01-0.04 mm
Hyphae	Hyphae, with frequent clamp- connection Diameter 1.5 – 3.5μm	Branched hyphae, with frequent clamp-connections Diameter 2.5 – 4.0μm	Hyphae rarely lack clamp connections, hyphal cells contain granular cytoplasm. Diameter 3.0 – 8.0μm	Hyphae rarely lack clamp connections hyphal cells contain granular cytoplasm Diameter 4.0 – 7.0μm
Mantle: surface	An interlocking irregular synenchyma of cells	Net prosenchyma, shortened, and hyphal cells shorter, broader and interlocking more closely.	Net prosenchyma compacted to form non –interlocking cells	Net prosenchyma compacted to form non-interlocking cells.
Mantle: inner	A synenchyma of cells	Net synenchyma of shortened cells	Net synenchyma- cells distinctly elongated	Net synenchyma- cells distinctly elongated
Species	Type1(sp.ind.)	*Thelephora sp*	*Amanita* sp1	*Amanita* sp2

Table 3-6 and Appendix 5 show that the mycorrhizal type was affected by the soil substrate. The numbers of mycorrhizal type 1, 2 and 3 are not significantly different in sandy loam and in sandy clay. The mycorrhizal type 4 differed with the substrates used. Those mycorrhizal types are presented in Fig. 3-8, 3-9 and 3-10. According to the root squash test however, the fungal genus involved in types 3 and 4 is the same.

Table 3-6 Effects of soil substrate on the number of mycorrhizal types per perforon on the roots of *S. leprosula* cuttings at 10 months after inoculation.

	N	Mean response			
Soil type		Type 1	Type 2	Type 3	Type 4
Clay	240	0.9 a	0.6 a	1.4 a	0.8 a
Sandy loam	235	1.2 b	1.4 b	1.8 b	0.9 c
Sandy clay	245	1.4 b	1.4 b	2.0 b	0.6 b

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

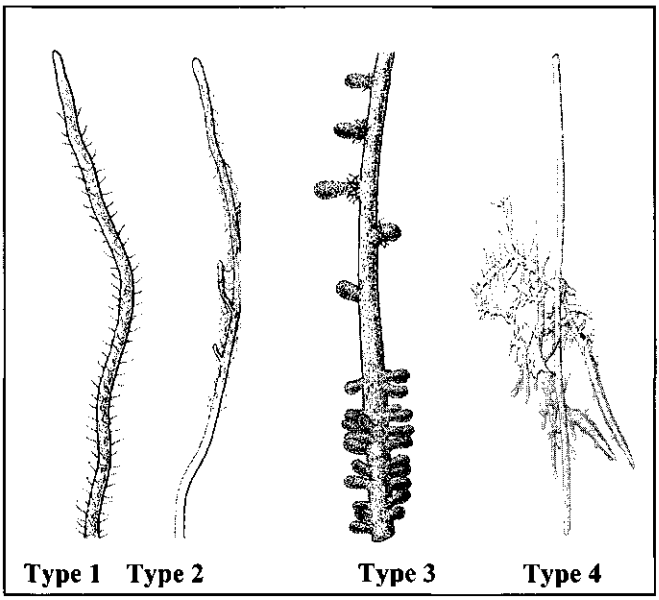


Figure 3-8 Different types of mycorrhizal colonization in roots of *S. leprosula* cuttings in perforons. Type 1 = sp indet., Type 2 = *Thelophora* sp, Type 3 = *Amanita* sp1 and Type 4 = *Amanita* sp2.

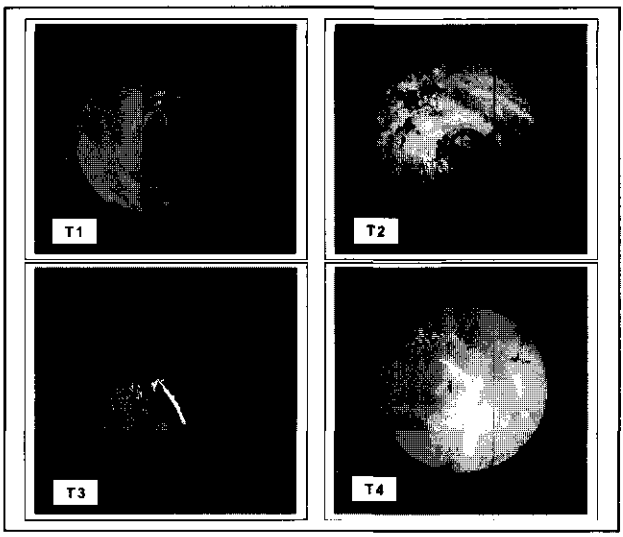


Figure 3-9 Different mycorrhizal root types of *Shorea leprosula* cuttings in perforons. Type 1 = sp indet., Type 2 = *Thelophora* sp, Type 3 = *Amanita* sp1 and Type 4 = *Amanita* sp2.

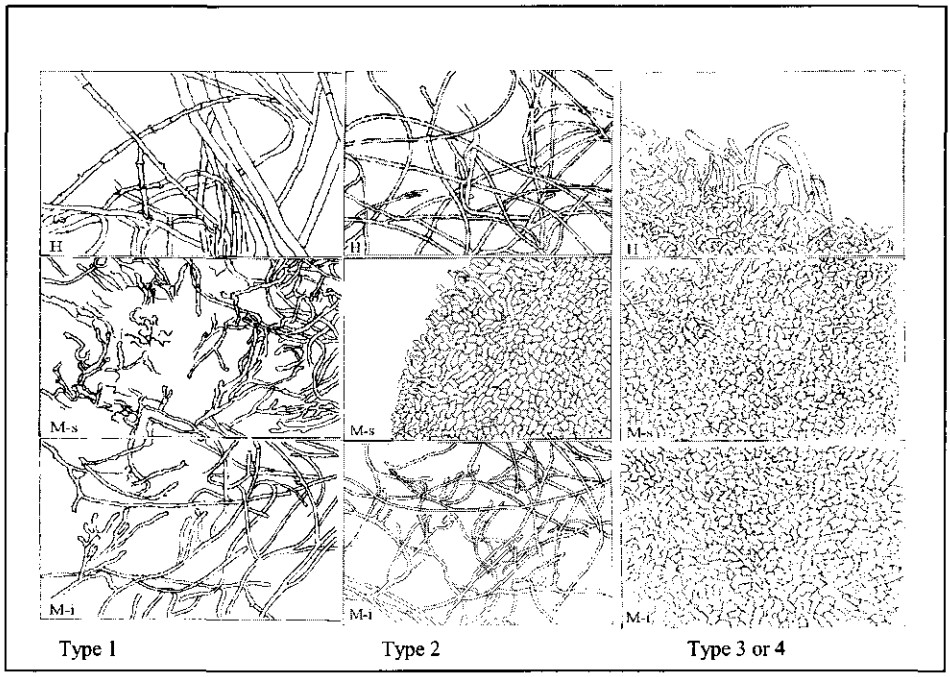


Figure 3-10 Root squashes of different mycorrhizal types. H = hyphae, M-s = mantle surface, M-i = inner mantle.

Fig 3-11 shows that during five months of observations the total number of type 3 (*Amanita* sp1) was higher than that of types 1, 2 and 4. Table 3- 6 and Fig 3-11 show that the development of type 3 in sandy loam and sandy clay was higher than in clay. Sandy loam and sandy clay are best suited to the development of mycorrhizal type 3.

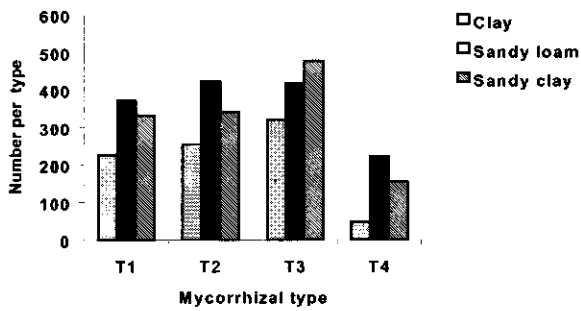


Figure 3-11 Number of mycorrhizal types in different soil substrates in perforon.

Fig. 3-12 shows that the number of mycorrhizal type 3 (*Amanita* sp1) was higher than that of the other types, while the number of mycorrhizal type 4 (*Amanita* sp2) was lower than that of the other ones. This would fit in with *Amanita* sp1 belonging to the early stage fungi, and *Amanita* sp2 belonging to later stage fungi (but see general discussion on ageing, par.4.2).

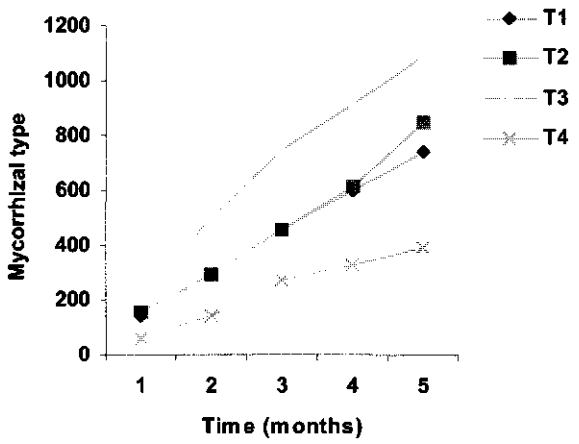
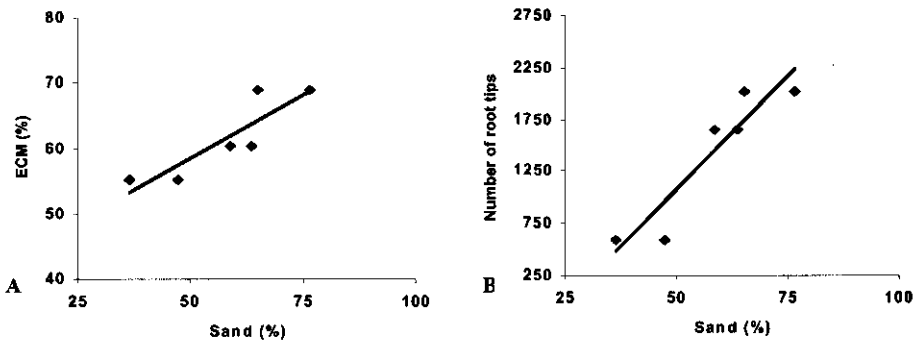


Figure 3-12 Cumulative numbers of different mycorrhizal types in the perforon.



Y = 0.38x + 39.27 (r = 0.95; P = 0.01) Y 43.459x – 1104.6 (r = 0.88; P= 0.01)

Figure 3-13 Correlation between sand fraction in percentages and percentages of mycorrhizal roots (A) and numbers of mycorrhizal root tips per perforon (B). P = significance (probability).

Fig. 3-13 shows that both the number of mycorrhizal root tips and the percentage of mycorrhizal roots were significantly affected by the percentage of sand in the soil substrate, with a correlation coefficient r = 0.88 (P = 0.01) and r = 0.95 (P = 0.01), respectively.

C. Nutrient uptake

Fig. 3-14 shows that nutrient uptake was affected by the soil substrate. The uptake of N, P, K, Ca, Mg and Fe was higher in sandy loam and sandy clay substrates than in clay. Fig. 3-11 and Table 3-4 show that, possibly, the number of mycorrhizal root tips is related to this nutrient uptake. Mycorrhizal development in sandy loam and sandy clay was higher than in clay.

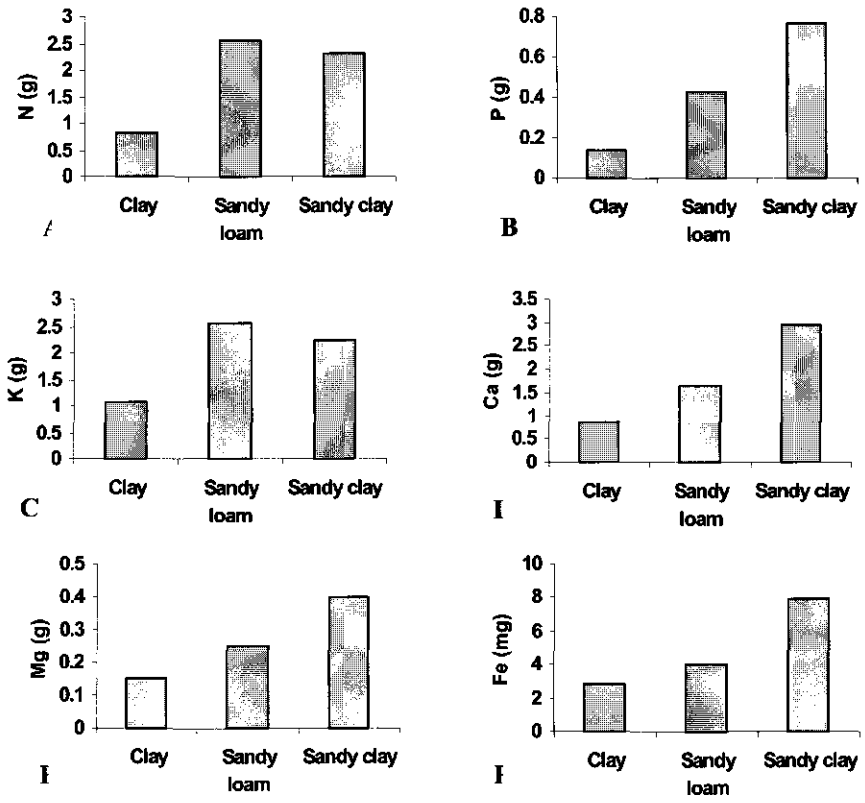


Figure 3- 14 Nutrient uptake (A) N-uptake: (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake in different soil substrates by *S. leprosula* cuttings.

Fig. 3-15, the correlation curve between the nutrient uptake and the dry weight of cuttings shows that the dry weight of cuttings tends to increase when the nitrogen uptake increases up to 40 mg/cutting. The biomass of cuttings decreases when the nutrient uptake of P, K, Ca, Mg and Fe rises to 5-7 mg, 40 mg, 30 mg, 5 mg and 0.002 mg/cutting, respectively.

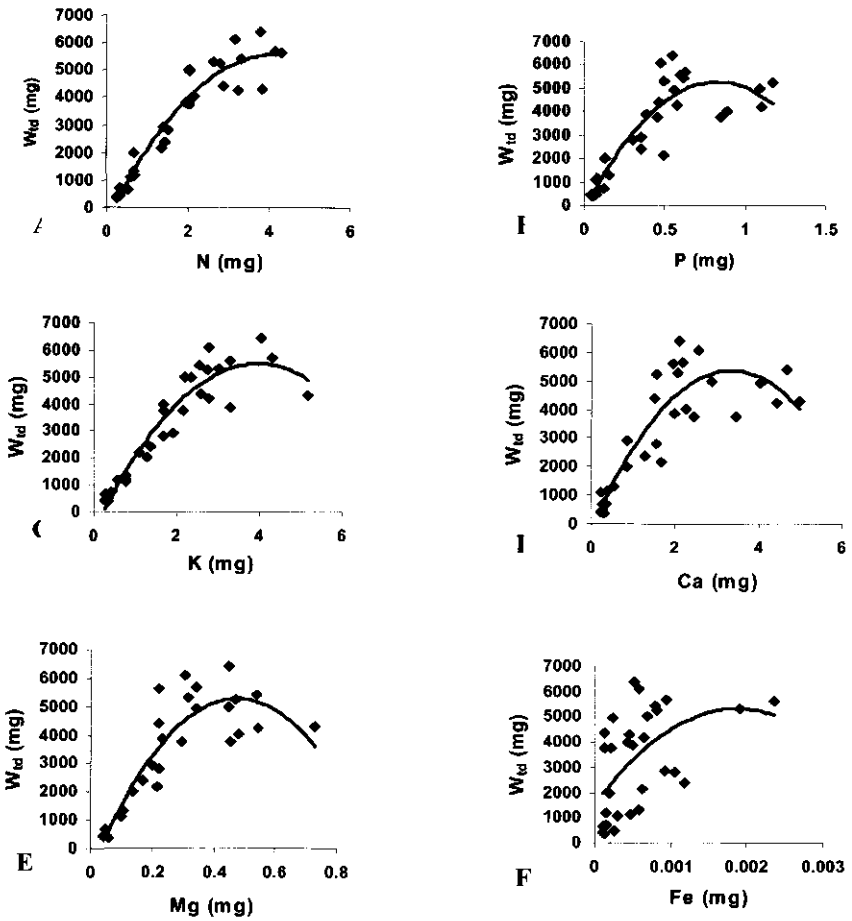


Figure 3- 15 Relationship between dry weight (W_{td}) and the nutrient uptake by *S. leprosula* cuttings in perforons. A) N-uptake (r = 0.96; P = 0.01); B) P-uptake (r = 0.90 ; P = 0.01); C) K-uptake (r = 0.95; P = 0.01); D) Ca-uptake (r = 0.91; P = 0.01); E) Mg-uptake (r = 0.91; P = 0.01) and F) Fe-uptake (r = 0.52 ; P = 0.01).

3.3.2 Effects of substrate autoclaving

A. Plant growth

The main purpose of substrate heat is to kill off pathogenic microorganisms and unknown mycorrhizal fungi, so that the pure effect of inoculated mycorrhizal fungi can be monitored. In this heat treatment, the soil substrate was autoclaved at 121°C for two hours. Nonetheless, wild mycorrhizal fungi sometimes contaminated the experiment, and soil heat changes soil chemical properties (see 3.2, material and methods). One negative effect of substrate sterilization is degradation of nutrient availability.

Table 3-7, Figure 3-16 and Figure 3-17 show the results of Duncan's test for the effect of heat treatment of soil substrates on some growth parameters of the cuttings. The growth of the cuttings was significantly and negatively influenced ($p<0.05$) by substrate sterilization, especially with regard to height and diameter growth, number of leaves and total dry weight. Perhaps the heat affected the nutrient balance, too.

Table 3-7 Effects of initial sterilization of soil substrates on several growth parameters of *S. leprosula* cuttings, 10 months after inoculation in perforons. Average height growth (ΔH), average diameter growth (ΔD), number of leaves (N_1), total dry weight (W_{td}).

		Mean response			
Treatments	N	ΔH (cm)	ΔD (mm)	N_1	W_{td} (g)
Not autoclaved (St0)	72	21.8 a	0.3 a	11 a	3.7 a
Autoclaved (St1)	69	18.3 b	0.2 b	9 b	2.5 b
Increment St1 vs St0 (%)		-16	-33	-18	-32

Values followed by the same letter in the same column (a or b) are not significantly different at 5% level as tested with Duncan's Multiple Range.

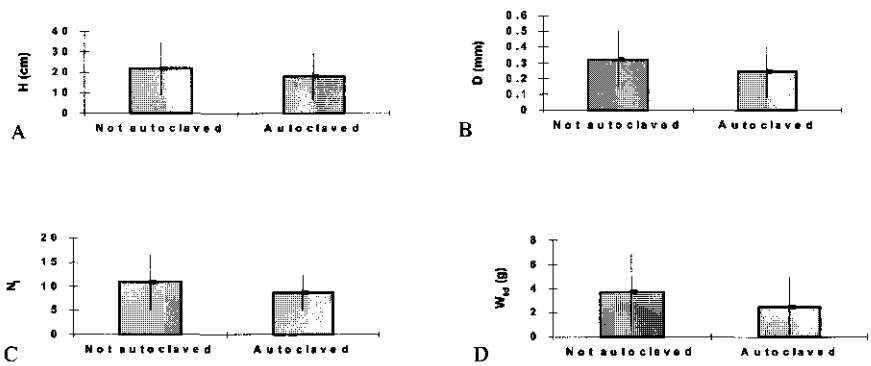


Figure 3-16 Sterilization of soil substrates (autoclaved versus not autoclaved). (A) Height growth; (B) diameter growth; (C) number of leaves and (D) total dry weight at harvest 10 months after inoculation. The length of the vertical line at the top of the bars indicates the uncertainty interval chance at 5 % level of significance (Duncan,s Test).

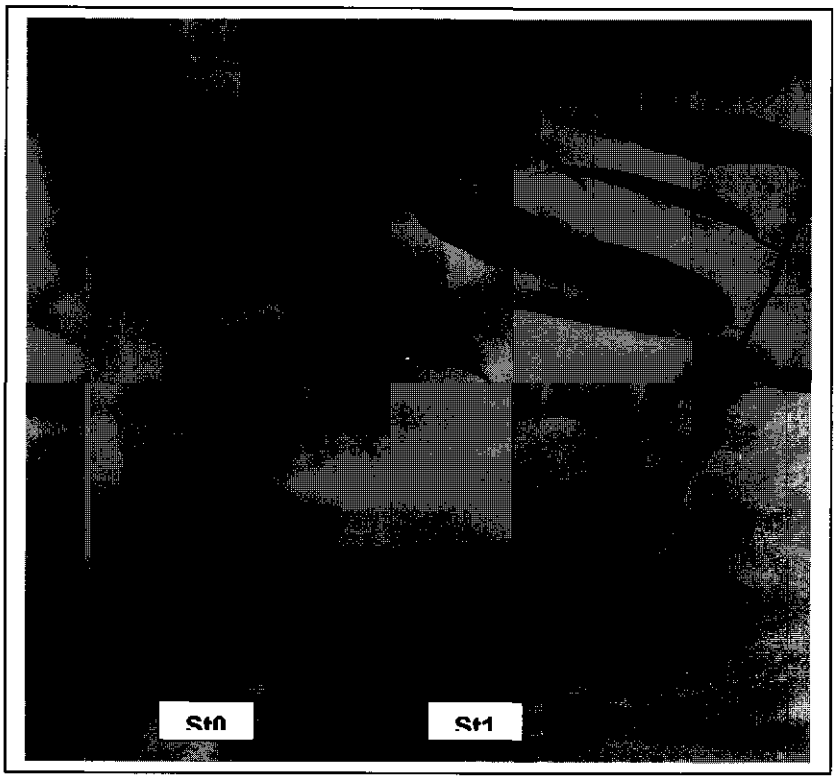


Figure 3-17 Effect of autoclaving on growth of *Shorea leprosula* cuttings
St0 = not autoclaved and St1 = autoclaved

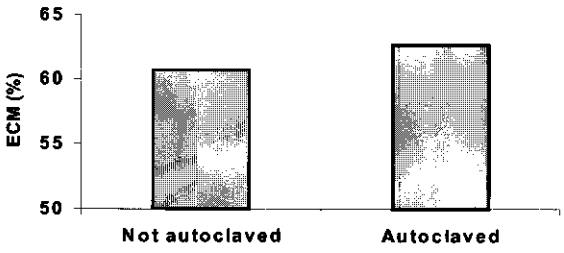


Figure 3-18 Percentage of mycorrhizal roots in not autoclaved and autoclaved soil substrates

B. Mycorrhizal development

Fig. 3-18 shows that the ECM % in a sterilized substrate is somewhat higher than in an unsterilized one (63 % vs. 61 %).

In general, the substrate contains natural mycorrhizal fungi if it is not autoclaved. It also contains their grazers, such as certain nematodes. Among the mycorrhizal types observed, only type 3 and 4 were significantly different in sterilized and unsterilized substrates (Table 3-8). The frequency of type 3 and 4 in unsterilized soil substrates was higher than in sterilized substrates (see 4.2., ageing).

Table 3-8 Effects of sterilization of the soil substrate on the frequency of mycorrhizal type 1, type 2, type 3 and type 4; see Fig. 3-8 on the roots of *S. leprosula* cuttings 10 months after inoculation.

		Mean response			
	N	Type 1	Type 2	Type 3	Type 4
Not autoclaved	365	1.2 a	1.8 a	1.2 a	0.9 a
Autoclaved	355	1.1 a	1.6 a	0.9 b	0.4 b

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

C. Nutrient uptake

Fig. 3-19 shows that the N, P, K, and Mg uptake in unheated substrates was higher than in heated substrates, except for the Ca and Fe uptake. Table 3-9 indicates that some nutrients in sterilised substrates were degraded, due to the heating process. There is no other possible cause.

Table 3-9 Chemical and physical properties of soil substrates before and after the sterilization process

Heat treatment	PH	C organic	N Total	C/N Ratio	P Available	K	Ca	Mg	Al	Sandy	Silty	Clay
Unheated	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
Clay	4.8	0.89	0.11	8.10	4.41	0.17	1.18	0.71	5.61	38.0	25.6	40.3
Sandy loam	4.9	1.13	0.09	12.60	4.18	0.19	2.36	0.56	1.24	57.3	19.2	23.5
Sandy clay	5.1	1.06	0.10	10.60	7.64	0.11	2.21	1.02	1.33	72.1	20.4	41.5
Heated												
Clay	4.7	0.92	0.08	11.50	6.22	0.19	1.54	0.80	4.26	37.8	26.4	39.5
Sandy loam	4.9	1.23	0.08	15.40	5.31	0.22	2.69	0.59	1.12	56.3	20.1	23.6
Sandy clay	5.0	1.08	0.09	12.00	8.92	0.15	2.47	1.32	1.28	71.3	19.6	43.1

Very hot conditions sometimes cause nutrient evaporation, fixation or, on the contrary, release of nutrients. Table 3-9 shows that the pH of the substrate after heating decreased by 0.1 unit. Nitrogen decreased, phosphorus increased, calcium increased, potassium increased, magnesium increased and aluminium decreased by the percentages shown in Table 3-9. After sterilization of the substrate, the nutrient content either decreased (N and Al) or increased (P, Ca, K and Mg).

Fig. 3-19 shows that Ca and Fe uptake in sterilized soil is closely correlated to the autoclaving process, but that this was not so for N, P, K and Mg.

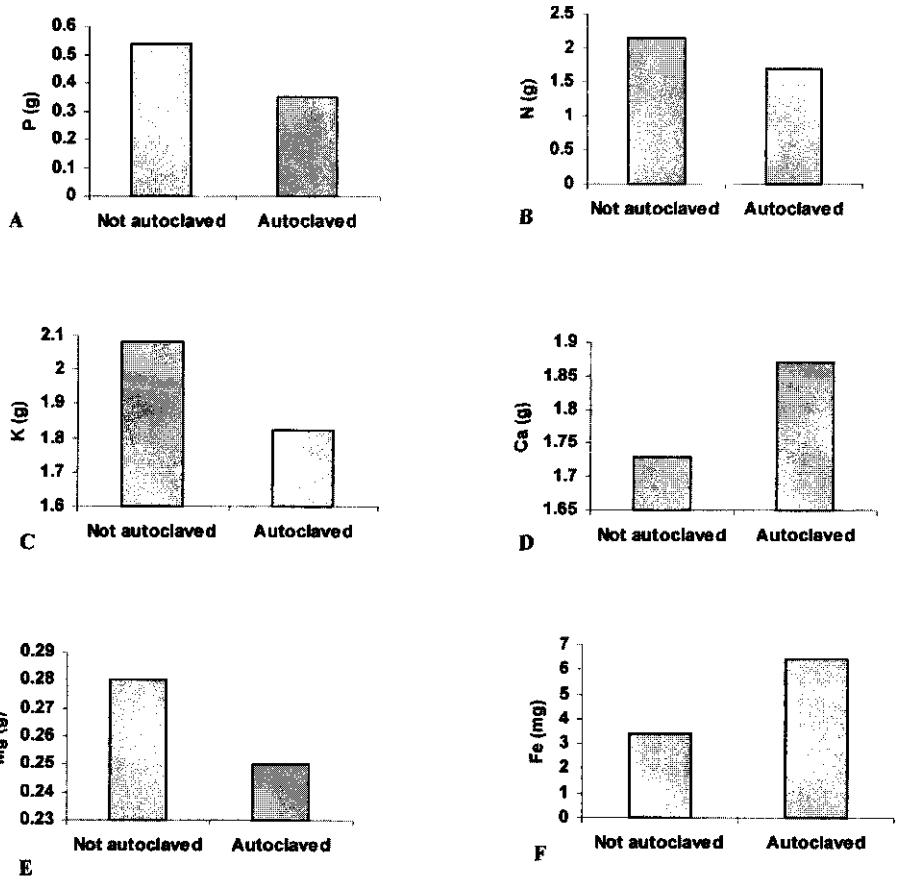


Figure 3-19 Nutrient uptake in not autoclaved and autoclaved substrate (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake.

3.3.3 Effects of inoculation

A. Plant Growth

Statistically, mycorrhizal inoculation with *Amanita* sp, *Russula* sp, *Scleroderma columnare* and the cocktail of fungi did not significantly affect the growth of the cuttings, but it did affect the percentage of mycorrhizal roots (ECM %) representing mycorrhizal development and nutrient uptake.

The growth of inoculated cuttings was not significantly different from the control. Perhaps unknown mycorrhizal fungi (Fig. 3-20) contaminated some cuttings under otherwise controlled conditions.

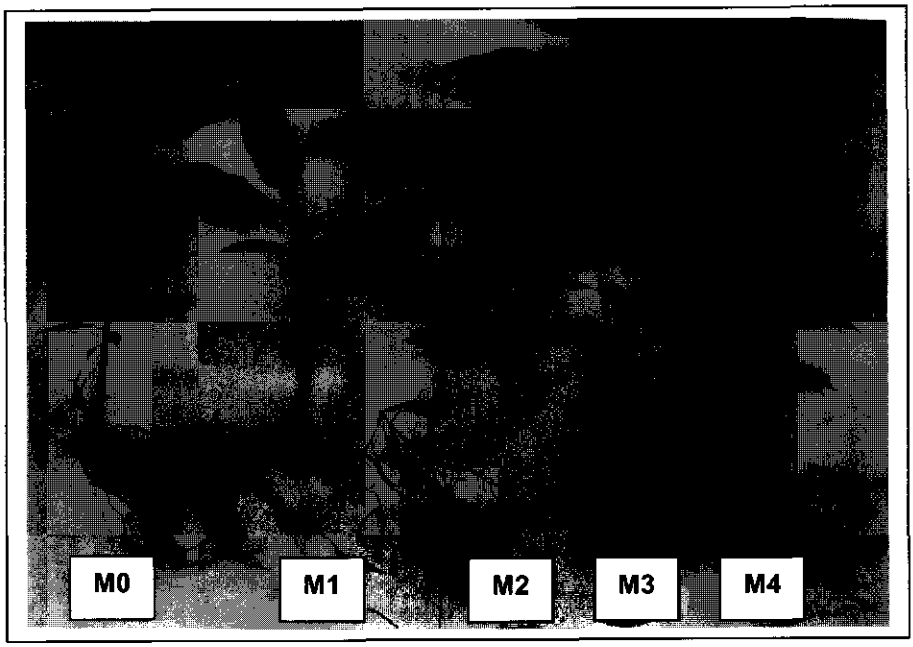


Figure 3-20 Some plants showing the effect of mycorrhizal inoculation on the growth of *Shorea leprosula* cuttings. M0 = without inoculation; M1 = inoculation with *Amanita* sp; M2 = inoculation with *Russula* sp; M3 = inoculation with *Scleroderma columnare* and M4 = cocktail of fungi.

B. Mycorrhizal development

Table 3-10 shows that the percentage of mycorrhizal roots in *S. leprosula* cuttings inoculated with *Amanita* sp, *Russula* sp, *Scleroderma columnare* and the fungal cocktail was significantly different from that of the cuttings without inoculation.

The percentage of mycorrhizal roots was 69% (*Russula* sp), 63 % (*Amanita* sp), 68% (*Scleroderma columnare*), 68 % (cocktail) and 41 % (no treatment). The percentage of mycorrhizal roots among the inoculations with the 3 fungi was not significantly different, ranging from 63 % to 69 %. The development of inoculated mycorrhizal fungi therefore was similar. They were all able to colonize the roots of *S. leprosula* cuttings.

Table 3-10 Effects of mycorrhizal inoculation on percentages of mycorrhizal roots (ECM %) of *Shorea leprosula* cuttings, 10 months after inoculation.

Mycorrhizal treatments	**N**	**ECM %**
No inoculation (M0)	29	41 b
Amanita sp (M1)	25	63 a
Russula sp (M2)	28	69 a
Scleroderma columnare (M3)	29	68 a
Cocktail of fungi (M4)	28	68 a
Increment M1 vs. M0 (%)		54
Increment M2 vs. M0 (%)		68
Increment M3 vs. M0 (%)		66
Increment M4 vs. M0 (%)		66

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

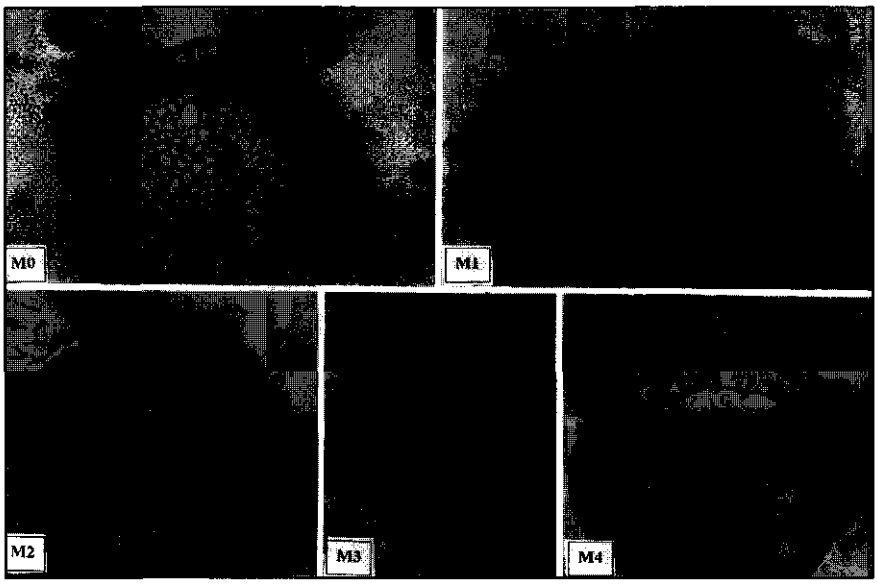


Figure: 3-21 Histological analysis of mycorrhizal and non-mycorrhizal roots in perforons. M0 = without treatment; M1= inoculated with *Amanita* sp; M2 = inoculated with *Russula* sp; M3= inoculated with *Scleroderma columnare* and M4 = inoculated with a cocktail of these fungi). M = mantle; Hn= Hartig-net; Ep= Epidermis; C = Cortex; Reec = Radially elongated epidermis cells.

Histological analysis (Fig. 3-21) shows that the mycorrhizal fungi that were used for inoculation are able to form a Mantle (M), and a Hartig-net (HN). The mycorrhizal roots formed Radially elongated epidermis cells (Reec).

The mantle thickness in each soil substrate is presented in Fig.3-22. The mantle thicker in sandy clay than in sandy loam and clay. In general, a substrate with a high sand fraction has a low bulk density and constitutes no barrier for mycorrhizal and root development. Mantle thickness can also be related to genetic factors (Smith and Read, 1997) and each mycorrhizal species has its own pattern. Table 3-4 shows that the number of mycorrhizal root tips and the percentage of mycorrhizal roots in sandy clay were higher than those in sandy loam and clay. In sandy clay the mycorrhizal development was very high.

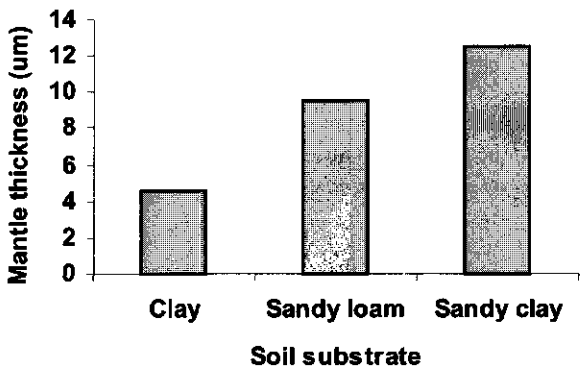


Figure 3-22 Effects of soil substrates on mantle thickness of mycorrhizal fungi.

Fig. 3-23 shows that the mantle layer and mantle thickness in the roots of cuttings inoculated with *Amanita* sp or *Scleroderma columnare* exceed those inoculated with other mycorrhizal fungi. The mantle layer ranged from 1 to 2 layers, which in figure are average, so the fraction are statistical, not real values. The mantle thickness ranged from 10 μm to 15 μm.

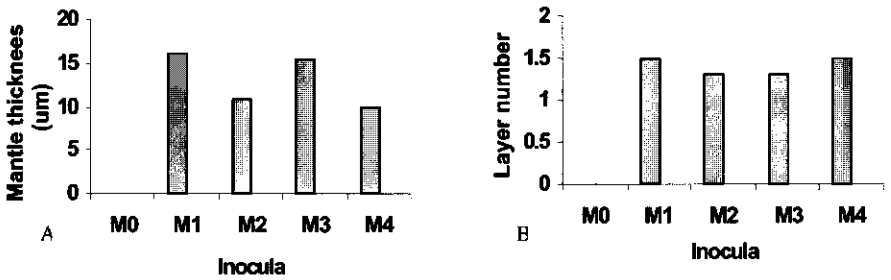


Figure 3-23 Effects of mycorrhizal inoculation on (A) avera ge mantle thickness and (B) mantle layers. M0 = no treatment; M1= inoculated with *Amanita* sp.; M2= inoculated with *Russula* sp.; M3= inoculated with *Scleroderma columnare* and M4= inoculated with a cocktail of these fungi.

The four mycorrhizal root types were shown in Table 3-11 and Fig. 3-8. Table 3-11 shows that inoculation affected the frequency of mycorrhizal type 3 (*Amanita* spl.). Fig. 3-12 showed that the cumulative number of type 3 roots was always higher than that for the other types during the period of observation.

Table 3-11 Effect of mycorrhizal inoculation on the numbers of mycorrhizal types 1, 2, 3 and 4 on the roots of *S. leprosula* cuttings 10 months after inoculation.

		Mean response of mycorrhizal type			
Treatments	N	Type 1	Type 2	Type 3	Type 4
M0 = No treatment	150	1.1 a	1.2 a	1.7 b	0.4 a
M1 = *Amanita sp*	135	1.2 a	0.9 a	1.5 a	0.4 a
M2 = *Russula sp*	145	1.2 a	1.1 a	1.5 a	0.6 a
M3 = *Scleroderma columnare*	145	1.8 a	1.1 a	1.6 a	0.6 a
M4 = *Cocktail of fungi*	145	1.1 a	1.2 a	1.8 c	0.6 a

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

C. Nutrient uptake

Nutrient uptake by different mycorrhizal fungi is presented in Fig. 3-24 (see also Fig. 2-23). The nitrogen uptake after inoculation with fungi used for inoculation (M1, M2, M3 and M4) was higher than without inoculation (M0), but the nutrient uptake of P, K, Ca, Mg in non-inoculated cuttings was higher than in inoculated cuttings. The highest Fe uptake was found in cuttings inoculated with *Russula* sp (M3). This supports the assumption that each species of mycorrhizal fungus has a specific aptitude for absorbing certain nutrients more than others.

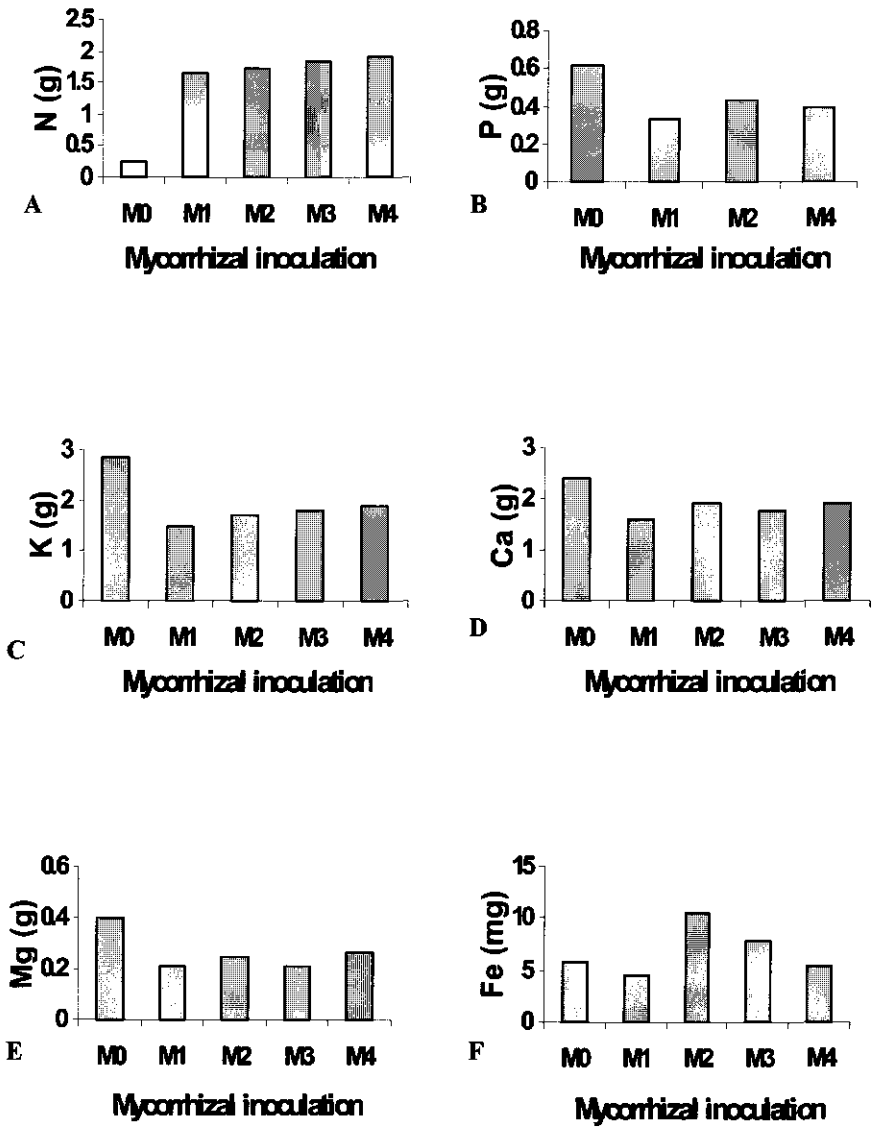


Figure 3-24 The effect of the composition of mycorrhizal inocula on the uptake of mineral nutrients. M0 = no treatment; M1= inoculation with *Amanita sp*; M2 = inoculation with *Russula sp*; M3 = inoculation with *Scleroderma columnare* and M4 = inoculation with a cocktail of these fungi.

3.3.4 Effects of light intensity

A. Plant growth

The effect of light intensity was not originally designed as a special treatment, but during the execution of the experiment the light intensity in the experiment was discovered to be less homogeneous than planned. Five blocks could be distinguished. The biological meaning of this way of grouping does not reside in mere light intensity but also takes into account the photosynthetic behaviour of the plants.

Light intensity plays an important role in growth of cuttings and mycorrhizal development. The average light intensity ($\mu mol.m^{-2}$) in the perforon experiment in the five groups is presented in Table 3-12.

Table 3-12 Average intensity of Photosynthetically Active Radiation ($\mu mol.m^{-2}$) measured above the perforon experiment during 10 months of observation.

	PAR measurement ($\mu mol.m^{-2}$)				
Block	9.00 (a)	12.00 (b)	16.00 (c)	Average (a+b+c)	Average (a+c)
I (PAR_1)	25.85	48.65	25.30	33.27	25.58
II (PAR_2)	21.85	76.99	27.80	42.21	24.43
III (PAR_3)	20.52	58.38	18.34	32.41	19.34
IV (PAR_4)	16.62	37.94	17.78	24.11	17.20
V (PAR_5)	17.67	51.30	16.06	28.34	16.87

The average light intensity at three moments of measurement (9.00 hrs 12.00 hrs and 16.00 hrs) was different among the groups, ranging from 24.11 to 41.21 $\mu mol.m^{-2}$. For *S.leprosula*, photosynthetic saturation occurs around 12.00 h. Therefore, it is treated separately from that for 9.00 hrs and 16.00 hrs, the latter two in one group. As a consequence, light intensity (PAR) per group was also different between $PAR_{(a+b+c)}$ and $PAR_{(a+c)}$.

The results of Duncan's Multiple Range test at a significance level of 5 % ($P < 0.05$) for the effect of light intensity on a number of growth parameters are presented in Fig. 3-25. The position of the plants indeed had a significant effect on height growth, number of leaves, total fresh weight and total dry weight. Table 3-13 and Fig. 3-25 show that the cuttings planted in PAR_1 and PAR_2 are not significantly different in height growth. PAR_1 was significantly different from PAR_3, PAR_4 and PAR_5 in number of leaves, total fresh weight and total dry weight. The largest growth responses were found with cuttings in PAR_1. The values for its growth parameters are 24 cm (ΔH), 12 (N_l), 16 g (W_{tf}), and 5 g (W_{td}).

Table 3-13 Effects of Photosynthetically Active Radiation (PAR) on various parameters of growth of *S. leprosula* cuttings during 10 months of observation. Average height growth (ΔH); number of leaves (N_l); total fresh weight (W_{tf}); total dry weight (W_{td}). a,b and c see Table 3-12.

		Mean Response			
Light intensity (a+b+c) μ mol/m^2	N	ΔH (cm)	N_l	W_{tf} (g)	W_{td} (g)
PAR_1 (33.27)	28	24.4 a	12.0 a	16.0 a	4.6 a
PAR_2 (42.21)	30	21.8 ab	9.4 b	10.3 b	3.3 b
PAR_3 (32.41)	29	17.8 b	9.6 b	9.9 b	3.1 b
PAR_4 (24.11)	29	17.5 b	8.9 b	8.3 b	2.4 b
PAR_5 (28.34)	25	18.9 b	9.2 b	8.8 b	3.0 b

Values followed by the same letter (a or b) in the same column are not significantly different at 5 % level as tested with Duncan's Multiple Range Test.

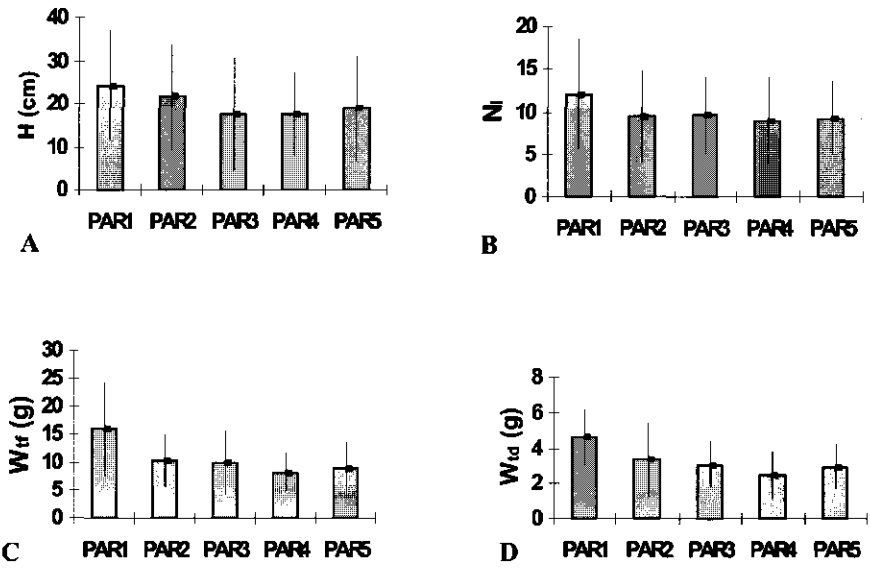


Figure. 3-25 Effect of PAR on the average value of some growth variables: (A) height growth; (B) number of leaves; (C) total fresh weight); (D) total dry weight at harvest 10 after inoculation. The length of the vartical line at the top of the bars indicates the amount due to chance at 5 %-level of significance (Duncan's Test)

Photosynthesis fixes $C0_2$ and energy in carbohydrates, which are fixed in the plant biomass. The correlation between dry weight and light intensity that logically ensues at different moments of measurement is presented in Fig. 3-26.

Fig 3-26A shows that the biomass of *S. leprosula* cuttings was correlated strongly with PAR at 9.00 hrs (r = 0.87, P = 0.01). The dry weight at 12.00 hrs and 16.00 hrs are affected moderately by the light intensity (Fig. 3-26B, r = 0.25 and P = 0.79; Fig. 3-26C, r = 0.66 and P = 0.24). When the effects of PAR at 9.00 hrs and 16.00 hrs are grouped, the dry weight of *S. leprosula* cuttings was also affected strongly by the light intensity (Figure 3-26D, r = 0.87 and P = 0.07).

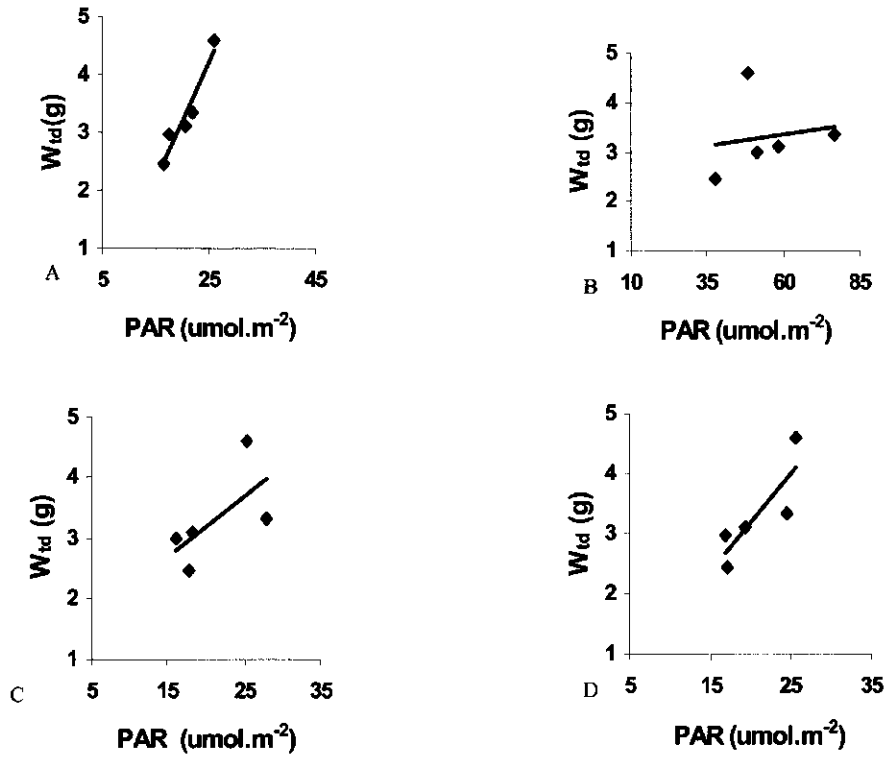


Figure 3-26 Relationship between dry biomass (W_{td}) and PAR ($\mu mol.m^{-2}$) measured weekly at three moments in time. A = PAR (9.00 hrs); B = PAR (12.00 hrs); C = PAR (16.00 hrs); D = $PAR_{(a+c)}$

During the seedling stage, *S. leprosula* is very much affected by light intensity. *S. leprosula* belongs to the shade tolerant species, most often defined as "capable of living in the shade". This was caused, in *Shorea* particularly, by a special mycorrhizal circuit between trees and seedlings (Yasman, 1995). High light intensity causes injury to the seedlings or kills them, so they are not shade-tolerant but shade-requiring. The relationship between light intensity and height growth is presented in Fig. 3-27. Fig. 3-27A and C show that the light intensity affected height growth of *S. leprosula* cuttings strongly at 9.00 hrs and 16.00 hrs with a high correlation coefficient and low incertitude. The height growth at 12.00 hrs was not affected much by light intensity. Probably, at 12.00 hrs, the light intensity was too strong to cause height growth of *S. leprosula* cuttings or even inhibited it. If the effects of light intensity at 9.00 hrs and 16.00 hrs are grouped, the height growth of *S. leprosula* cuttings was affected very strongly by light intensity with a high correlation coefficient and low incertitude (Fig. 3-27 D).

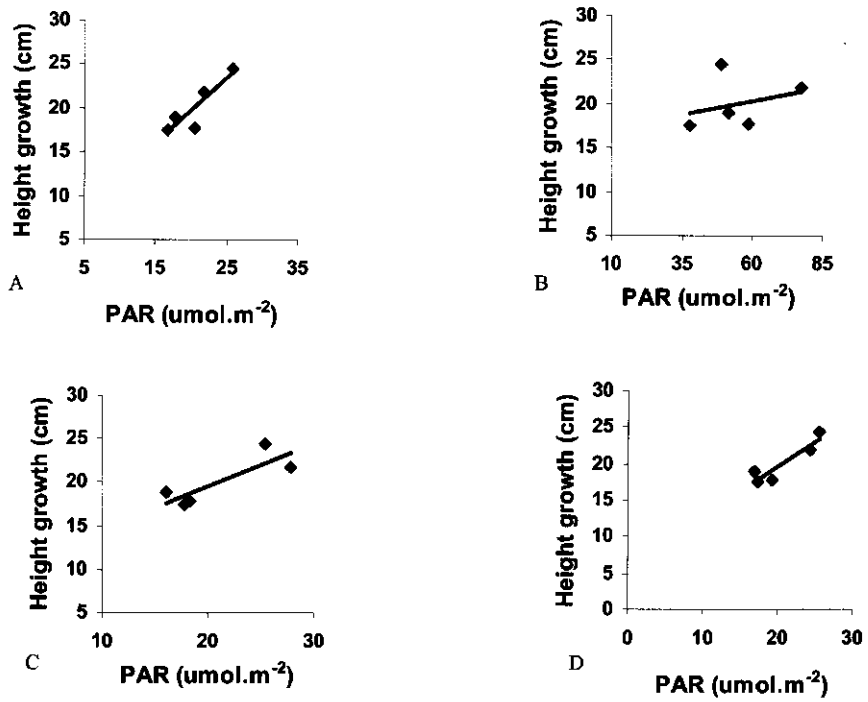


Figure 3-27 Relationship between height growth and PAR ($\mu mol.m^{-2}$) measured weekly at three moments in time. A = PAR (9.00 hrs); B = PAR (12.00 hrs); C = PAR (16.00 hrs); D = $PAR_{(a+c)}$.

B. Mycorrhizal development

Environmental factors affect mycorrhizal development. Among them are light intensity, soil heat and soil humidity.

The average soil temperature, humidity and air temperature are presented in Table 3-14. The critical soil heat for the survival and development of mycorrhizae on Dipterocarp roots is at 33° C (Noor and Smits, 1987; Yasman, 1995). The average soil temperature in the perforons was below 30° C.

Table 3-14 Average soil temperature, humidity and air temperature, measured in the perforons during 10 months of observation

	Average environmental conditions		
Block	Soil temp. (^{0}C)	Air humidity (%)	Air temperature (^{0}C)
I	26	83	31
II	29	87	33
III	29	85	33
IV	29	84	33
V	27	79	32

Table 3-15 shows by relative value that mycorrhizal roots were moderately affected by light intensity, soil heat and air temperature. The atmospheric humidity also influenced mycorrhizal roots somewhat (Fig. 3-28).

Table 3- 15 Correlation between light intensity (PAR), humidity, air temperature, soil temp erature and percentage of mycorrhizal root infection (ECM %).

Correlation	Equation	r	P
PAR (μmol.m^{-2} vs. ECM %	Y = 0.22 x + 59.31	0.54	0.34
Soil temperature vs. ECM %	Y = 0.86 x + 42.13	0.52	0.45
Atmospheric humidity vs ECM %	Y = 0.17 x + 52.04	0.17	0.76
Air temperature vs. ECM %	Y = 1.60 x + 14.39	0.53	0.36

Note: r = coefficient correlation, P = significant (probability)

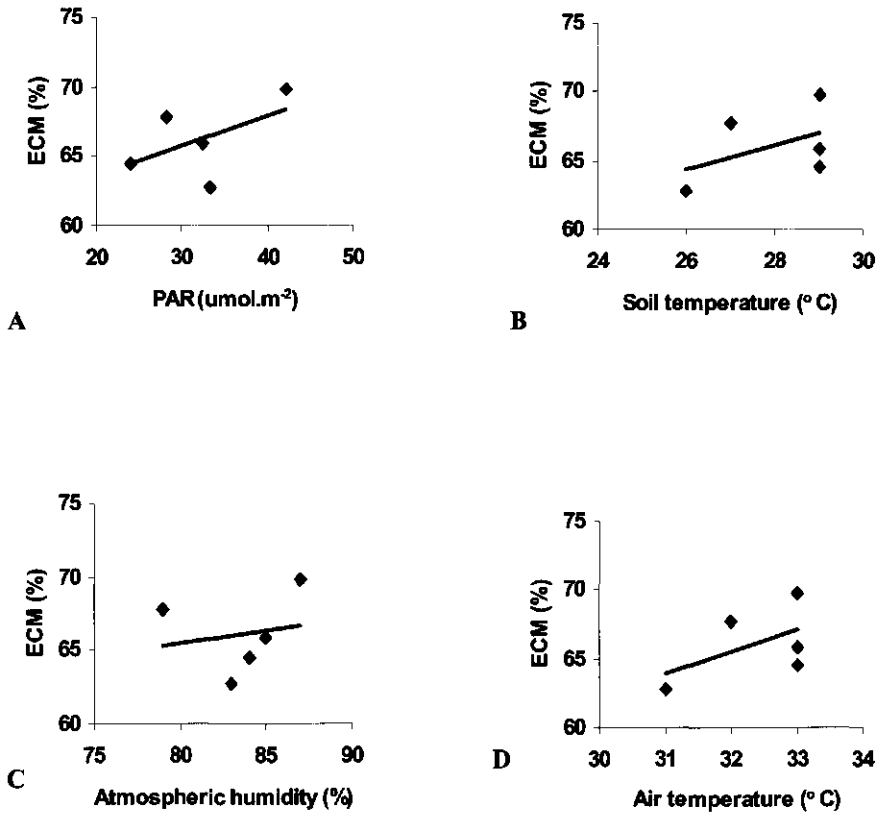


Figure 3- 28 Relationship between ECM (%) and (A) PAR; (B) soil temperature; (C) atmospheric humidity and (D) air temperature.

3.3.5 Effects of interaction between soil substrate and sterilization

A. Plant growth

Table 3-2 showed that the single factor of soil substrate influenced the growth of *S. leprosula* cuttings, where the highest growth was found in sandy substrates. Table 3-7 showed that the single factor of sterilization also influenced the growth of *S. leprosula* cuttings significantly.

Table 3-16 Interactions between soil substrate and heat treatment on various parameters of growth of *S. leprosula* cuttings, 10 months after inoculation. Average height growth (ΔH); average diameter growth (ΔD); number of leaves (N_l); leaf area (A_l); total fresh weight (W_{tf}); total dry weight (W_{td}).

		Mean response					
Treatments	N	ΔH (cm)	ΔD (mm)	N_l	A_l (cm2)	W_{tf} (g)	W_{td} (g)
Unsterilized clay (St0S1)	24	9.5 a	0.2 d	6.3 d	4.8 d	3.1 e	1.0 c
Unsterilized sandy loam (St0S2)	24	32.4 a	0.5 a	14.0 a	26.5 a	18.8 a	6. I a
Unsterilized sandy clay (St0S3)	24	23.5 b	0.3 bc	12.3 ab	18.4 bc	13.6 bc	4.2 b
Sterilized clay (St1S1)	22	11.7 d	0.1 d	5.9 d	5.6 d	5.1 de	1.0 c
Sterilized sandy loam (St1S2)	23	17.9 c	0.3 c	8.9c	16.2 c	9.0 cd	3.0 b
Sterilized sandy clay (St1S3)	24	24.7 b	0.4 b	11.2 bc	21.4 b	14.4 ab	4.4 b
Increment St0S2 vs. St0S1 (%).		241	150	122	452	506	510
Increment St0S3 vs. St0S1 (%).		147	50	95	283	339	310
Increment St1S1 vs. St0S1 (%).		23	- 50	- 6	17	65	0
Increment St1S2 vs. St0S1 (%).		88	50	41	238	190	200
Increment St1S3 vs. St0S1 (%).		160	100	78	346	365	340

Values followed by the same letter (a, b, ab, c, bc, d) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

With regard to the combination of sterilization and soil substrate, the growth of cuttings in unsterilized sandy loam was significantly higher than in all other combinations as judged by height and diameter growth, leaf area and total dry weight. The largest growth was found in cuttings that had been planted in unsterilized sandy loam. The cuttings in both unsterilized and sterilized clay did not grow very well compared with sandy loam and sandy clay. The growth of *S. leprosula* cuttings was stronger in unsterilized substrates than in sterilized substrates. The growth increment of cuttings planted in unsterilized sandy loam was about one to five times that in unsterilized clay, depending on the parameter (Table 3-16).

B. Mycorrhizal development

The mycorrhizal development was affected by soil substrate (Table 3-4). The number of mycorrhizal roots increased significantly when the *S. leprosula* cuttings had been planted in sandy clay or sandy loam. The number of mycorrhizal roots increased by 9 % and 25 %, respectively, as compared to clay. Fig. 3-18 shows that the ECM % in sterilized substrate was a little higher than in unsterilized substrate (63 % versus 61 %).

Fig. 3-29 shows the interaction between soil substrate and sterilization on the mycorrhizal development. The highest percentage of mycorrhizal roots was found in sterilized sandy clay (72 %). Autoclaving of clay increases the percentage of mycorrhizal roots from 53 % to 58 %, while in sandy loam and sandy clay it increased from 58 % to 63 % and from 66 % to 72 %. Soil sterilization increased the percentage of mycorrhizal roots in the perforons by about one tenth.

In squashes made of mycorrhizal roots in this experiment, mycorrhizal type 1, 2, 3 and 4 were all found. Statistical analyses show that mycorrhizal type 3 and 4 were significantly affected by soil substrate and sterilization (Table 3-17). The frequency of mycorrhizal type 3 and 4 in unsterilized sandy loam increased by 62 % and 110 % as compared to unsteriliuzed clay. Mycorrhizal type 3 and 4 indeed developed well in unsterilized sandy loam.

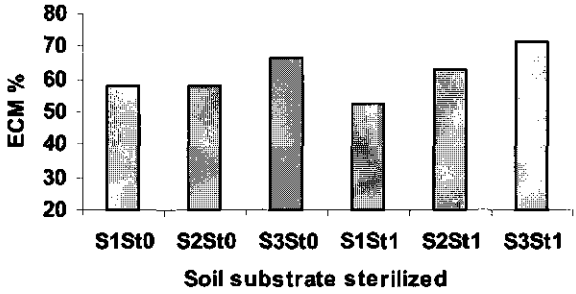


Figure 3- 29. Effect of soil substrate and sterilization on the percentage of mycorrhizal roots. S1= clay; S2= sandy loam; S3= sandy clay; St0 = unsterilized; St1= sterilized.

Table 3-17 Interactions between soil substrate and sterilization and the frequency of mycorrhizal types 1, 2, 3 and 4 in the roots of *S. leprosula* cuttings 10 months after inoculation.

		Mycorrhizal type			
Treatments	N	Type 1	Type 2	Type 3	Type 4
Unsterilized clay (S1St0)	120	1.1 a	1.3 a	1.3 a	0.1 a
Unsterilized sandy loam (S2St0	125	1.1 a	1.5 a	2.1 b	1.2 c
Unsterilized sandy clay (S3St0)	120	1.4 a	1.5 a	1.9 b	0.8 b
Sterilized clay (S1St1)	120	0.8 a	1.7 a	1.5 a	0.1 a
Sterilized loam (S2St1)	120	1.3 a	1.2 a	1.4 a	0.5 b
Sterilized sandy clay (S3St1)	125	1.3 a	1.3 a	2.0 c	0.5 b
Increment S2St0 vs. S1St0 (%)		0	15	62	110
Increment S3St0 vs. S1St0 (%)		27	15	46	70
Increment S1St1 vs. S1St0 (%)		- 27	31	10	0
Increment S2St1 vs. S1St0 (%)		18	- 8	8	40
Increment S3St1 vs. S1St0 (%)		18	- 0	54	40

Values followed by the same letter (a, ab, c) in the same column are not significantly different at 5 % level if tested with Duncan's Multiple Range Test.

C. Nutrient uptake

Fig. 3-14 showed that the nutrient uptake of N, P, K, Ca, Mg and Fe in sandy loam and sandy clay was higher than in clay substrate. In comparison, Fig. 3-19 shows that the N, P, K and Mg uptake in unsterilized substrate has increased, while the Ca and Fe uptake descended. The combination treatment of soil substrate and autoclaving is shown in Fig. 3-30. The highest N and K uptake was found in

unsterilized sandy clay (Fig. 3-30A, C – cf code S2St0). The highest Ca and Fe uptake was found in heated sandy clay (Fig. 3-30D, F - cf code S3St1). It seems that N uptake was accompanied by K uptake, while Mg and Ca uptake were accompanied by P uptake, and Fe uptake was stimulated by the Ca uptake. It is possible that there was some change in the nutrient availability due to the substrate heating (see Table 3-10).

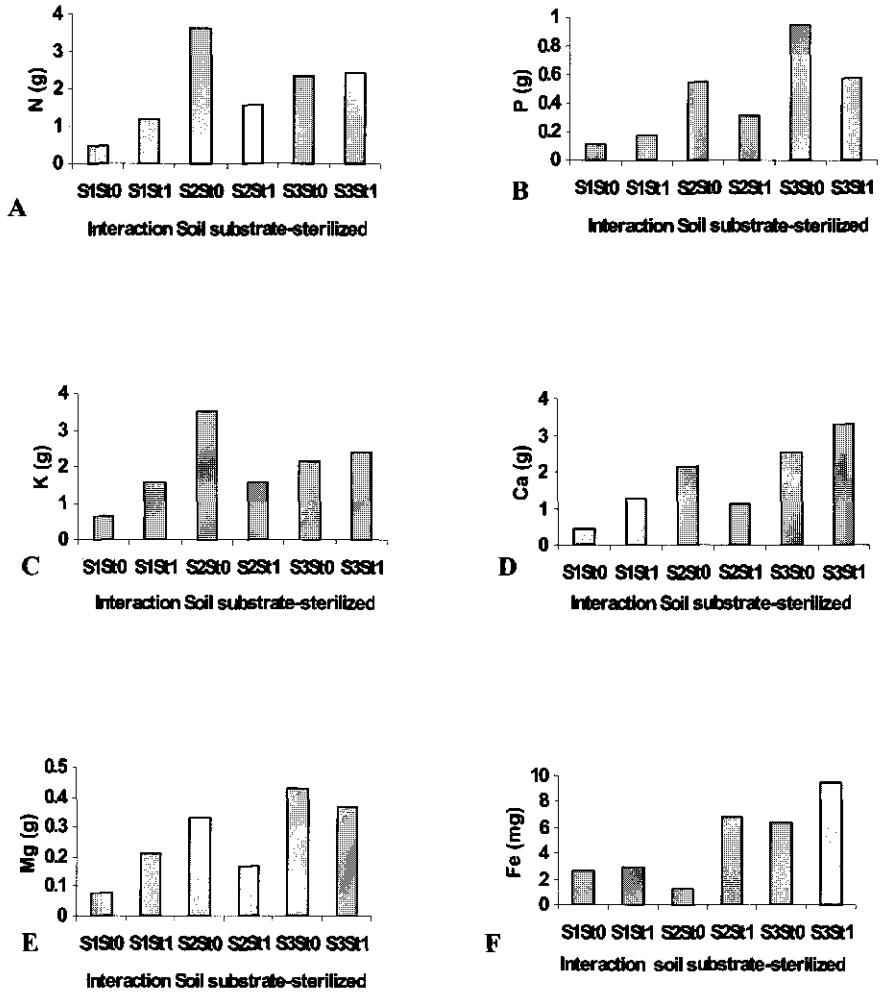


Figure 3-30 Interaction between sterilized soil substrates and nutrient uptake. (A) N-uptake; (B) P-uptake; (C) K-uptake; (D) Ca-uptake; (E) Mg-uptake and (F) Fe-uptake. S1 = clay, S2 = Sandy loam, S3 = Sandy clay, St0 = not sterilization and St1 = sterilized.

3.4 DISCUSSION

The effect of soil substrate, clay, sandy loam or sandy clay, on the growth of cuttings, mycorrhizal development and nutrient uptake is discussed below. *Shorea leprosula* cuttings were inoculated with *Amanita* sp., *Russula* sp., *Scleroderma columnare,* and a cocktail of those mycorrhizal fungi. The heating of the soil substrate by autoclaving justified some conclusions on the importance of spontaneous indigenous mycorrhizal fungi in the soil substrate.

3.4.1 Growth of *S. leprosula* cuttings

The heat of the soil by autoclaving affects the chemical properties of the soil substrate. Soil heat decreased the availability of some nutrients particularly N, Na and Al. The availability of P, K, Ca and Mg increased (Table 3-9). However, none of these correlations were highly significant. The effects of autoclaving on available soil nutrients can therefore be neglected when comparing inoculated with non-inoculated treatments. This facilitates an estimation of the importance as inoculum potential as the naturally occurring indigenous fungi.

In any successful ectomycorrhizal research program on the effects of mycorrhizal inoculation, soil heat by autoclaving is therefore important. Soil must be either chemically or physically treated to eliminate or significantly reduce populations of fungi, bacteria, nematodes, and possibly insects that could reduce the efficacy of the introduced ectomycorrhizal fungus, or cause malfunction of feeder roots (Marx and Ruehle, 1991).

According to the parameters used (see Fig. 3-6 and Fig. 3-7), the growth of *S. leprosula* cuttings was higher in sandy loam and in sandy clay than in clay (Table 3-2). An important parameter for plant growth is total dry weight (biomass). The increment of total dry weight in sandy loam and in sandy clay was 350 % and 330 % of the increment on clay (100 %). The different growth rates in different soil substrates are related to the porosity of the soil (see section 2.4.1).

Table 3-7 shows that the growth rates of *S. leprosula* cuttings were influenced by soil substrate heating. The growth response of cuttings in an unsterilized medium was higher than in a sterilized substrate. The growth increment of *S. leprosula* cuttings in a sterilized substrate was negative, from – 16 % to – 32 %, as compared with unsterilized substrate. In other words, the cuttings planted in non-autoclaved substrate grew better than in autoclaved substrate. Growth reduction in autoclaved soil was at least co-determined by change in the availability of some nutrients. Indeed, soil heat affected the growth of *S. leprosula* cuttings by decreasing the nutrient availability by 0.01 to1.35 % (Table 3-9). According to Chen et al. (1991), soil heating affects plant growth by changing chemical and physical soil properties.

Sterilization and soil substrate in interaction (Table 3-16) made that growth of *S. leprosula* cuttings was higher in unsterilized sandy loam than of in other interaction treatments. The increment of cuttings planted in unsterilized sandy loam compared with that in unsterilized clay was 241 % (ΔH), 150 % (ΔD), 122 % (N_l), 452 % (A_l), 506 % (W_{tf}) and 510 % (W_{td}). Cuttings of *S. leprosula* did not grow very well in both non-autoclaved and autoclaved clay, as compared with sandy loam and sandy clay. The lowest growth response was recorded in heated clay. The bulk density of clay increases with heat treatment. Hence, hyphae of inoculated mycorrhizal fungi can develop at the surface only. Their ability to penetrate the soil decreases significantly with higher bulk density (Nurida, 1999).

In this regard the mycorrhizal potential of indigenous fungi is very important. The development of indigenous mycorrhizal fungi was shown to be as effective as that of the inoculated mycorrhizal fungi in terms of growth and percentage of mycorrhizal roots. Most probably, contaminating mycorrhizal fungi the vector of which is air or water, also infected non-inoculated cuttings. In this experiment, some fruiting bodies of *Inocybe* sp. rose above the soil surface in the perforons during the observation period. Smits (1992) reported aggressive fungi, like *Thelophora terrestris* to be present in a dipterocarp nursery. In temperate regions, infection by common mycorrhizal nursery fungi, such as *Cenococcum geophilum* and *Thelophora terrestris* is particularly frequent (Mason, et al. 1982).

Table 3-13 and Figure 3-25 show that the largest growth of *S.leprosula* cuttings was found when they were planted in PAR_l (33 $\mu mol.m^{-2}$). The relationship between biomass and light intensity at 9.00 h in Fig. 3-26A showed a strong correlation ($r = 0.87$). However, correlation between biomass and light intensity at 12.00 h and 16.00 h was moderate, namely $r = 0.25$ and $r = 0.66$, respectively (Fig. 3-26B and 3-2C). The light intensity at 9.00 h and 16.00 h in all groups strongly affected the biomass of *S. leprosula* ($r = 0.87$; see Fig. 3-26D). The height growth at 12.00 h was not affected ($r = 0.33$) by light intensity, but at 9.00 h and 16.00 h the light intensity affected height growth very much (Figures 3-27B and 3-27D). Hence *S. leprosula* clearly is shade-tolerant, a tolerant species that does not profit from the high light intensity around noon. This points to a low photosynthetic saturation point for PAR input. The evidence (par. 3.3.4.a), showing that stronger light damage the plant shows that, when young, *S. leprosula* is not only shade-tolerant, but is shade-requiring. In terms of temperaments (see Oldeman, 1990) this is new.

3.4.2 Mycorrhizal development

Mycorrhizal development starts with spore germination, followed by mycelium growing into the soil, mycelial colonization of the root system, formation of mantle sheets and finally formation of the Hartig net. The symbiotic activities of mycorrhizae include many ecological and physiological mechanisms, all influencing the success of the symbiosis. Melin (1962) wrote that tree roots stimulate spore germination by producing exudates, which fits in with the rhizosphere-phyllosphere

diagram by Ruinen and Oldeman (1993 ex Oldeman, 2001). Especially basiodiospores of "early stage" fungi. i.e. *Thelophora* sp., *Hebeloma* sp. and *Scleroderma* sp. respond to seedling roots. Infection of seedlings by " late stage" fungi can occur from mycelial strands in the forest (Fleming, 1983).

The number of root tips and the percentage of mycorrhizal roots of *S. leprosula* cuttings were higher in sandy loam and sandy clay than in clay. The increment of mycorrhizal roots in sandy loam and in sandy clay was 9 % and 25 % higher than in clay. The percentages of mycorrhizal roots and number of root tips were related to physical and chemical properties of the substrate (see Appendix 7 and Table 3-3).

Intense solar radiation heating up the soil surface influences mycelial growth (Smits, 1994). Table 3-15 shows that effective light intensity was not correlated to the percentage of mycorrhizal roots (r = 0.54). The surface heating therefore was not transmitted deeper down. On this basis, it may be assumed that soil temperature was moderately correlated with the percentage of mycorrhizal roots and indeed, r = 052. The correlation between relative atmospheric humidity and percentage of mycorrhizal roots was very low (r = 0.17). Correlation with air temperature was also moderate (r = 0.53). Climatic conditions as a whole were correlated moderately or not at all.

Four types of mycorrhizal colonization were found, resulting in four morphological mycorrhizal types shown in Figs. 3-8 and 3-9. The mycorrhizal roots were identified following Mason and Ingleby (1997). Type 1 was brown-black, where type 2 was brown. Type 3 and 4 had exactly the same silver-white colour. Mycorrhizal type 3 dominated the mycorrhizal development after inoculation with the cocktail of 3 fungi. In other words, type 3 was able to use the environment more thoroughly than type 1 and type 2.

Table 3-5 and Figure 3-10 show detailed anatomical differences among the four types. Following the method of Mason and Ingleby (1997), type 2 was shown to be induced by *Thelophora* sp. Type 3 and Type 4 were induced by *Amanita* sp1 and *Amanita* sp 2, Type 1 was difficult to identify. On the basis of a provisional determination by Noor (pers. comm.), it must have been induced by *Russula* sp.

The early fungus *Thelophora* sp was not inoculated in the experiment, but it was nonetheless found in mycorrhizal roots. The species hence was able to colonize the root system of *Shorea leprosula* spontaneously. It came perhaps from the unsterilized soil substrate (indigenous fungi) or was airborne. On the other hand, the pattern of mycorrhizal development for *Scleroderma columnare* and *Russula* sp. were not found. It is possible that the spores of *S. columnare* and *Russula* sp. did not germinate, and were supplanted by other indigenous mycorrhizal fungi such as *Thelophora* sp., in accordance with Smits (1992). Lu et al. (1998) obtained 22 % mycorrhizal roots with spore inocula of *Scleroderma* sp., while inoculation with

spores of *Russula* sp. did not form any ectomycorrhizae. It is possible that in this experiment *Russula* sp. and *S. columnare* spores were replaced by propagules of the aggressive mycorrhizal fungus of *Thelophora* sp. *Amanita* sp also seems to be an aggressive mycorrhizal fungus, since mycorrhizal structures of *Amanita* sp1 and *Amanita* sp2 were found consistently. According to the squash test, mycorrhizal type 3 and 4 belong to the same species of *Amanita* (see par. 4.2. "Ageing"). This would cast a new on ecological temperament of this species or perhaps this genus.

The mycorrhizal type was affected by the kind of soil substrate. The number of root tips with mycorrhizal types 1, 2, 3 and 4 was higher in sandy loam and sandy clay than in clay (see Fig. 3-11). In sandy loam, mycorrhizal type 4 more frequently than type 1, 2 and 3. Appendix 7 and Fig. 3-13 show that the sand fraction in sandy clay is higher than that for other soil substrates and correlation between percentage of sand fraction and percentage of mycorrhizal roots (number of root tips) was very strong $r = 0.95$ and $r = 0.88$ respectively. Probably, mycorrhizal type 3 needs much more aeration.

In general, unsterlized soil contains many ectomycorrhizal fungi. However, among the mycorrhizae observed, type 3 and type 4 were more frequent in sterilized soil than in unsterilized soil. The highest percentage of mycorrhizal roots and number of root tips of *S. leprosula* cuttings was obtained after inoculation with *Russula* sp (69 %); see Table 3-10.

3.4.3 Nutrient uptake

Nutrient uptake of N, P, K, Ca, Mg and Fe by *S. leprosula* cuttings was affected by the soil substrate. Fig. 3-11 shows the nutrient uptake of these elements in sandy loam and sandy clay to be higher than in clay. The nutrient uptake from the soil substrate, due to mycorrhizal development, as represented by the percentage of mycorrhizal roots was higher in sandy loam and sandy clay than in clay (Table 3-4). Fig. 3-13A shows that the correlation between percentage of mycorrhizal roots and percentage of sand fraction Table 3-3 was very strong ($r = 0.95$). Clearly, nutrient uptake in the soil is strongly determined by mycorrhizal development (see 3.4.2).

The beneficial role of mycorrhizae for both symbionts, particularly with respect to the uptake of phosphorus, appears to be related to the nutrient depletion zone that surrounds the roots. The extent of the depletion zone varies from one nutrient element to another, depending on the solubility and mobility of the element in the soil solution (Hopkins, 1995, Kahn, 1982 ex Oldeman, 1990, 2001). The depletion zone for nitrogen, for example, extends some distance from the root because nitrate is readily soluble and highly mobile. Phosphorus, on the other hand, is less soluble and relatively immobile in soils, so the depletion zone for phosphorus is smaller. A farther outreach of extramatrical hyphae enhances the uptake of phosphorus, nitrogen and other nutrients.

P uptake is also regulated by soil pH, Al and Fe. If soil pH is less than 6.8, the predominant form of phosphorus is a monovalent orthophosphorus anion ($H_2PO_4^-$). Plant roots readily absorb the orthophosphate. Between pH 6.8 and pH 7.2, the predominant form is HPO_4^{-2}, which is readily absorbed. In alkaline soil (pH greater than 7.2), phosphorus is virtually unavailable for uptake by plants (Hopkins, 1995).

In this experiment the pH of clay, sandy loam and sandy clay was 4.8, 5.0 and 5.1 respectively. Most of the phosphorus in the soil hence was in the form of orthophosphate. In neutral pH, the Fe, Al, Ca and Mg precipitate the phosphorus, in the form of insoluble phosphate. Extramatrical hyphae of mycorrhizal fungi are able to "digest" the insoluble phosphate by producing an enzyme, acid-basic phosphatase. The orthophosphate is transported through the hyphae and the sheath of ectomycorrhizae and bound to inorganic polyphosphates (Harley, 1989). It was also shown that phosphate stored in the sheath is mobilized and transported to the host by a mechanism depending on oxygen supply, heat and the absence of a metabolic inhibitor.

In the present experiment, phosphorus uptake in sandy clay was higher than in clay. It is plausible that the increase of P uptake is related to either soil porosity, or increased oxygen supply. P uptake in clay was lower than in other substrates. Table 3-9 shows that the Al contents were 5 times higher in clay than in either sandy loam or sandy clay. The Al present favored precipitation of the phosphorus in clay. According to Cumming and Weintein (1990), the fungal symbiont modulates ionic relations in the rhizosphere, and so reduces Al-P precipitation reactions, Al uptake, and subsequent exposure of root and shoot tissue to Al.

The importance of Fe in plant nutrition is highlighted by the strategy of plants when having to develop under iron stress (Marschner, 1986). A plant is able to exudate caffeic acid around the root system. Acidification of the rhizosphere encourages chelation of Fe^{3+} with caffeic acid, which then moves to the root surface where the iron is reduced to Fe^{2+} at the plasma membrane. Reduction to Fe^{2+} causes the caffeic acid to release the iron, which is immediately taken up by the plant, before it has the opportunity to form insoluble precipitates. Fig. 3-14 shows that the Fe uptake by *S. leprosula* cuttings in sandy loam and sandy clay was increased, because sandy clay and sandy loam were more aerated than clay. The production by plants of caffeic acid is affected by oxygen supply, because caffeine is derived from xanthine, this being an oxigenated purine (cf. Lawrence, 1996). The ability of caffeic acid to transform the Fe^{3+} to Fe^{2+} in sandy clay was higher than in sandy loam and clay, thus explaining the higher Fe uptake in that soil.

Nitrogen taken up by plants is translocated in the form of NO_3^-. Aluminum toxicity is suspected to be the cause of deficient nitrification in acid soils. Soil moisture and oxygen supply go hand-in-hand and little nitrification occurs in water-saturated soils because of the limited oxygen supply (Hopkins, 1995).

Potassium (K) is absorbed by the plant in the form of K_2O. Fig. 3-14 shows that more K was taken up in sandy loam and sandy clay than in clay, because sandy loam and sandy clay are more porous than clay. The oxygen supply was therefore higher than in clay. Also K uptake in sandy loam and sandy clay was higher than in clay. A higher sand fraction certainly facilitates oxygen supply by increasing soil porosity.

Magnesium is required in large amounts by the plant as a building block of chlorophyll molecules. Plants absorb it as a divalent cation (Mg^{2+}). Fig. 3-14 shows that Mg uptake by *S. leprosula* cuttings was higher in sandy clay and sandy loam than in clay. Mg uptake in sandy loam was practically the double of that in clay. This heightened Mg uptake was also reflected by the large, dark green leaves of *S. leprosula* cuttings planted in sandy loam and sandy clay. *S. leprosula* in clay, on the contrary, showed small, thick, light green leaves. Dark green, large leaves contain more chloroplasts than light green, small leaves, and more chloroplasts contain more magnesium (Fig 3-7D).

Calcium uptake by *S. leprosula* cuttings in sandy clay and sandy loam was higher than in clay. Ca affects the soil pH. As said above, pH in clay, sandy loam and sandy clay was 4.8, 5.0 and 5.1 respectively. This pH affected the solubility of some nutrients such as P and Fe. Plants take up calcium as a divalent cation (Ca^{2+}). It is involved in protein phosphorylation. Ca^{2+} may be an important factor in regulating the activities of a number of enzymes (Hopkins, 1995).

On the one hand, Fig. 3-19 shows that N-, P- and Mg-uptake by *S. leprosula* cuttings were higher in unsterilized soil than in sterilized soil. On the other hand, Ca- and Fe-uptake were lower in unsterilized than sterilized soil. Moreover, Table 3-9 shows that the process of heating the soil induces a growth reduction in those cuttings. Reduced nutrient uptake in heated soil is almost certainly due to lowered availability of certain nutrients. Combining the nature of the substrates and the heat treatment, the data show that N^+ and K^+ uptake were highest in unsterilized sandy clay. Accumulation of Ca in the rhizosphere and rhizoplane occurs when input in the root zone of Ca by mass flow exceeds plant uptake of Ca. In contrast, P and K usually are present in the soil solution in low concentrations. Soil depletion in the rhizosphere is a typical feature and may lead, for example, to release of K by weathering of the non-exchangeable fraction of clay minerals (Marschner, 1986).

The biomass of the cuttings was found to increase with nitrogen uptake up to a limit of 40 mg/ cutting. Lower values of biomass were found at uptakes of 5 to 7 mg/cutting P, 40 mg /cutting K, 5 mg/cutting Mg and 20 mg/cutting Fe. Mycorrhization of the root affects the value of the biomass per cutting. The mechanisms explaining this ecological and metabolic fact we examined above, and will be considered more closely in the feedback diagram of the sap-stream in the general discussion (Fig. 4-1). In its turn, the general environment influences the

development of mycorrhizal associations. Factors involved are, for instance, soil fertility, nitrogen and phosphorus availability, soil aeration, and the light climate.

3.5 CONCLUSIONS

As whole, the data support the modulation of nutrient uptake by mycorrhizal symbiosis; in particular by hyphae bridging the distance of the depletion zone, the roots and the soil beyond the immediate rhizosphere and modifying the chemical form, i.e. the availability of nutrients by exudation mechanisms.

The growth of cuttings, the mycorrhizal development and the nutrient uptake were higher in sandy loam and sandy clay. One known case supports aeration playing an important part in this phenomenon, i.e. the oxygen sink constituted by root synthesis of caffeine, this being an oxygenated purine. Caffeine then plays a part in iron uptake.

The biological regulation of nutrient uptake is supported by the fact that soil heating in an autoclave at 121° C during two hours, that killed most soil organisms, also affected the nutrient availability to the plants.

Four morphological types of mycorrhizal complexes were found. They belong to *Telophora* sp., *Amanita* sp1, *Amanita* sp.2, and one unidentified species that might belong to the genus *Russula*. Moreover, indigenous fungi that "contaminated" the experiment by arriving borne by air or by soil water, did not negatively affect the growth of *S. leprosula* cuttings. The proportions among the species differed according to the treatment. This is ascribed to a varying efficacy of the use soil of resources among the fungus species, or a varying degree of "aggressivity". The concept of "aggressivity" requires more study to define it closely enough. As it is, it has no very precise biological meaning.

The growth performance of the cuttings is influenced by their photosynthetic capacity, which is saturated during a period around noon. The variation of photosynthetic response at low light intensity (also see Chapter 2) was surprising and had not been included in the original research proposal. It is explains biomass variations and will be considered in more depth in feedback diagram of the sap-stream in general conclusions (Fig 4-1).

The above biological conclusions lead to a recommendation to use unautoclaved sandy loam or sandy clay as the preferred substrate in nursery practice.

CHAPTER 4
GENERAL DISCUSSION AND CONCLUSIONS

4.1 THE ROLE OF SOME ENVIRONMENTAL FACTORS IN *SHOREA LEPROSULA* GROWTH AND DEVELOPMENT

Growth and differentiation, resulting in development are part of the life cycle of every plant. Growth is a term related to quantifiable changes in volume and mass, while differentiation is a term referring to other differences that arise among cells, tissues and organs at a higher organization level (Oldeman, 1990; Hopkins, 1995).

Important environmental factors for the mycorrhizal development such as light intensity, soil heat, soil fertility, pH, relative humidity, nutrients and soil substrate were discussed in Chapters 2 and 3. In these experiments soil substrate, mycorrhizal inoculation, NPK fertilization, and light intensity affected the growth of *S. leprosula* cuttings.

Physical and chemical soil properties affected mycorrhizal development and growth in the root system of *Shorea leprosula* cuttings. In this experiment, cuttings in sandy loam and sandy clay showed a stronger growth than in clay, It is assumed that this is due to a high percentage of sand providing an oxygen supply and water potential, more apt to further development and survival of mycorrhizae. This confirms the findings by Oldeman and Iriansyah (1993) that symbiosis and root growth depend on an adequate oxygen supply.

A sufficient flow of carbohydrates from the host plant is well known to be required to maintain long-term mycorrhizal symbiosis (e.g. Harley, 1989). For this flow to exist, carbon bound by photosynthesis must exceed carbon expelled by respiration. In graphs of photosynthesis and respiration against light intensity, this happens after the compensation point, where both CO_2 flows are equal. The results of the present study showed that photosynthesis peaks at quite a low light intensity in cuttings of *S. leprosula* (see 2.3.1.1). The species hence is shade-tolerant, even shade–requiring, which is a tree temperament unmentioned in the literature consulted (e.g. Oldeman, 1990; Oldeman and van Dijik, 1991; Rossignol et al., 1998). Indeed, height growth of these cuttings was stronger in controlled conditions under low light intensity than in semi-controlled conditions under higher light intensity. When the light doubled, growth was retarded. Photosynthesis peaked at about 12 $\mu mol.m^{-2}$. This is borne out by our experiments showing that photosynthesis is maximal at 9.00 hours and 16.00 hours and that the saturation point is reached or even exceeded around noon.

Fitter and Hay (1981) as well as Land and Gower (1997) wrote that plants growing under low light intensity adapt their physiological processes by modifying the transpiration rate or increasing the efficiency of the photosynthetic process. In

addition, Scholes et al. (1997) stated that a higher light intensity is able to cause photoinhibition, dissipation of excitation energy and the transition to a phase of relaxation in the shoot of dipterocarp seedlings, especially *Dryobalanops lanceolata, Shorea leprosula, Hopea nervosa* and *Vatica oblongifolia.*

In the experimental inoculation of *S. leprosula* cuttings with spores of *Amanita* sp, *Russula* sp, *Scleroderma columnare* and a cocktail of these fungi, the highest growth of the cuttings and the highest percentage of mycorrhizal roots were obtained by applying a cocktail of fungi. It is plausible that at least a few mycorrhizal fungi in the cocktail of fungi have a specific role in promoting the growth of *S. leprosula* cuttings, as predicted by Smits (1982). The results in Chapter 2 (Fig. 2-23E) confirm this role for *Amanita* sp in relation to Mg absorption.

Supriyanto (1999) reported an important growth stimulation of *S. leprosula* seedlings by inoculation with *Scleroderma columnare, Amanita umbronata,* and *Discomyces* sp., while inoculation with *Scleroderma columnare, Russula* sp. and *Laccaria lacata* did not promote growth. Indeed, the results of a mycorrhizal dependency test between *Shorea leprosula* and each of a series of mycorrhizal fungal species varied greatly.

The movements, accumulations and biochemical processing of soil minerals are part of a physiological network with multiple feedbacks. Many other factors, such as light, water, heat, acidity and micro-life are playing different parts in such a network, as shown in sap-stream diagrams (see Oldeman 1974, 1990 pp. 67-83). The significant effect of NPK fertilization in one particular dosage under one particular kind of partial experimental control therefore can not be explained without further research, and for the moment NPK fertilization can not be applied with predictable results in dipterocarp forestry practice.

In Chapter 3 it was shown that the process of heating soil substrate by autoclaving at 121° C for two hours decreased the availability of some nutrients, such as P, K and Mg, but not Ca and Fe. The nutrient uptake by cuttings of *S. leprosula* planted in sandy loam and in sandy clay was higher than in clay. There are elements of proof, particular in Fe absorption physiology, that different amounts of the nutrient uptake by *S. leprosula* cuttings are due to higher porosity and thereby better aeration of the first two soils. This in turn provides for better mycorrhizal development and thus for enhanced nutrient uptake via mycorrhizae in naturally stimulating feedback loop.

4.2 AGEING OF ECTOMYCORRHIZAL COMPLEXES

Mycorrhizal development was influenced by the physiological age of the host plant, physical and chemical soil properties, nutrient stress and ecological change. In Chapter 3 the frequency of mycorrhizal type 3 (*Amanita* sp1) was shown to be

higher than that of mycorrhizal types type 1, 2 and 4 (see Fig. 3-11). The squash test says, however, that type 3 and 4 belong to the same species of *Amanita* (par. 3.3.1.b).

This experiment showed on the one hand that the relationship between the percentage of mycorrhizal roots (ECM %) and the nutrient uptake was not important, but that certain species of mycorrhizal fungi did have an effect on the uptake of nutrient, sometimes specific nutrient.

Physiological ageing of mycorrhizal hyphae is known to exist. Irawan (1997) showed that *Cenococcum geophyllum* changed colour from black to dark grey, and the colour of the hyphae also changed from black to dark pinkish at high concentrations of N or fungicide. Wulandari (1999) observed a colour change in cultures of *Scleroderma sinnamariense*, from pale to dark yellow, turning to dark brown when the hyphae died. Moreover, all observations indicate that the physiological state of the host plant influenced the physiological of ageing of mycorrhizae. In our experiments, observations on mycorrhizal roots showed different of mycorrhizal hyphae (Table 3-5 and Fig. 3-10). If indeed the *Amanita* fungi forming type 3 and 4 belong to one species, this would be a spectacular example of polymorphism. Moreover, it is known that behaviour under stress is quite similar to behaviour ageing plants (Oldeman, 1990; Rossignol et al., 1998). The comportment of type 3 and 4 after autoclaving support this (par. 3.3.2.b and 3.3.5.b), if soil heating diminished stress by grazers on mycorrhizae (e.g. nematodes) and other organism, such as insect or bacteria.

In the present experiment, several inoculated fungi formed a Hartig net and a mantle. The thickness and number of cells of the mantle is species- dependent. The highest number of layers was found in *Amanita* sp., followed by the cocktail of fungi, *S. columnare* and *Russula* sp. The mantle layer probably plays a role in the defence of roots against pathogenic microbes (Smits, 1994). Mantle thickness also was correlated with the percentage of sand in the substrate, i.e. with air and water in the soil pores.

Sterilization of the soil substrate by autoclaving was only partial, because some bacteria in particular can survive harsh treatment. However, most populations of both pathogenic and beneficial microbes, such as mycorrhizal fungi, are reduced by heat. In the present experiment, the general mycorrhizal contaminant *Thelephora terrestris* became common and raised the growth performance of cuttings. This emphasizes the importance of such contaminant species in explaining the behaviour of young plants in greenhouses and nurseries.

Moldenke et al. (1994) stresses the fact, that microorganisms fix nutrients at certain places in the soil. The shift in nutrients in our autoclaving experiment (3.3.2) fits in with this image. The resulting decreased availability of N, P, K and Mg caused

stunted growth of the cuttings, with small, thin, pale green leaves and chlorosis. However, the treatment also caused increase in Ca and Fe. In the form of Fe^{3+}, iron is toxic. Mycorrhizae can transform Fe^{3+} in Fe^{2+} and translocate this to the phytobiont (Hopkins, 1995). It was shown above (par. 3.4.3) that iron processing is a very delicate process, dependent also on aeration because of the involvement of caffeine produced by the phytobiont.

Chapter 3 (discussion) showed that caffeine, belonging to the group of nitrogenous purines, energy-rich metabolic compounds (Lawrence, 1996), is part of the explanation of the potential of roots to profit from aeration by having more oxygen to burn. There also was a link with dynamics of phosphorus, which a key atom in energy carrying phospahate such ATP and ADP. The data of present experimental study suggest that iron uptake and transport are part of, or determined by these complex metabolic dynamics.

Therefore it would important in the future to study these iron flows and processes closely, with the objective to obtain parametric values for these and other organic soil processes. No other element than Fe appears to be so ecologically sensitive in the experiments.

The soil pH could not have been raised much by such small increases of calcium as found after autoclaving. Acidity therefore can be excluded as an explanation for the shifts observed in the amounts of nitrogen, phosphorus, potassium and iron.

4.3 OVERVIEW OF THE EFFECTS OF ENVIRONMENTAL INPUTS ON THE GROWTH OF *SHOREA LEPROSULA*

Every producing system depends on inputs originating from the environment. The relative atmospheric humidity was higher under controlled conditions than under semi-controlled conditions, so the water budget of the plants growing under these two conditions was influenced differently. The output in terms of total dry weight of the cuttings under controlled conditions was also higher than under semi-controlled conditions. According to Kentaro et al. (1996), the water supply from roots to leaves in *Shorea leprosula* is higher than in *S. seminis* and *S. pauciflora.*

A higher water supply causes a higher turnover of the sap-stream in the whole plant. The motor of the sap-stream is transpiration by leaves (Zimmermann (1983). Our data and the results of Kentaro et al. (1996) therefore point to a strong metabolism, including photosynthesis and able to handle relatively large amounts of water.

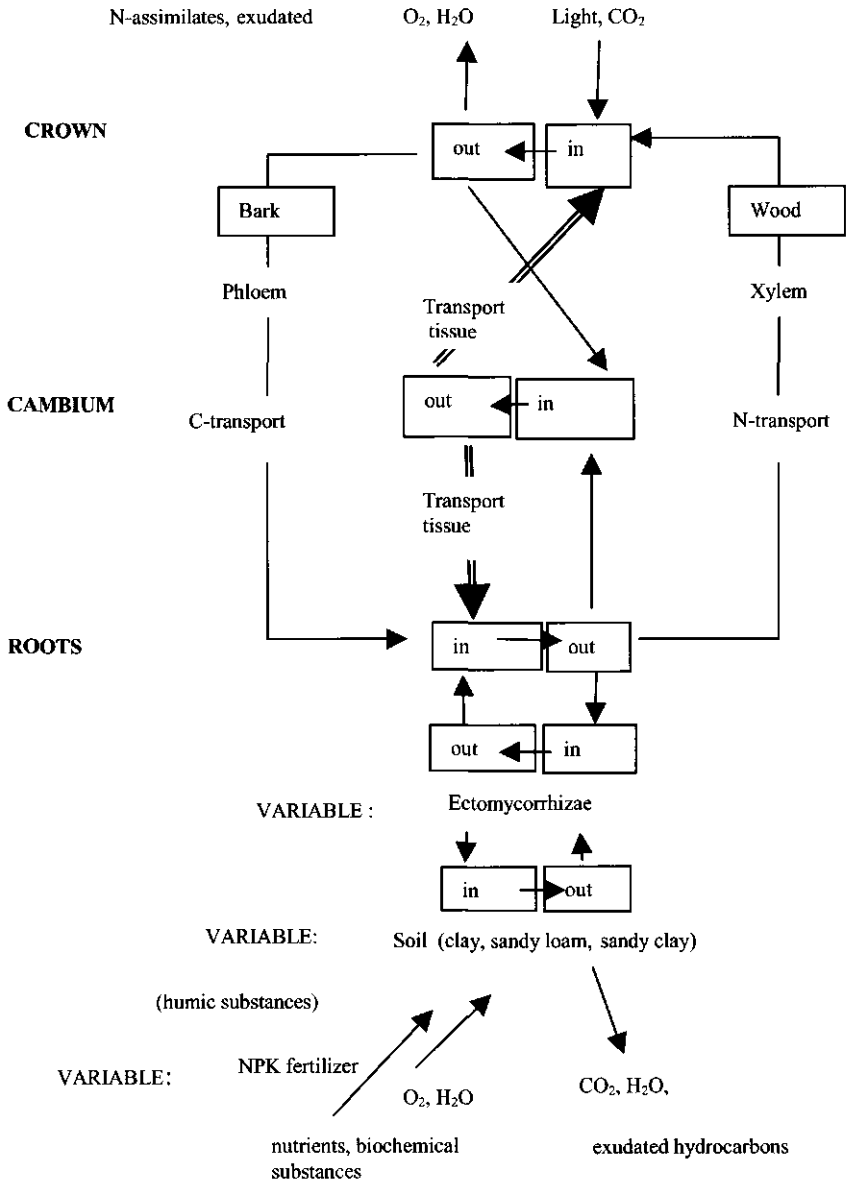


Figure 4-1 The sapstream of *Shorea leprosula*. Humic substances between brackets: not present in experiment. Inspired by Oldeman (1990, p 76). Note that soil and ectomycorrhizae are shown here as "filters" determining the inputs into the tree roots.

Stronger photosynthesis points to higher production of biomass, i.e. also to higher dry matter production.

The importance of mycorrhizae for plant growth has been illustrated in Fig. 4-1 as follow:

Mycorrhizae serve as an intermediary between the plant roots and the soil. The fungus obtains water and mineral nutrients by means of hyphae in the soil and shares them with the plant root by means of intercellular hyphae.

In exchange it receives sugars from the host plant. The sugars are produced in the tree crown by the photosynthesizing leaf mass. The latter process depends on inputs of water and mineral nutrients coming from below (soil, roots, fungi send up, water and nutrients) and CO_2 and light from above (air, sunlight).

This explains the importance of the light input for mycorrhizal development, boosted through feedback. Being functional in the input of water and bound nutrients to the tree, mycorrhizae also supply vitamins, hormones and other essential organic nitrogen compounds to the phytobiont, where they are carried everywhere by the sap-stream.

Vessels and tubes, built dry matter, contain sap, i.e. water. Dry weight represents structure, whereas fresh weight represents structures plus the amount of water they contain. This is correlated with environmental humidity (Fig. 2-6E, F). The structures are part of the architecture of the plant, determining the distribution of the sap-stream inside the plant body. Plant architecture is built by meristem (Hallé et al., 1978), which receive their energy from sunlight by photosynthesis.

In this way, the hydric dynamics of the sap-stream are linked to photosynthesis and input of solar radiation. In the beginning of the present discussion, it was already shown that photosynthesis of cuttings peaks at a light intensity of 12 $\mu mol.m^{-2}$, so that the young *S. leprosula* is shade-requiring. The sap-stream diagram (Fig. 4-1) explains this fact by a balance use of inputs, in which photosynthetic overproduction would damage the hydric system. In other terms, the input of water is a limiting factor. This input is linked to increased inputs of solutions through mycorrhizae, another element in the balance of production. The data from Yasman (1995) support this, although this author did not explicitly place them in context of the sap-stream.

Input from sandy clay and sandy loam through the mycorrhizae into the sap-stream was higher than from clay. This is explained by the higher oxygen supply, which favoured mycorrhizal development and survival and boosted metabolism, i.e. respiration. The input of NPK fertilizer in the sap-stream did not effect the growth of the cuttings and mycorrhizal development.

The link between water availability and size and quality of the soil pores may also influence the growth of mycorrhizal hyphae; more water, easier passage through the pores (Van der Wal, pers.comm., 2002).

The effect on the sap-stream of inoculation with a cocktail of mycorrhizal fungi is a higher number of leaves and higher total fresh and dry weight. The sap-stream in this case hence stimulates photosynthesis (leaf number), because the structural mass increases (dry weight). The difference between dry and fresh weight indicates higher water content and thus an increased sap-stream, as we saw.

The right environmental conditions indeed are important for the mycorrhizal development as well as for the growth of *S. leprosula* cuttings in the nursery. Mycorrhizal development and growth affected the combination treatment between controlled conditions and sandy loam or sandy clay. Mycorrhizal inoculation with a cocktail of fungi stimulated the growth of *S. leprosula* cuttings. The nutrient uptake of N, P, K, Ca, Mg and Fe was influenced by the development of mycorrhizae, which developed stronger in sandy loam or sandy clay than in clay.

The percentage of mycorrhizal roots increased with light intensity, as long as the soil temperature soil did not rise above 30° C. The feedback diagram (Fig.4-1) shows how this works by the production and distribution of photosynthetic sugars, which is different according to light inputs and sugar outputs.

4.4 PRACTICAL APPLICATION OF THE PRESENT FINDINGS IN INDONESIAN SILVICULTURE

From mere knowledge of the flowering and fruiting patterns in the dipterocarp population, it is very difficult to predict the stocks of plants in the nursery. Indeed, the pattern of flowering and fruiting of dipterocarp species is irregular (Burgess, 1972). According to Smits (1994) the production of stock plants of dipterocarps by vegetative propagation provides higher survival chances and a better quality in stock plants. The risk of pests and diseases is low, the quality is relatively homogeneous, and vegetative propagation is also a way to overcome the problem of an irregular planting stock supply from seeds or wildlings due to mast flowering and genetic diversity.

To obtain healthy and vigorous seedlings of a high quality, growth factors must be considered. Among these are soil, compatible mycorrhizal fungi, and if appropriate, fertilizers. The choice of the appropriate technology fully depends on the soil composition, the species of trees and fungi, and available materials in the nursery. In terms of application, the nursery managers prefer the simplest technology, low cost and optimizing growth for silviculture by using strong planting stock.

The technique that has been developed for the production of stock plants of dipterocarp species for vegetative propagation consists of taking cuttings of juvenile orthotropic stems of dipterocarps and treat them for rooting with an auxine hormone in either solid or aqueous media. In the present experiments, cuttings from hedge orchards were used, treated with Rootone F at a dosage of 5 mg/cutting.

Important elements for a successful vegetative propagation of dipterocarp from cuttings are a high atmospheric humidity maintained during the rooting process, the appropriate technique of taking cuttings from hedge orchards, the right dosage of hormones, the proper kind of medium (solid media or aqueous media), the optimal age of plant material and light reduce to suitable intensities. As already mentioned in the introduction (section 1.1), the juvenility of cuttings can be assessed by various parameters, viz. leaf size, size ratio (length to width, shape of leaf), presence of a drips point, presence of green glands in the leaf, leaf thickness and the presence of a thick cuticula. This method of producing cuttings has been extensively described in various publications (Leppe, 1995; Smits et al., 1990) and in a manual by Yasman and Smits (1988)

The overall survival cuttings are slightly higher when rooted in solid media than in aqueous media. Rooting percentages differ slightly between the two methods, depending upon the tree species considered. Some species developed roots faster in aqueous media (*Hopea rudiformis, Shorea ovalis, S. balangeran, S. cf. polyandra),* others faster in sand (*S. lamellata*) or vermiculite. *S. leprosula* grows justs as well in aqueous media as in vermiculite (Omon, 1994; Tolkamp and Aldrianto, 1996).

The success rate of the method, from the moment the cuttings are rooted and ready to be planted in pot in the nursery till the moment they are ready to be planted in the field, varies between species, but amounts to an average of some 60 to 70 % (Aldrianto et al., 2001).

The success of transplanting cuttings to the field also depends on whether or not they have obtained compatible mycorrhizal fungi in the nursery. The techniques for inoculation with mycorrhizal fungi that can be used are the use of either spore inocula, vegetative inocula or soil inocula. The method of inoculation using soil collected from underneath or nearby the mother tree is practical, often used, and applicable in large-scale dipterocarp nurseries (Yasman, 1995).

Smits (1992) stated that the use of soil inocula encounters the following practical problems.

1. The method is expensive and bulky. More experiments on selecting the appropriate soil are needed.
2. The potential of mycorrhizal fungi and their species is most often unknown.
3. The risk of pathogenic microorganisms infecting the nursery is quite high.

4. The viability of the soil inoculum has decreased drastically after 8 days of storage.
5. It is quite difficult to use this technique on a large scale.

Considering these difficulties, a new inoculation technique using spores or mycelia is needed. The viability of some spores is high enough and can sometimes be prolonged to one year. A mixed inoculum would be preferable, as was also shown by the experiments in these pages.

For the acquisition of soil inoculum, topsoil is removed down to a depth of between 10 and 15 cm, denuding the forest land over larger surfaces. Improvement of the technology for producing soil inoculum in large quantities and of good quality should be studied and monitored continuously. Inoculation using spores as inoculum is the easiest way to inoculate the seedling, but spore production is limited by seasonal conditions (Brundrett, 1998 and Supriyanto, 1999). In our experiments it was shown that inoculation with a cocktail of fungal spores gave a higher percentage of root infection and better growth of *S. leprosula* cuttings than inoculation with spores of a single species. Therefore, such cocktails should be used in the nursery and further developed as to their fungal species composition and viability.

Another factors in successful mycorrhizal survival and development is the growing medium. In our experiments the medium was forest soil, and it was shown that the use of sandy loam and sandy clay, containing a sand fraction of between 50 % to 70 % was more suited to mycorrhizal survival and development than clay, poor in sand. The use of NPK fertilizer in our experiments did not affect the growth and mycorrhizal development. In view of the very complex set of interactions between one tree species, more than four fungal species, various soil and different nutrient application, no general conclusion can be drawn from the present experiments as to the optimization of fertilization in the nursery. Composing the substrate by mixing soil for *S. leprosula* nurseries should be carried out with this information in mind.

4.5 CONCLUSIONS

The introductory Chapter 1 ended with two hypotheses that were to be tested in the present study. Indeed, supporting evidence is found for both, whereas nothing in data of Chapter 2 and 3 support rejecting these hypotheses.

1. Different mycorrhizal associations indeed were shown to result in different growth performances of cuttings of *Shorea leprosula.* This is supported by the significant correlation between a number of growth parameters and the inoculation of the cuttings with different species of mycorrhizal fungi in different experimental environments.

It was to be expected that the correlation was not significant for all parameters. On the one hand this is due to the inherent "noise" in the web of multiple interactions. Even among 12 variations of factors tested (Ch. 2) there are potentially $12^2 = 144$ interactions, many of which obscure the results of others, while not all could be tested. On the other hand, the experiments were not carried out in fully isolated chambers or vats, but were only partly isolated form the chaotic environment of the tropical rain forest around the greenhouse. The "natural" interactions added "natural noise" to the statistics.

2. Environmental conditions indeed were shown to affect the interaction between soil mycorrhizal and *S. leprosula* plants. There was a particularly strong influence of soil granulometry, growth mounting as more sand was present. Aeration and hydrology of soil pores explained this influence. Incoming light had a clear, non-linear influence, with a peak value of light intensity for optimum effect on growth performance. The sap-stream diagram shows this to be related to the amount of photosynthetic sugars recieved by fungus from the plant.

   Chemical soil fertility played a dubious role. It never showed a consistent correlation with the pattern of growth performance and inoculation. A few, very complex biological mechanisms of absorption and processing of minerals could be partly reconstructed by combining the present experimental results with data from literature. They show chemical soil fertility in itself to be separated from the green plant by many web of biological regulation. Iron uptake is one example that could be best placed physiologically, because previous research had shown the physiological involvement of caffeine.

   A very general conclusion may be drawn before closing this book. The present study confirms that guidance of natural processes for use of forests and trees, like the wild *orangutan* do, is a more efficient way towards sustainable forestry than the reliance on artificially simplified theory and techniques, like many forestry expert do. In Indonesian, the latter would *orang hutan.*

SUMMARY

"Dipterocarpaceae: *Shorea leprosula* cuttings, mycorrhizae and nutrients" discusses the mycorrhizal development in conditions of different dosage of NPK fertilizer, on different soil substrates and under different environmental conditions (controlled conditions and semi-controlled conditions).

This research was conducted in a greenhouse at the Research Station Samboja (WANARISET), East Kalimantan Indonesia. The book consists of four chapters.

Chapter 1, with a general introduction, provides an overview of the literature on Dipterocarpaceae, mycorrhizae, fertilization, and soil substrates with special reference to environmental factors.

Chapter 2 describes an experiment on the influence of environmental factors, mycorrhizal inoculation, fertilization, soil substrates and their interactions per treatment. The results show that those environmental factors, soil substrates and mycorrhizal inoculation affected both the growth of *S. leprosula* cuttings and the mycorrhizal development. NPK fertilizer did not significantly affect the growth of *S. leprosula* cuttings. The strongest growth of *S. leprosula* cuttings was obtained under controlled conditions.

The environmental factors, especially light intensity, significantly affected both the growth of *S. leprosula* cuttings and the mycorrhizal development. The light intensity maximizing the growth of *S. leprosula* cuttings was 12 $\mu mol.m^{-2}$, occuring at 9.00 hrs and 16.00 hrs. As confirmed by other studies, photosynthetic activities are more significant at 9.00 hrs and 16.00 hrs, while at 12.00 hrs the photosynthetic activity is lower than those at 9.00 hrs and 16.00 hrs.

Sandy loam and sandy clay favour the growth of *S. leprosula* cuttings and the mycorrhizal development as compared with clay. Sandy clay and sandy loam are more aerated than clay. The higher oxygen supply favours the mycorrhizal development.

Chapter 3 discusses an experiment on mycorrhizal development and the inoculum potential in various soil substrates, observed by an intrascope in perforons (root-boxes). The results show that an unidentified and unsterilized soil inoculum advances the growth of *S. leprosula* cuttings and the mycorrhizal development. Autoclaving of the soil causes a decrease in nutrient availability in the soil, especially of N, P, K, and Mg, whereas Ca and Fe increase. In sterilized substrates a new mycorrhizal development, either from spores that had survived the heat, or from airborne spores, was found. Sterilization by autoclaving at 121° C for two hours

clearly affected the nutrient availability negatively. The physiological state and age of cuttings also affected the mycorrhizal development.

Chapter 4 includes the general discussion and conclusion. Aeration or oxygen supply in the soil substrates indeed affects the growth of *S. leprosula* and the mycorrhizal development. The potential inoculum plays an important role in promoting the growth of *S. leprosula* cuttings and the mycorrhizal development. Light intensity affected the growth of *S. leprosula* cuttings. In this Chapter, also the physiological effects of the mycorrhizal development were discussed. The combined effect of all experimental inputs was explained in a sapstream model of the whole cutting. This also highlighted the role played by *Amanita* sp in mobilizing magnesium, which as the main component of chlorophyll, boosted the photosynthesis.

Several new facts emerged. When young, *Shorea leprosula* proves to be shade requiring, not shade-tolerant, because high light intensity damages it. This is a new temperament. If the squash test is right, two distinct morphological types of mycorrhizae are caused by one *Amanita* species, providing a striking case of dimorphism probably caused by stress. Finally Fe-uptake and processing may well be a parameter for very complex root processes, and should be the subject of through research.

At the end of the Chapter, the application of knowledge obtained by the implementation in nursery techniques was discussed, especially the use of soil inocula and fertilization aspects for the *S. leprosula* and the necessity of light management by means of adequate roofing.

SAMENVATTING

"Dipterocarapceae: *Shorea leprosula* stekken, mycorrhizen en voedingsstoffen" gaat over de mycorrhizen-ontwikkeling onder proefondervindelijke omstandigheden met divers gedoseerde NPK-meststof, op verschillende bodemsubstraten en in een verschillende omgeving, te weten gecontroleerde tegenover ongecontroleerde omstandigheden. Het onderzoek werd uitgevoerd in een kas in het Onderzoeksstation Samboja (WANARISET) in Oost-Kalimantan, Indonesië.

Het eerste hoofdstuk bevat de algemene inleiding. Het biedt een literatuuroverzicht over dipterocarpen, mycorrhizen, bemesting en bodemsubstraten, met bijzondere aandacht voor andere omgevingsfactoren.

Hoofdstuk twee beschrijft een proefneming over de invloed van omgevingsfactoren, inoculatie met mycorrhizen, kunstmestgiften, bodemsubstraten en hun interacties per behandeling. De resultaten tonen dat de omgevingsfactoren, het bodemsubstraat en de inoculatie met mycorrhizenschimmels zowel invloed hebben op de groei van *S. leprosula*-stekken als op de mycorrhizen-ontwikkeling. NPK-kunstmest beinvloedde de groei van de stekken neit op voorspelbare wijze.

Bij de omgevingsfactoren beinvloedde vóór alle andere de lichtintensiteit de groei van de stekken en de mycorrhizen-ontwikkeling. De lichtsterkte voor een maximale groei van de stekken was 12 $\mu mol.m^{-2}$ van vol daglicht, en wel vooral om 9.00 uur des morgens en 16.00 uur des namiddags. Hoogstwaarschijnlijk, en zoals ook uit neotropische studies blijkt, is er actieve fotosynthese in de morgen en de late middag, terwijl ze rond het middaguur laag is of zelfs wegvalt.

Zandig leem en zandige klei bevorderen de groei van stekken van *S. leprosula* en de mycorrhizen-ontwikkeling meer dan klei. Zandige substraten zijn beter doorlucht dan klei. Het zuurstofaanbod is daardoor hoger, hetgeen mycorrhizen-ontwikkeling bevordert en de wortelrespiratiwe versterkt.

Hoofdstuk drie bespreekt een proefneming over mycorrhizen-ontwikkeling en het potentiëel van het inoculum in diverse bodemsubstraten, zoals *in vivo* waargenomen via een intrascoop door de gaten van een perforon ("worteldoos"). De waarnemingen tonen dat ongeïdentificeerde en ongesteriliseerde resten van natuurlijk bodem-inoculum de groei van de stekken en de ontwikkeling van de mycorrhizen vooruit hielpen. Sterilisatie deed de beschikbaarheid van voedingsstoffen in de bodem afnemen, in het bijzonder van N, P, K en Mg, terwijl Ca en Fe juist toenamen. In gesteriliseerde substraten werd eveneens nog ontwikkeling van schimmels gevonden, hetzij uit sporen die de verhitting hadden overleefd, hetzij door ingewaaide sporen. Sterilisatie door verhitting tot 121° C in een autoclaaf gedurende twee uur bracht duidelijk de hoeveelheid beschikbare

voedingsstoffen omlaag. De fysiologische staat en de leeftijd van de stekken beïnvloedden eveneens de ontwikkeling der mycorrhizen.

Hoofdstuk 4 bevat de algemene discussie en conclusies. Beluchting, ofwel zuurstofrijkdom in het bodemsubstraat bevorderen inderdaad de groei van stekken en de ontwikkeling van mycorrhizen. Het potentiëel aan inoculum speelt een voorname rol bij de bevordering van stekgroei en mycorrhizen-schimmelontwikkeling. De lichtintensiteit was sterk bepalend voor de groei van *S. leprosula*-stekken. In dit hoofdstuk staat ook een discussie over het fysiologische effect van mycorrhizen. Het gecombineerde effect van de experimentele inputs en hun interactie en terugkoppelingsrelaties wordt verklaard met een sapstroomdiagram voor de stekken als hele planten. Hieruit blijkt bij voorbeeld het belang van de specialistische schimmel *Amanita* sp. bij het mobiliseren van Mg, dat als bestanddeel van chlorophyll de fotosynthese helpt vergroten.

Verscheidene nieuwe feiten werden ontdekt. Jonge *Shorea leprosula*-planten zyn schaduweisend, niet shaduwminnend, te hoge lichtinsiteit beschadig ze. Dit is een nieuw boom-temperament. Volgens de squash test maakk één *Amanites*-soort twee verschillende vormen mycorrhizen, een treffend en nieuw voorbleeld van dimorfisme bij schimmels, mgelŷk wŷzend op stress. Tenslotte zyn ijzeropname en-omzetting waarschijnlijk een parameter voor zeer complexe metabolische wortelprocessen die met zuurstof te maken hebben. Dit zou moeten worden onderzocht.

Tenslotte wordt de toepassing van de verkregen kwekerijwetenschap in de praktijk belicht. In het bijzonder komen daarbij de aard van het te vervaardigen of te kiezen substraat, de regulatie van het invallend licht door schermen, en de samenstelling van het mycorrhizen-inoculum als belangrijke technische aspecten naar voren.

RINGKASAN

"Dipterocarpaceae: *Shorea leprosula* cuttings, mycorrhizae and nutrients" membahas perkembangan mikoriza pada berbagai dosis pupuk NPK dan tipe tanah pada kondisi lingkungan yang terkontrol dan semi terkontrol.

Penelitian ini dilakukan di rumah kaca stasiun penelitian Wanariset Samaboja, Kalimantan Timur Indonesia. Buku ini terdiri dari 4 bab yang masing-masing isinya akan dijelaskan sebagai berikut :

Bab pertama berisi pendahuluan dan tinjauan pustaka mengenai Dipterocarpaceae, mikoriza, pemupukan, dan media tanam, dikaitkan dengan faktor lingkungan.

Bab dua menjelaskan hasil penelitian pengaruh perlakuan kondisi lingkungan, inokulasi mikoriza, pemupukan dan tipe tanah termasuk interaksi antar perlakuan. Hasil penelitian menunjukkan bahwa faktor lingkungan, media tanam, inokulasi mikoriza telah mempengaruhi pertumbuhan stek *S. leprosula* dan perkembangan mikoriza. Sedangkan perlakuan pemupukan NPK tidak berpengaruh nyata terhadap pertumbuhan stek *S. leprosula*. Pertumbuhan stek *S. leprosula* yang terbaik diperoleh pada stek yang ditanam pada kondisi terkontrol.

Faktor lingkungan telah memberikan pengaruh yang sangat nyata terhadap pertumbuhan stek *S. leprosula* dan perkembangan mikoriza, khususnya intensitas cahaya. Intensitas cahaya yang dapat mempengaruhi pertumbuhan stek *S. leprosula* adalah 12 $\mu mol.m^{-2}$ dari cahaya penuh, terutama pada jam 9.00 dan 16.00. Dengan demikian diduga dari informasi penelitian yang lain bahwa fotosintesis yang efektif terjadi pada jam 9.00 dan 16.00 sedangkan pada jam 12.00 proses fotosintesis rendah.

Media tanam yaitu pasir berlempung dan pasir berliat meningkatkan pertumbuhan stek *S. leprosula* dibandingkan stek yang ditanam di media tanah liat. Hal ini dikarenakan aerasi pada pasir berlempung dan pasir berliat lebih baik dibanding dengan liat. Dengan demikian faktor oksigen sangat berpengaruh terhadap perkembangan mikoriza.

Bab tiga membahas hasil penelitian perkembangan mikoriza dan potensi inokulum dalam tanah yang diamati dengan menggunakan alat intrascope di perforons (root-box). Hasil penelitian menujukkan bahwa potensi inokulum dalam tanah cukup baik dan berpengaruh terhadap pertumbuhan stek *S. leprosula* dan perkembangan mikoriza. Hal ini dikarenakan sterilisasi media tumbuh menyebabkan penurunan ketersediaan zat hara seperti N, P, K dan Mg dalam tanah, sedangkan unsur Ca dan Fe yang tersedia menjadi meningkat. Akibat dari sterilisasi tersebut muncul type ektomikoriza baru yaitu *Thelophora sp* yang berasal dari inokulum tanah, air, dan

atau udara. Dengan demikian sterilisasi dengan autoklap pada suhu 121° C selama dua jam kurang baik terhadap zat hara yang tersedia. Selain itu terjadi suksesi mikoriza yang diakibatkan oleh faktor umur tanaman, fisiologi dari mikoriza itu sendiri dan lingkungan.

Bab empat berisi pembahasan umum dan kesimpulan dari hasil penelitian pada bab-bab terdahulu. Disimpulkan bahwa pertumbuhan stek *Shorea leprosula* dan perkembangan mikoriza dipengaruhi oleh faktor aerasi tanah atau oksigen dalam media tumbuh. Selain itu pula potensi inokulum berperan terhadap pertumbuhan stek *Shorea leprosula* dan perkembangan mikoriza. Intensitas cahaya berpengaruh terhadap pertumbuhan stek *Shorea leporosula.* Didalam bab ini juga dibahas pengaruh fisiologis dan ekologis dari perkembangan mikoriza. Kombinasi semua perlakuan sebagai masukan yang dijelaskan dalam model aliran nutrisi untuk stek. Jamur *Amanita* sp mempunyai peranan yang sangat penting dalam penyerapan unsur Mg, yang digunakan sebagai komponen klorofil untuk mendorong fotosintesis.

Beberapa fakta baru yang diperoleh bahwa pada waktu anakan *S. leprosula* terbukti membutuhkan naungan , bukan toleran terhadap naungan, karena dengan tingginya cahaya menyebabkan kematian. Disini menunjukan temparmen baru untuk jenis ini. Selain itu jika hasil dari identifikasi betul bahwa terjadi dua type morphology dari satu jenis jamur *Amanita* hal ini mungkin disebabkan oleh kondisi stres. Terakhir mungkin bahwa penyerapan besi oleh tanaman merupakan prsoses complek oleh akar, sehingga perlu penelitian lebih lanjut.

Pada akhir dari tulisan ini dibahas juga aspek kepentingan praktis/aplikasi dari hasil penelitian dalam hubungannya dengan penyedian bibit yang berkualitas baik di persemaian. Hal ini dilakukan dengan penggunaan inokulum tanah (*potential inoculum*). Untuk perlakuan pemupukan masih perlu dipertimbangkan, khususnya dari jenis *Shorea leprosula.* Pengaturan intensitas cahaya dilaksanakan dengan penggunaan naungan.

GLOSSARY

Amphimycorrhizae :	Mycorrhizae in which plant roots are infested by intracellular hyphae, often with distinctive clamp connections and minute haustoria-like organs, and all of the root surface being covered with a dense mantle of hyphae, at certain times bearing cystidia-like structures, without any apparent change in root morphology or cortex cell morphology (Smits, 1994).
Architecture (of tree):	The visible morphological expression of the genetic blueprint of a tree at any one time (Hallé et al., 1978).
Autoclave:	Closed vat in which steam under pressure.
Branching:	Formation of lateral axes from meristems on an axis.
Cortex:	The tissue, formed by the cambium in stem and root, surrounding and not being part of the vascular bundles.
Cotyledon:	First leaf or leaves of a seed plant found in the embryo and which may form the first photosynthetic leaves, have often particular forms or may remain below the ground.
Cutting :	Part of a plant cut off for vegetative reproduction of that plant.
SEAMEO-BIOTROP:	South East Asian Ministers of Education Organization/ Regional Center for Tropical Biology
ECM :	See Ectomycorrhizae
Ectomycorrhizae (ECM):	Mycorrhizae characterized by an external fungal sheath or mantle around the plant root, where the hyphae penetrate between the epidermal and often the cortical cells, so forming a Hartig net.
Endomycorrhizae:	Mycorrhizae, which do not form external sheaths or mantles but the hyphae of which penetrate both between and into the living cell or cortex cell, often by means of haustoria.

Fruiting body:	Vernacular expression for sporocarp (see sporocarp).
Hartig net :	The net-like structure, visible on cross-sections of ectomycorrhizal roots, and consisting of fungal hyphae entering between epidermis and/or cortex and filling the intercellular spaces.
Hyphae :	Filamentous multicellular structures that constitute the mycelium of the fungi.
Hedge orchard:	Young trees pruned back to hedges, so that they reiterate numerous orthotropic shoots apt to serve for vegetative propagation by means of stem cuttings.
Inoculum :	Reproductive material of micro-organisms brought into a host organism to contaminate it.
Inoculation:	Introduction of biological material (inoculum) into a medium such as a living organism, synthetic substrate or soil to start a new culture.
Mycorrhizae:	A symbiotic most often mutual and obligate association between a fungus and the root of higher plants in which the fungus lives on or within the root.
Mycobiont :	The fungal partner in mycorrhizal symbiosis with a higher plant
Orthotropic	Property of a vertical axis, most often with leaves in a spiral, functionally exploring, ensuring height growth, penetrating higher "empty" vegetation layers and overtopping.
PAR:	Photosynthetic Active Radiation (measured by instrument types LI-250, LI-COR Inc).
Phytobiont :	The green plant partner in the mycorrhizal symbiosis with another organism.
PT. KEM :	Kalimantan Equatorial Mining Company.
Ramification :	The process of branching.
Rhizomorph :	A root-like aggregation of hyphae.

Rootone F:	Industrial mix of rooting hormones containing 0.057 % IBA, 0.113 % of three chemical derivatives of NAA (0.067 % 1-Naphthaline Acetamida, 0.033 % 2-Methyl-1-Naphthalene Acetic Acid, 0.013 % 3-Methyl-1-1-Napthalena Acetamida) and 4 % fungicide, thiram (Tetra methyl thiram disulphate), mixed in talcum (95.85 %).
SEAMEO:	South East Asian Ministers of Education Organization.
Sylleptic:	Axes, built by a meristem without a preceding resting period; quick-profit organs, allowing the vegetative tree body to expand in one continuing sweep when the external conditions are right, particularly in non seasonal climates.
Solarization :	Heating, using solar radiation or pasteurization of the culturing media.
Sporocarps:	Structure inside which spores are produced.
Sterilization	Extermination of micro-organisms, often by heating to high temperatures in an autoclave.
Succession:	The chronological sequence of different organisms colonizing a particular substrate.
TPTI:	Tebang Pilih Dan Tanam Indonesia (The Indonesian Selective Felling and Replanting System).
VAM:	Vesicular Arbuscular Mycorrhizae, a subdivision of the Endomycorrhizae.

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APPENDIX 1
FREQUENCY DISTRIBUTION OF WIDTH AND LENGTH OF LEAVES OF THE *S. LEPROSULA* CUTTINGS

Width Class of Leaf	**Length Class of Leaf**									**Total**
	1.9-3.5	3.6-5.2	5.3-6.9	7.0-8.6	8.7-10.3	10.4-12.0	12.1-13.7	13.8-14.4	14.5-15.3	
0.8-1.4	1	2	-	-	-	-	-	-	-	3
1.5-2.1	1	4	3	1	-	-	-	-	-	9
2.2-2.8	1	2	4	4	-	-	-	-	-	11
2.9-3.5	-	1	1	3	1	1	-	-	-	7
3.6-4.2	-	-	1	2	1	1	-	1	-	7
4.3-4.9	-	-	-	2	-	-	1	-	-	3
5.0-5.6	-	-	-	-	-	-	-	-	1	1
Total	3	9	9	12	2	2	1	1	1	41

Note: the form factor of leaf = 0.69 (0.7)

APPENDIX 2
SUMMARY OF THE SIGNIFICANCE LEVELS (%) OF EACH PARAMETER CALCULATED FOR THE EFFECTS OF THE VARIOUS INTERACTIONS STUDIED IN EXPERIMENT I

	Significance level (F value) per measured variable (%)							
Treatments	H	D	N_l	A_l	W_{tf}	W_{td}	N_{rt}	ECM
E=(C+SC)	99 **	99 **	99**	99 **	99 **	99 **	11 ns	99 **
Soil (S)	99 **	99 **	97 **	99 **	99 **	99 **	99 **	99 **
Fertilizer (F)	63 ns	78 ns	3 ns	30 ns	54 ns	59 ns	41 ns	83 ns
Mycorrhizae (M)	54 ns	74 ns	96 *	89 ns	98 *	98 *	41 ns	99 **
S + F	75 ns	72 ns	92 ns	78 ns	92 ns	89 ns	90 ns	86 ns
S + M	44 ns	80 ns	61 ns	47 ns	49 ns	72 ns	19 ns	34 ns
F + M	33 ns	39 ns	85 ns	12 ns	56 ns	50 ns	52 ns	33 ns
SFM	19 ns	3 ns	33 ns	5 ns	15 ns	15 ns	87 ns	27 ns
E + S	99 **	99 **	81 ns	99 **	99 **	99 **	33 ns	99 **
E + F	95 *	72 ns	97 *	99 **	99 **	98 **	90 ns	71 ns
E + M	38 ns	32 ns	68 ns	77 ns	13 ns	19 ns	70 ns	88 ns
E+ S + F	11 ns	4 ns	87 ns	58 ns	68 ns	61 ns	68 ns	64ns
E +S+M	27 ns	40 ns	23 ns	2 ns	3 ns	5 ns	1 ns	76 ns
E+F + M	73 ns	70 ns	9 ns	3 ns	22 ns	15 ns	35 ns	56 ns
E + S + F + M	56 ns	56 ns	7 ns	7 ns	2 ns	7 ns	1 ns	10 ns

Note : E = controlled and semi controlled conditions; S = soil substrate; F = NPK fertilizer; M = mycorrhizal inoculation; H = height growth; D = diameter growth; N_l = number of leaves; A_l = leaf area; W_{tf} = total fresh weight; W_{td} = total dry weight; N_{rt} = number of root tips; ECM % = percentage of mycorrhizal roots.

Remarks: *= 5 % level of significance ; ** = 1 % level of significance, ns = not significant

APPENDIX 3
RESULTS OF CHEMICAL AND PHYSICAL SOIL ANALYSES OF *S. LEPROSULA* CUTTING AFTER HARVESTING OF THE EXPERIMENTS UNDER CONTROLLED CONDITIONS

Soil substrate	pH	C organic	N total	C/N ratio	P available	K	Ca	Mg	Al	Sandy	Silty	Clay
	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
Clay												
0 mg + M0	5.0	0.71	0.09	7.89	3.48	0.15	0.75	0.85	5.37	38.43	21.66	39.91
+ M1	4.8	0.68	0.08	8.50	3.78	0.15	1.05	0.99	5.37	34.95	23.97	41.08
+ M2	4.7	0.82	0.08	10.25	3.16	0.11	0.77	0.95	0.49	23.06	38.47	38.47
+ M3	4.8	0.73	0.07	10.43	3.70	0.15	0.79	1.03	5.27	34.27	27.07	38.66
+ M4	5.0	0.68	0.08	8.50	3.48	0.13	1.02	0.75	5.17	28.44	35.08	36.48
50 mg + M0	4.9	0.85	0.08	10.63	3.44	0.21	1.29	0.87	8.72	29.38	34.61	36.01
+ M1	4.7	0.53	0.09	5.89	5.03	0.09	1.01	0.78	5.16	27.85	41.20	30.95
+ M2	4.9	0.65	0.08	8.13	3.65	0.15	0.75	0.77	5.17	29.05	29.36	41.59
+ M3	4.7	0.67	0.08	8.38	3.14	0.10	1.07	0.94	5.27	33.37	12.81	53.82
+ M4	4.7	0.55	0.08	6.88	2.92	0.08	0.90	0.89	0.90	27.98	29.37	42.65
100 mg+ M0	4.9	0.75	0.08	9.38	3.24	0.15	2.00	1.43	4.41	34.54	23.38	42.08
+ M1	5.0	0.83	0.08	10.38	3.59	0.13	1.60	0.73	5.09	36.88	22.28	40.84
+ M2	4.9	0.66	0.09	7.33	3.49	0.17	1.31	1.21	4.96	34.07	25.47	40.46
+ M3	4.7	0.77	0.08	9.63	3.09	0.09	1.87	1.48	1.32	27.78	38.14	34.08
+ M4	4.6	0.67	0.08	8.38	3.03	0.10	1.92	0.86	1.65	35.75	33.31	30.94
200 mg + M0	4.5	0.58	0.08	7.25	3.04	0.10	1.81	1.13	6.79	32.21	34.96	32.83
+ M1	4.9	0.83	0.09	9.22	3.12	0.16	2.35	1.61	4.51	32.89	26.85	40.26
+ M2	4.2	0.56	0.08	7.00	3.23	0.13	1.35	0.82	0.99	24.84	39.61	35.55
+ M3	4.8	0.70	0.08	8.75	3.20	0.09	0.82	0.79	3.64	22.33	37.28	40.39
+ M4	4.8	0.76	0.09	8.44	2.83	0.12	1.65	1.17	1.97	23.66	37.41	38.93
Average	**4.8**	**0.70**	**0.08**	**8.56**	**3.38**	**0.13**	**1.30**	**1.00**	**4.11**	**30.59**	**30.61**	**38.80**
Sandy loam												
0 mg + M0	5.0	0.99	0.09	11.00	3.44	0.09	1.49	0.51	2.41	56.59	18.09	25.32
+ M1	5.2	0.94	0.10	9.40	3.40	0.15	2.44	1.02	2.30	55.85	19.62	24.53
+ M2	5.0	1.19	0.11	10.81	3.31	0.15	2.16	0.59	1.91	52.14	20.86	27.00
+ M3	5.0	1.05	0.10	10.50	3.73	0.12	2.00	0.84	2.77	55.16	21.24	23.60
+ M4	4.9	1.07	0.07	15.29	4.03	0.14	2.09	0.81	2.49	57.18	21.41	21.41
50 mg + M0	5.1	1.05	0.10	10.50	3.06	0.11	2.13	0.53	1.44	75.50	14.00	10.50
+ M1	4.8	1.07	0.10	10.70	3.54	0.15	2.35	1.07	2.30	32.74	26.56	40.70
+ M2	5.0	1.04	0.11	9.46	2.62	0.26	2.53	0.90	1.39	55.19	18.67	26.14
+ M3	5.1	1.14	0.09	12.67	3.62	0.10	2.15	0.54	1.25	56.69	18.05	25.26
+ M4	5.1	1.00	0.11	9.09	3.78	0.13	2.09	1.02	2.49	56.67	16.85	26.48

Appendix 3 (cont'd)

Soil substrate	pH	C organic	N total	C/N ratio	P available	K	Ca	Mg	Al	Sandy	Silty	Clay
	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
100 mg+ M0	5.1	0.96	0.09	10.67	3.45	0.16	1.98	1.28	1.64	56.15	23.61	20.24
+ M1	5.0	0.91	0.09	10.11	3.89	0.13	1.85	1.16	1.91	54.14	17.55	28.31
+ M2	5.2	1.14	0.10	11.40	2.75	0.12	2.66	0.74	1.25	58.37	18.73	22.89
+ M3	5.0	1.19	0.10	11.90	3.21	0.09	2.06	1.07	1.83	53.86	14.17	31.97
+ M4	4.7	1.10	0.10	11.00	2.86	0.05	2.04	1.35	1.34	55.61	13.91	30.48
200 mg + M0	5.1	0.95	0.10	9.50	4.55	0.16	3.39	1.72	1.74	58.22	17.90	23.87
+ M1	4.8	0.90	0.10	9.00	3.54	0.09	2.42	0.87	2.56	60.11	11.47	28.42
+ M2	5.1	0.87	0.09	9.67	3.80	0.08	2.62	1.56	1.83	58.32	9.87	31.81
+ M3	5.0	0.82	0.10	8.20	3.24	0.09	2.84	1.38	0.49	57.94	11.48	30.58
+ M4	5.3	0.92	0.09	10.22	3.94	0.14	3.02	1.60	1.91	52.21	15.15	32.64
Average	**5.0**	**1.02**	**1.10**	**10.55**	**3.49**	**0.13**	**2.32**	**1.03**	**1.86**	**55.93**	**17.46**	**26.61**
Sandy clay												
0 mg + M0	5.2	0.84	0.07	12.00	9.34	0.06	0.89	0.43	0.77	74.86	13.97	11.18
+ M1	5.0	0.95	0.07	13.57	11.73	0.06	0.98	0.20	0.58	74.02	8.66	17.32
+ M2	5.0	0.93	0.07	13.29	10.21	0.04	1.23	0.59	0.76	71.54	8.36	20.10
+ M3	5.4	0.88	0.06	14.67	8.31	0.04	1.17	0.19	0.38	68.25	11.26	20.49
+ M4	5.3	0.89	0.08	11.13	9.58	0.08	1.21	0.41	0.48	76.73	15.51	7.76
50 mg + M0	5.1	0.97	0.07	13.86	8.13	0.06	1.27	0.42	0.99	72.58	10.33	17.09
+ M1	5.4	0.88	0.07	12.57	10.38	0.04	1.12	0.44	0.57	77.16	9.79	13.05
+ M2	5.1	0.92	0.08	11.50	7.99	0.01	1.38	0.54	0.41	72.59	12.61	14.80
+ M3	5.4	1.00	0.07	14.29	10.43	0.05	0.86	0.24	0.29	72.79	16.33	10.88
+ M4	5.1	1.01	0.07	14.43	8.69	0.03	0.64	0.35	1.47	74.32	6.42	19.26
100 mg+ M0	5.5	0.97	0.07	13.86	10.69	0.07	1.41	0.33	0.38	76.98	11.51	11.51
+ M1	5.5	0.86	0.09	10.67	8.08	0.07	1.60	0.60	0.29	76.26	15.10	8.63
+ M2	5.5	0.99	0.08	12.38	11.81	0.08	1.81	0.58	0.29	78.40	12.00	9.60
+ M3	5.4	0.74	0.08	9.25	8.04	0.07	1.65	0.75	0.29	73.49	19.88	6.63
+ M4	5.6	0.96	0.07	13.71	8.59	0.07	1.46	0.52	0.19	74.85	8.38	16.77
200 mg+ M0	5.4	0.83	0.08	10.38	10.03	0.09	1.87	1.02	0.19	72.91	9.25	17.84
+ M1	5.3	1.02	0.08	12.75	8.60	0.09	2.25	1.07	1.40	38.29	21.49	40.22
+ M2	5.3	0.80	0.07	11.42	9.54	0.10	2.52	1.17	0.29	35.41	24.50	40.09
+ M3	5.2	1.02	0.09	11.33	10.23	0.10	1.99	1.09	1.45	65.44	16.17	18.39
+ M4	5.3	0.97	0.08	12.13	10.41	0.08	1.38	0.64	0.39	77.09	15.27	7.64
Average	**5.3**	**0.92**	**0.08**	**12.46**	**9.54**	**0.06**	**1.43**	**0.58**	**0.59**	**70.20**	**13.34**	**16.46**

Note : 0 mg ; 50 mg; 100 mg ; 200 mg = dosage NPK fertilizer , M0 = without inoculation ; M1 = inoculation with *Amanita sp*; M2 = inoculation with *Russula sp* ; M3 = inoculation with *Scleroderma columnare* and M4 = inoculation with a cocktail of the three fungi (M1+M2 +M3)

APPENDIX 4
RESULTS OF CHEMICAL AND PHYSICAL SOIL ANALYSES OF THE *S. LEPROSULA* CUTTING AFTER HARVESTING OF THE EXPERIMENTS I UNDER SEMI-CONTROLLED CONDITIONS

Soil substrate	pH	C Organic	N Total	C/N ratio	P available	K	Ca	Mg	Al	Sandy	Silty	Clay
	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
Clay												
0 mg + M0	4.7	0.68	0.07	9.70	8.60	0.18	0.41	0.91	5.18	33.11	24.44	42.45
+ M1	4.6	0.80	0.10	8.00	3.43	0.10	0.75	0.91	6.52	51.46	20.64	27.90
+ M2	4.6	0.81	0.09	9.00	3.63	0.09	0.82	0.56	4.23	55.87	19.47	24.66
+ M3	4.8	0.79	0.08	9.88	3.10	0.15	0.76	0.85	5.47	31.53	27.90	40.57
+ M4	4.4	0.80	0.09	8.90	3.57	0.12	1.01	0.62	4.27	32.83	33.85	33.32
50 mg + M0	4.8	1.17	0.08	14.63	3.21	0.14	0.78	0.42	4.98	35.12	21.25	43.63
+ M1	4.6	0.65	0.08	8.13	3.03	0.15	0.80	1.08	6.20	34.88	30.29	34.83
+ M2	4.8	0.68	0.07	9.71	2.95	0.17	0.58	0.78	5.21	33.55	29.24	37.21
+ M3	4.8	0.72	0.08	9.00	3.54	0.20	0.66	1.01	5.63	34.16	21.95	43.89
+ M4	4.8	0.51	0.08	6.38	3.72	0.13	0.85	0.36	3.28	80.65	8.35	11.00
100 mg+ M0	4.8	0.90	0.08	11.25	3.56	0.14	1.03	0.49	4.33	35.21	30.69	34.10
+ M1	4.6	0.77	0.08	9.63	3.55	0.17	0.75	0.93	5.06	38.48	23.33	38.18
+ M2	4.8	0.96	0.08	12.00	2.76	0.20	0.74	1.02	5.07	34.48	28.56	36.96
+ M3	4.9	0.96	0.08	12.00	2.95	0.15	1.30	0.59	3.82	34.00	31.16	34.83
+ M4	5.0	0.68	0.07	9.70	3.18	0.19	0.79	1.27	5.08	33.68	31.32	35.00
200 mg + M0	4.8	0.91	0.08	13.38	3.40	0.22	1.23	0.60	0.22	32.91	32.25	34.83
+ M1	4.8	0.58	0.08	7.25	3.45	0.19	1.40	1.29	0.19	34.12	26.01	39.87
+ M2	4.7	0.73	0.08	9.13	3.43	0.19	1.00	1.17	0.19	35.73	27.06	37.21
+ M3	4.9	1.00	0.07	14.29	3.29	0.15	1.63	0.81	0.15	33.87	25.29	40.85
+ M4	4.7	1.01	0.08	12.63	3.34	0.18	1.56	0.65	0.18	38.66	22.30	39.04
Average	**4.8**	**0.81**	**0.08**	**10.23**	**3.58**	**0.16**	**0.94**	**0.82**	**3.76**	**38.72**	**25.77**	**35.52**
Sandy loam												
0 mg + M0	4.8	0.86	0.10	8.60	3.38	0.12	2.24	0.55	0.12	52.74	18.55	28.71
+ M1	5.3	0.87	0.06	14.50	4.12	0.17	4.05	1.23	0.17	71.96	22.99	5.05
+ M2	4.8	1.05	0.10	10.50	3.59	0.11	2.07	0.74	0.11	60.24	17.66	22.10
+ M3	4.9	1.05	0.11	9.55	4.20	0.11	2.05	0.83	0.11	56.28	14.57	29.15
+ M4	4.8	1.12	0.10	11.2	3.16	0.11	2.10	0.67	0.11	46.82	21.34	31.84
50 mg + M0	5.2	0.98	0.12	8.17	3.54	0.12	2.38	1.31	0.12	65.42	14.41	20.17
+ M1	4.8	1.02	0.11	9.27	3.76	0.12	2.38	0.86	0.12	63.82	17.35	18.83
+ M2	4.7	1.12	0.10	11.20	3.52	0.11	2.42	1.00	0.11	59.21	18.36	22.43
+ M3	5.2	1.09	0.09	12.11	3.47	0.16	2.72	1.04	0.16	56.19	17.21	26.60
+ M4	4.7	1.04	0.10	10.40	3.05	0.24	3.48	1.00	0.24	54.29	26.78	18.93
100 mg+ M0	4.9	1.02	0.11	9.27	3.27	0.14	2.14	0.78	0.14	81.59	8.91	9.50
+ M1	5.1	1.02	0.11	9.27	3.08	0.18	2.58	0.72	0.18	73.58	11.74	14.68
+ M2	4.9	1.13	0.10	11.30	3.64	0.09	2.16	1.26	0.09	50.19	28.27	21.54
+ M3	5.1	1.28	0.09	14.22	2.75	0.15	2.62	0.78	0.15	55.58	19.60	24.82
+ M4	4.7	1.05	0.12	8.75	3.43	0.09	1.89	0.73	0.09	47.26	28.97	23.77

Appendix 3 (cont'd)

Soil substrate	pH	C Organic	N Total	C/N ratio	P available	K	Ca	Mg	Al	Sandy	Silty	Clay
	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
200 mg + M0	5.1	0.93	0.11	8.46	3.45	0.19	2.74	1.18	0.19	55.87	19.96	24.17
+ M1	4.8	1.06	0.10	10.60	3.16	0.13	2.46	1.14	0.13	51.01	29.30	19.69
+ M2	4.8	1.13	0.11	10.27	3.14	0.15	2.48	0.90	0.15	80.01	7.86	12.13
+ M3	5.1	1.10	0.07	15.71	4.14	0.18	2.19	0.85	0.18	57.50	18.59	23.91
+ M4	4.9	0.86	0.10	8.60	4.57	0.12	2.53	1.14	0.12	48.70	29.32	21.98
Average	**4.9**	**1.04**	**10.6**	**3.52**	**0.14**	**2.48**	**0.94**	**0.14**	**0.12**	**59.41**	**19.50**	**21.00**
Sandy Clay												
0 mg + M0	5.2	1.21	0.09	13.44	11.27	0.13	1.39	0.27	0.58	74.07	9.72	16.21
+ M1	4.9	1.01	0.08	12.63	9.60	0.07	2.00	0.76	1.23	71.95	10.52	17.53
+ M2	5.1	1.02	0.07	14.57	10.51	0.08	1.23	0.24	0.82	69.24	12.33	18.43
+ M3	5.2	1.17	0.08	14.63	9.40	0.12	1.33	0.32	0.77	71.09	25.29	3.62
+ M4	5.3	1.17	0.08	14.63	10.88	0.07	1.44	0.37	0.76	73.44	15.94	10.62
50 mg + M0	5.0	1.03	0.08	12.88	9.84	0.05	1.43	0.55	0.57	71.48	18.24	10.28
+ M1	5.4	1.13	0.08	14.13	11.04	0.06	1.29	0.43	0.48	72.12	15.93	11.95
+ M2	5.4	1.11	0.09	12.33	11.53	0.13	1.65	0.42	0.58	70.26	19.61	10.13
+ M3	4.9	1.00	0.08	12.50	10.14	0.08	1.48	0.81	1.31	75.93	9.03	15.04
+ M4	5.1	0.98	0.08	12.25	9.51	0.07	1.77	0.57	1.23	68.36	21.44	10.20
100 mg+ M0	5.1	1.09	0.08	13.63	10.59	0.05	1.71	0.66	1.89	71.24	17.62	11.14
+ M1	5.2	0.96	0.08	12.00	10.18	0.04	1.41	0.50	0.74	69.14	18.77	12.09
+ M2	5.4	0.91	0.08	11.38	11.42	0.09	1.62	0.46	0.48	71.98	16.35	11.67
+ M3	5.2	1.05	0.08	13.13	9.39	0.33	2.62	0.83	1.31	71.65	17.50	10.85
+ M4	5.3	1.15	0.08	14.38	13.06	0.47	1.51	0.50	0.77	76.92	12.59	10.49
200 mg + M0	5.0	0.97	0.08	12.13	11.56	0.05	1.50	0.82	0.82	73.88	18.28	7.84
+ M1	5.0	1.13	0.09	12.56	10.62	0.04	1.43	0.62	0.66	71.24	18.66	10.10
+ M2	5.5	1.05	0.08	13.13	9.34	0.09	1.57	0.72	0.76	60.20	17.06	22.74
+ M3	5.5	1.19	0.09	13.22	12.93	0.22	1.91	0.62	0.38	57.16	19.04	23.80
+ M4	4.9	0.84	0.08	10.50	9.64	0.03	1.33	0.73	2.69	57.14	18.36	24.50
Average	**5.2**	**1.06**	**0.08**	**13.00**	**10.62**	**0.11**	**1.58**	**0.56**	**0.94**	**69.92**	**16.61**	**13.46**

Note: 0 mg ; 50 mg; 100 mg ; 200 mg = dosage NPK fertilizer , M0 = without inoculation; M1 = inoculation with *Amanita sp*; M2 = inoculation with *Russula sp*; M3 = inoculation with *Scleroderma columnare*; and M4 = inoculation with a cocktail of the three fungi (M1+M2 +M3)

APPENDIX 5
SUMMARY OF THE SIGNIFICANT LEVEL (%) EACH MYCORRHIZAL TYPE CALCULATED FOR THE EFFECTS OF THE VARIOUS INTERACTIONS STUDIED IN EXPERIMENT II. (PERFORON)

Treatments	**Significance level (F value) per measured variable (%)**			
	Type 1	Type 2	Type 3	Type 4
Soil (S)	99**	99 **	99 **	99 **
Sterilized (St)	50 ns	98 **	71 ns	55 ns
Mycorrhizal (M)	2 ns	68 ns	95 *	99 **
Interaction S+St	77 ns	28 ns	93 ns	8 ns
Interaction S+M	59 ns	93 ns	83 ns	8 ns
Interaction St+M	58 ns	56 ns	19 ns	16 ns
Interaction S+St+M	86 ns	69 ns	93 ns	21 ns

Note : S = Soil substrate, St = sterilized soil substrate, M = mycorrhizae
** = 1 % level of significance, ns = not significant

APPENDIX 6
SUMMARY OF THE SIGNIFICANT LEVEL (%) EACH PARAMETER CALCULATED FOR THE EFFECTS OF THE VARIOUS INTERACTIONS STUDIED IN EXPERIMENT II. (PERFORON)

Treatments	**Significance level (F value) per measured variable (%)**							
	H	D	N_l	A_l	W_{tf}	W_{td}	N_{rt}	ECM
Soil (S)	99 **	99 **	99 **	99 **	99 **	99 **	99 **	99 **
Sterilized (St)	99 **	99 **	99 **	92 ns	95 *	95 *	82 ns	16 ns
Mycorrhizal (M)	69 ns	83 ns	55 ns	71 ns	91 ns	82 ns	65 ns	99 **
Interaction S+St	99 **	99 **	99 **	99 **	99 **	99 **	67 ns	77 ns
Interaction S+M	60 ns	47 ns	54 ns	60 ns	17 ns	29 ns	49 ns	62 ns
Interaction St+M	9 ns	22 ns	5 ns	11 ns	10 ns	8 ns	49 ns	52 ns
S+St+M	52 ns	61 ns	37 ns	90 ns	66 ns	63 ns	91 ns	73 ns

Note: S = Soil substrate, St = sterilized soil substrate, M = mycorrhizae ; h = height growth; d = diameter growth; Nl = number of leaves; Al = leaf area; Wtf = total fresh weight; Wtd = total dry weight; Nrt = number of root tips; ECM % = percentage of mycorrhizal roots, *= 5 % level of significance; ** = 1 % level of significance, ns = not significant

APPENDIX 7
RESULTS OF CHEMICAL AND PHYSICAL SOIL ANALYSES OF *S. LEPROSULA* CUTTING GROWN IN PERFORONS AFTER HARVESTING AT THE END OF EXPERIMENT II

Soil substrate	PH	C organic	N Total	C/N Ratio	P available	K	Ca	Mg	Al	Sandy	Silty	Clay
	H_2O	%	%	%	Ppm	Me/100 g				%	%	%
Clay												
Unsterilized+M0	4.8	1.02	0.08	12.75	3.42	0.17	0.92	0.41	5.01	35.05	29.69	35.26
+ M1	4.7	0.98	0.08	12.25	3.02	0.20	0.98	0.49	3.17	58.06	22.14	19.80
+ M2	4.6	0.97	0.08	12.13	3.95	0.14	0.57	1.26	0.99	74.37	10.99	14.64
+ M3	4.6	0.90	0.08	11.25	3.11	0.18	0.21	0.58	5.20	35.81	24.35	39.84
+ M4	4.8	1.03	0.08	12.87	3.29	0.15	0.98	0.47	3.86	39.75	24.11	36.14
Sterilized + M0	4.8	0.81	0.09	9.00	2.93	0.17	0.75	0.64	4.62	34.04	28.48	37.48
+ M1	4.7	0.71	0.07	10.14	3.34	0.19	0.73	0.43	1.55	37.60	25.69	36.71
+ M2	4.7	0.87	0.09	9.70	4.32	0.16	1.11	0.68	6.06	37.42	26.78	35.80
+ M3	4.8	0.80	0.08	10.00	3.22	0.16	0.21	0.53	5.06	33.42	25.36	41.22
+ M4	4.7	0.96	0.08	12.00	3.45	0.16	0.90	0.34	2.96	39.71	20.10	40.19
Average	**4.7**	**0.91**	**0.08**	**11.21**	**3.41**	**0.17**	**0.74**	**0.58**	**0.85**	**42.52**	**23.77**	**33.71**
Sandy loam												
Unsterilized+M0	4.8	1.22	0.11	11.10	3.67	0.13	1.90	0.42	1.45	56.99	20.48	22.53
+ M1	4.8	1.16	0.10	11.60	3.21	0.13	2.05	0.60	0.91	53.47	21.38	25.15
+ M2	4.8	1.12	0.10	11.20	2.43	0.11	2.48	1.20	1.45	54.95	28.16	16.89
+ M3	4.4	1.13	0.10	11.30	3.62	0.11	2.13	1.25	1.64	71.00	12.43	16.57
+ M4	5.0	1.10	0.11	11.60	3.48	0.18	2.36	1.06	1.71	57.30	19.92	22.78
Sterilized + M0	4.8	1.34	0.10	13.40	3.17	0.13	2.14	1.30	2.02	58.46	20.34	21.20
+ M1	4.7	1.14	0.08	14.25	3.42	0.16	1.82	1.13	1.82	73.04	17.98	8.98
+ M2	4.8	1.22	0.10	12.20	3.11	0.16	2.03	0.52	0.87	71.45	16.52	12.03
+ M3	4.9	1.15	0.08	14.38	3.45	0.20	2.17	0.52	1.15	58.83	18.87	22.30
+ M4	4.9	1.17	0.09	13.00	2.92	0.05	1.94	1.00	0.98	56.23	19.64	24.13
Average	**4.8**	**1.18**	**0.10**	**12.40**	**3.25**	**0.14**	**2.10**	**0.90**	**1.40**	**61.17**	**19.57**	**19.26**
Sandy clay												
Unsterilized+M0	5.4	0.81	0.08	10.13	3.08	0.09	1.58	0.30	0.29	55.27	21.36	23.37
+ M1	4.9	0.85	0.08	10.63	3.40	0.07	1.39	0.27	0.38	57.78	19.35	22.87
+ M2	4.7	0.84	0.08	10.50	9.04	0.10	1.44	0.67	1.23	67.67	26.27	6.06
+ M3	5.3	1.03	0.07	14.72	9.94	0.07	1.88	0.39	0.48	65.43	27.11	7.46
+ M4	4.9	0.98	0.08	12.25	8.86	0.05	1.52	0.44	0.82	79.79	14.91	5.30
Sterilized + M0	4.9	0.96	0.09	10.67	9.58	0.07	1.37	0.40	0.87	79.39	13.74	6.87
+ M1	4.6	0.88	0.08	11.00	10.04	0.03	1.34	0.34	0.58	77.95	11.03	11.02
+ M2	4.9	0.86	0.07	12.29	10.71	0.02	1.24	0.57	0.82	74.32	13.46	12.22
+ M3	4.6	0.91	0.08	11.38	12.58	0.05	1.56	0.52	0.67	73.61	9.58	16.81
+ M4	5.1	1.09	0.07	15.57	10.41	0.07	1.47	0.27	0.77	78.17	8.73	13.10
Average	**4.9**	**0.92**	**0.08**	**11.91**	**8.76**	**0.06**	**1.48**	**0.42**	**0.69**	**70.94**	**16.55**	**12.51**

Note: M0 = without inoculation; M1 = inoculation with *Amanita sp*; M2 = inoculation with *Russula sp*; M3 = inoculation with *Scleroderma columnare* and M4 = inoculation with cocktail of the three fungi.

CURRICULUM VITAE

R. MULYANA OMON, was born in Bogor, West Java, Indonesia on December, 29th, 1950. He finished the Primary School (SR) in 1963, the Junior School (SMP) in 1966 and the Senior School (SMA) in 1969 in Bogor, West Java. Then he studied at the College of Forestry (Akademi Ilmu Kehutanan) in Bandung, West Java and graduated in 1974. After graduating from College of Forestry, he worked at the Forest Research Institute Bogor (BOSBOUW) from 1974 to 1980. In 1981 he moved to the Sub Forestry Research Institute Samarinda as head of Wanariset (Research Station) at Samboja, East Kalimantan and he studied at the Faculty of Forestry, UNMUL (Mulawarman University) in Samarinda East Kalimantan and finished his studies there 1982. In 1983 Ministry of Forestry was established, and he moved to Bogor to the Forest Research and Development Center (FRDC) Bogor where he worked from 1983 to 1985. From 1986- 1988 he worked in Irian Jaya as Project Leader at the Forestry Research Institute Manokwari, Irian Jaya. In 1989 he moved to Bogor (FRDC) and in 1991 he studied at Bogor Agricultural University (IPB) where he obtained his MSc degree in 1994. He worked at the Tropenbos Kalimantan Project as Project Manager from 1994 to 2000 and has been working as a researcher in Forest Rehabilitation and Stand Establishment group from 2000 until now. During all this time he has given many lectures to students and trainees concerning nursery techniques for Dipterocarpaceae, especially concerning the method of reproducing Dipterocarpaceae by cuttings and mycorrhiza inoculation. He supervised students of Mulawarman University Samarinda (UNMUL) and Bogor Agricultural University (IPB) in the field. He was admitted to a Ph.D sandwich programme at Wageningen University and Research (WUR), the Netherlands on November 1996. During his research, he was supervised by his promotor Prof. Dr.Ir. R.A.A.Oldeman (Wageningen University) and the co-promotors Dr.W.T.M. Smits (Director Gibbon Foundation) and Dr.Ir. Supriyanto (Bogor Agricultural University). In 1976 he married Nurul Chotimah and they were blessed with three sons. The oldest son's name is Eka Permana, the second is R. Fajar Dwi Permadi and the youngest is Aria Tri Peryoga.