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MIOMBO TREES AND MYCORRHIZAE

Ecological strategies, a basis for afforestation

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MIOMBO TREES AND MYCORRHIZAE

Ecological strategies, a basis for afforestation

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PROPOSITIONS

The survival of miombo seedlings hinges upon their ability to feed upon themselves during periods of drought and to withdraw water and energy from themselves in order to resume growth towards the end of the long dry period.

A forest ecosystem is a living system in which trees can not be claimed to be superior to other organisms, except in biomass.

Deficiency symptoms in biological and socio-economic systems in the tropics do not always indicate the absence of the apparently lacking element.

The majority of Africans have never seen a tropical forest.

Whereas the cold war has apparently finished, the wars seem hotter than ever.

Root pruning of miombo seedlings for the purpose of planting them in the miombo zones, is producing disabled subjects abandoned to the care of nobody.

Although preservation of biological diversity is now high on the agenda in nature management, in human society the extinction of ethnic and cultural diversity is proceeding stronger than ever.

He who takes a hasty decision to cut down a huge canopy tree while living in the neighbourhood of its phyllosphere and rhizosphere is not wise.

All living organisms will once escape life but none will escape death.

The strenght of symbiotic bonds in ecosystems is like social ties in human society, in that the amount of food available to each organism plays a crucial role.

In miombo seedlings lateral roots and rhizomorphs are to the taproot, what water streams are to the river passing through a desert.

Survival of just germinated seedlings in tropical forests, apart from physical factors, depends upon the way in which the seedlings fit in an existing network of roots and hyphae.

'Love' is a bottomless pit: millions have fallen inside; if at all anyone reemerges, injuries are severe.

The liberation of man today must seek a way to free him not from external enemies, but from himself.

Seeds of division preserve their viability for a long period and once germinated, they survive periods of drought, floods and frosts.

PREFACE

This study has been guided and supported by many persons and institutions to whom I would like to express my gratitude. I would like to express my sincere appreciation to Prof Dr Ir R.A.A. Oldeman, my promoter and the promoter of the work discussed in this book. Professor Oldeman has guided this work from its embryo to its completion. His contribution is partly reflected in the number of donors whom he made aware of the existence and the needs of this project and who financially supported the implementation of the project. Professor Oldeman has been a promoter indeed. I would like to convey my gratitude to Dr Th. W. Kuyper, my co-promoter. Despite his daily booked agenda, Dr Kuyper made my study one of the priorities. Regular discussions conducted with him and literature and materials provided by him, greatly improved the quality of the work discussed here. I am highly indebted to the Sokoine University of Agriculture (SUA), especially to the Dean of the Faculty of Forestry, the heads of Department of Forest Biology, Forest Management and Mensuration, Wood Utilisation and Forest Engineering for their cooperation and for providing the available facilities for my work. I thank Dr Lulandala, Dr Temu and Dr Maganga of the department of Forest Biology to which I was attached, for their scientific and technical advise. I would like to thank Dr Maganga in a special manner for his kindness and the amount of time he devoted to me. I want to thank the heads of the Department of Crop Science and of the Department of Soil Science for allowing me to use their facilities during my greenhouse and laboratory work. The chapter on iron deficiency is based on discussions I conducted with Dr Semoka. I would like to thank him for his advice. Mr Kitojo, Mrs Mtengeti, Mr Msalilwa, Mr Malekela, Mr Somitera, Mr Rumwecha, Mr Pius and their colleagues have offered their technical skills during my laboratory and nursery work. I would like to thank Dr Termorshuizen and Dr Jonkers for reading and commenting upon some of my manuscripts. Dr Termorshuizen has also helped in

drawing graphs and making a Dutch summary translation. Dr Arnolds has been one of the authors of my interest in mycorrhizae. I would like to thank him. I am thankful to my Tanzanian colleagues, Dr Madoffe, Mr Nsolomo, Mr Mattaba, Mr Ndossi, Mr Sirito, Mr Mrecha and Mr Makupa for their cooperation. I would like to thank the manager and the personnel of the DANIDA/TANZANIA National Tree Seed Project (NTSP). The NTSP has generously offered almost all seeds used in this study. I am grateful for the cooperation I received from the Department of Ecological Agriculture, the Biological Station, the Department of Forestry and the department of Phytopathology of Wageningen Agricultural University (WAU) and the cooperation of their personnel during the preparation of the research proposal and the writing up of this book. This study could not have been realised without the financial support from a list of institutions and persons. The International Foundation for Science (IFS) generously offered me a grant to cover all costs incurred in the field and in the laboratory during my stay in Tanzania. The IFS grant covered transport, casual labour, equipment, laboratory and nursery materials, literature and miscellaneous. I would like to thank Dr Sabine Bruns, Scientific Secretary and her colleagues for administering the grant. The WAU offered me a scholarship, which covered a ticket to Tanzania and back, the costs of living, study and University fees in the Netherlands while preparing the research proposal and processing data presented and discussed in the present work. Dutch friends and institutions contributed to my living allowance in Tanzania. This was done through opening at Wageningen an account "Studiefonds E. Munyanziza". Since I do not know all names of the contributors, I would like to request Dr Jonkers and Mrs Angélique Bosch-Jonkers to convey my thanks to all those contributors who wish to remain anonymous. Dr Jonkers and Mrs Bosch have administered the account. I thank them in a special manner. My special thanks also go to the 'Stichting Thurkow Fonds' and the Foundation Pronatura, the 'Fundatie van de Vrijvrouwe van Renswoude te Delft' and the 'Stichting

Wageningenfonds' of the WAU for their financial support. Mr Ledoux and his wife and the Lions Club also contributed to my living allowance in Tanzania and in the Netherlands. The costs of the multiplication of the book have been met by the LEB-fonds, the Treubmaatschappij and the subsidy from the WAU. I would like to convey to them my gratitude. There are many other friends who directly or indirectly supported my study. I convey to them a word of thanks. I want to thank Ir G. Pennings for helping in analyzing data with the computer. Yvonne Claassen, my wife, has actively participated in data collection, processing and writing up. She took all photos of the present work and took care of my daily life after we married. To her I am indebted. My thanks also go to Alexandra van der Sleesen for correcting part of this work and for advising how I could improve the rest regarding English language. The family Ledoux, fam. Hennink, fam. Haverkort, fam. Oldeman, fam. Claassen, and fam. Lukwaro made my stay outside Rwanda enjoyable. The community of Adventists of SUA and that of Ede created for me a weekly rest during which I could recharge myself for the weekly load. I finally want to express my love to my parents Samuel Sebarame and Genaroza Nyiragatanga for their priceless sacrifice for more than 20 years that I spent on the school benches and especially the 9 years I spent studying outside Rwanda.

Esron Munyanziza

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Chapter 1

GENERAL INTRODUCTION

1.1 Miombo vegetation

1.1.1 Plant ecological aspects and distribution

The majority of Africans have never seen a tropical forest. However, most African people have seen shrubs, trees or at most woodlands. The coming generation in many African countries may not even see woodlands unless immediate action is taken. The term *woodland* refers to stands of trees reaching a height of 15 to 20 m, with their crowns just touching each other and forming a light canopy. This contrasts strongly with the lofty evergreen rain forest and its complex, interlaced deep canopy. Miombo woodlands are dominated by the genera *Brachystegia* and *Julbernardia* (Lind and Morrison, 1974). The architecture of the miombo woodlands is structured by three layers. A dominant tree layer of 14-18 m, sometimes up to 21 m in height, has a density of approximately 65 trees per ha; a secondary tree layer of 8-12 m but sometimes up to 14 m in height has a density of about 80 stems per ha and a shrub layer, less than 8 m tall has 375-500 stems per ha (Malaise ex Celandier, 1983).

The miombo woodlands constitute the largest single biome in Tanzania (Temu, 1979). They cover about two thirds of the country and extend 1000 miles southwards through Zambia and Mozambique into Zimbabwe and westwards to Angola and Zaïre. In Tanzania they extend from sea level up to 1600 m in areas receiving 500 to 1200 mm of rain (Lind and Morrison, 1974). The miombo woodlands constitute a supply of timber to wood industries. Important timber tree species harvested from the miombo woodlands are (Abeli, 1990) *Pterocarpus angolensis* D.C. (Leguminosae-Papilionoideae), *Afzelia quanzensis* Welw. and species of the genera *Julbernardia* and *Brachystegia*

(Leguminosae-Caesalpinioideae). *P. angolensis* and *A. quanzensis* have very valuable timber. *P. angolensis* is known as the premium of East Africa (Bryce, 1967). Other main products harvested from the miombo woodlands include charcoal, beeswax (Lind and Morrison, 1974), food and medicines. (Celander, 1983; Härkönen et al., 1993).

1.1.2 Trees of the miombo woodlands and symbiosis

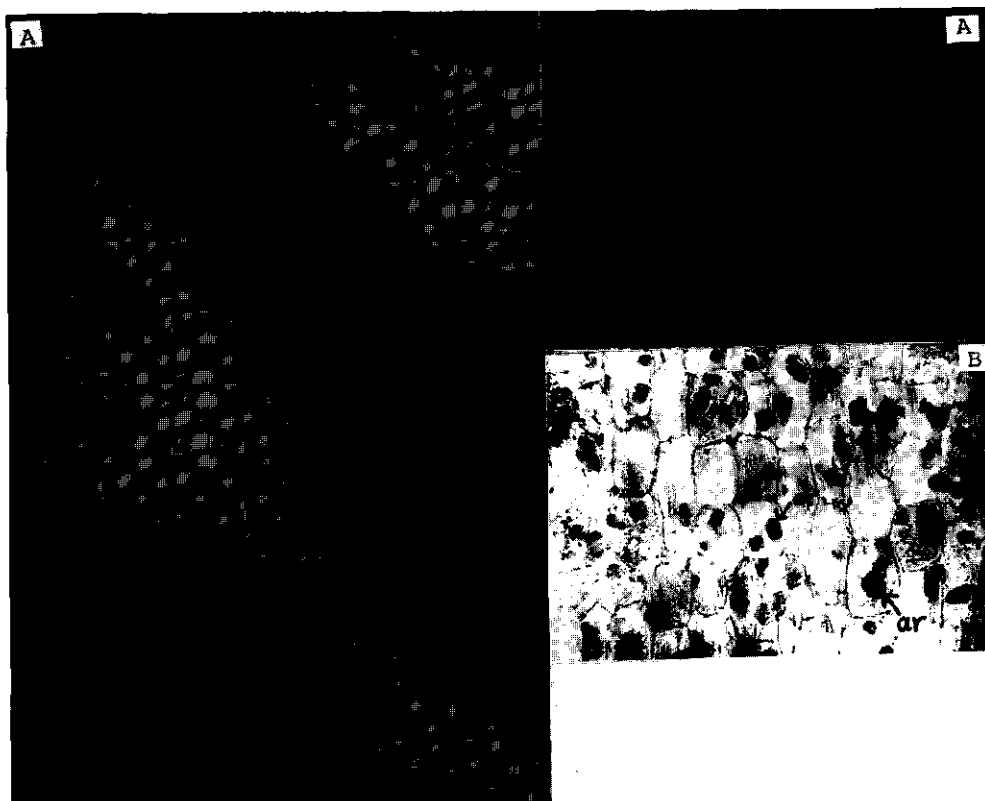


Fig. 1. The two dominant types of mycorrhizae associated with miombo trees. (A) ectomycorrhizal root and its cross section (this work) (B) VA mycorrhizal root section (ex Schenck, 1991).

Surveys carried out in various African countries indicate that most of the shrubs occurring in the miombo woodlands form one of two dominant types of mycorrhiza, namely vesicular-arbuscular (VA) mycorrhizae (fig. 1b) or ectomycorrhizae (fig. 1a). In addition, some plants of miombo woodlands biologically fix nitrogen.

Mycorrhiza is a symbiotic association between plants and fungi. This association takes place on the root level. A mycorrhiza is a root-fungus "organ" resulting from the association.

Ectomycorrhizae are characterized by the fungal sheath (sh) on the root surface and the penetration of fungal hyphae between the cortical cells where they form a net referred to as the Hartig net (hn). Usually hyphae and/or rhizomorphs emanate from the sheath and radiate in the surrounding soil. Ectomycorrhizal association prevails in trees of various families in the temperate countries (Harley and Smith, 1983). Available information on tropical mycorrhizae indicates that ectomycorrhizae also constitute an important feature of some tropical forests. For example, tropical pine forests (Ivory, 1980) and dipterocarp forests (De Alwis and Abeynayake, 1980; Smits et al., 1987) in South East Asia form ectomycorrhizae. Trees dominating the miombo woodlands, namely *Brachystegia*, *Julbernardia* and *Azelia* species, form ectomycorrhizae in nature (Högberg and Nylund, 1981; Högberg, 1982).

Vesicular-arbuscular mycorrhizae are found in the majority of plants growing in the miombo woodlands (Högberg, 1982; Högberg and Pearce, 1986). In this type of mycorrhiza, the fungal hyphae penetrate into the cortical cells where they branch, forming structures known as vesicles and/or arbuscles (ar; fig. 1b). VA mycorrhizae do not have a fungal sheath. *P. angolensis* forms endomycorrhizae in nature and nodulates. More than 90% of higher plants form VA mycorrhizae. VAM are common in crops, grasses, shrubs and most of tropical tree species.

VA mycorrhizal fungi are not saprophytic. Ectomycorrhizal fungi show varying levels of saprophytic ability. Almost all of their energy requirements are met by their tree partner. In

return, ectomycorrhizal fungi ensure a number of benefits for their host. These include increased water and nutrient uptake, resulting in increased growth and survival (Bowen, 1980). The advantage of mycorrhizal formation is great when plants are growing in adverse sites. Trees which fix nitrogen biologically have an additional advantage when they are growing in a site deficient in nitrogen.

1.2 Ectomycorrhizae in exotic pine plantations

Pine trees are naturally associated with ectomycorrhizal fungi (Harley and Smith, 1983). When pines were artificially introduced in various parts of the world, the role of mycorrhizae for their growth and survival was appreciated (Mikola, 1970). Plantation failures were experienced until mycorrhizal inoculum was also artificially introduced and mixed with the growing medium (Mikola, 1980). The first successful establishment of pines in tropical Africa dates from 1927. This was achieved after the introduction of mycorrhizal soil from Europe via South Africa (Kaumi, 1980). Thus most of ectomycorrhizal fungi observed in exotic pine plantations in the tropics are those reported from the temperate countries. Most of these fungi are basidiomycetes and include several species for instance, *Rhizopogon luteolus*, Fr.; *Suillus granulatus* (Fr.) O. Kuntze *Boletus* spp.; *Amanita* spp.; *Thelephora terrestris* (Ehr.) Fr.; *Russula* spp.; *Inocybe* spp., *Scleroderma* spp. (Ivory, 1980).

Comparison of reports on mycorrhizal survey in exotic pine plantations and nursery studies (Maghembe, 1982, Thoen and Bâ, 1989, Ivory and Munga, 1992) indicates that some fungi occur in both indigenous vegetation such as the miombo woodlands and exotic pine plantations.

1.3 Location of the study and site conditions

Most of the experiments carried out in this study took place in the miombo woodlands on the foot of Uluguru Mountains in Morogoro. The others were carried out in Sao Hill (Iringa), in Meru (Arusha) and Mazumbai within the Usambara Mountains in Tanga Region, Tanzania (fig. 2a). The miombo woodlands covered by this study are characterized by a harsh climate. Rainfall is limited and irregular. The annual rainfall varies from 500 to 1200 mm. There is a short hot rainy season from November to January and a long rainy season from the middle of March to end of May. However, in general, the miombo woodlands are characterized by one wet season and one dry season. The highest temperatures are reached in October or November although peaks may occur, for example in Tanzania, as late as January. The daily maximum temperatures often exceed + 30 °C. The average temperature per annum is around + 20 °C (Celander, 1983).

Most miombo soils have no organic layers. The litter is yearly burned or consumed by termites (Trapnell et al., 1976). Fires are often followed by rain, resulting in soil erosion (fig. 2b). Miombo soils are also characterized by low CEC, low nitrogen content, and little phosphorus. Högberg (1992) estimated that organic carbon is less than 1% and total nitrogen less than 0.1%.

Sao Hill, Arusha, and Mazumbai, have a cool climate and higher rainfall than Morogoro. According to Balmforth (ex Lundgren, 1978), the average temperature is between 7 and 29 °C. Exotic pine plantations have been established in Sao Hill and Arusha. Mazumbai has a montane rain forest.

1.4 Justification

For most African countries, the term tropical forest means woodlands. The miombo woodlands constitute the most complex vegetation of this type (Lind and Morrison, 1974).

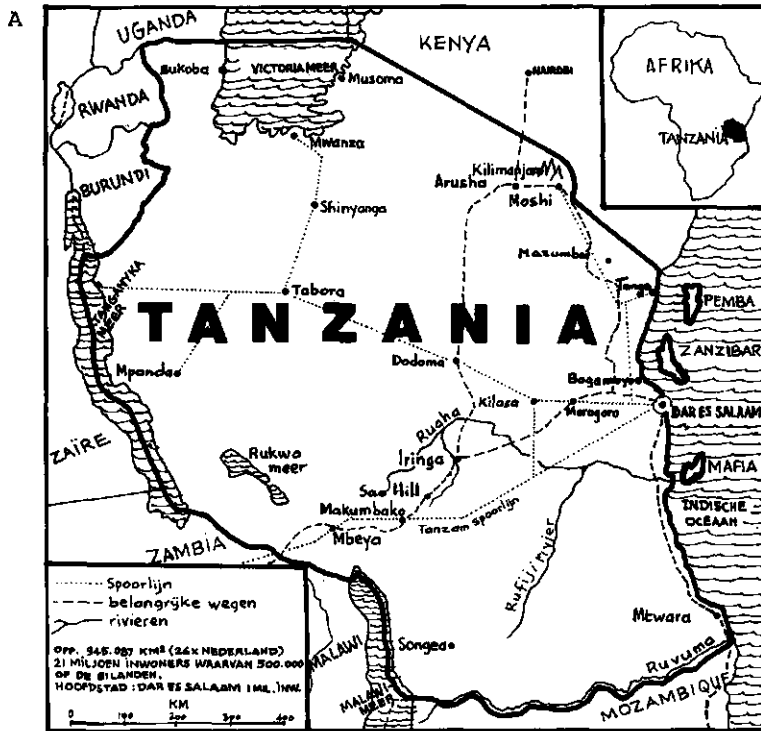


Fig. 2. Location of the study. (A) map of Tanzania showing Arusha, Sao Hill, Mazumbai and Morogoro (Bijeen et al., 1986/87) (B) eroded site in degraded miombo woodland, Morogoro.

Their wide distribution (Temu, 1979), together with recent rapid decline due to fires and cutting for various reasons (Burley et al., 1989), make a new management policy important. In the past in afforestation and agroforestry, emphasis has been put on exotic fast growing species, namely *Pinus*, *Eucalyptus* and *Leucaena* spp. (Ojo and Iyamabo, 1977). But today, there is a clear need to draft and implement a new forest policy. This should include the miombo woodlands for a number of reasons. (1) The miombo woodlands grow naturally in areas where conditions are unfavourable for pines. (2) In most African countries, the aim of planting is more than only production of wood. (3) The miombo woodlands need to be understood as an ecological entity, and preserved. (4) There are increasing outbreaks of disease and pests in exotic plantations (Chamshama et al., 1992).

There is, however, a lack of knowledge regarding the silviculture and management of miombo woodlands (Abeli, 1992). As Mgeni and Malimbwi (1990) and Refsdal (1992) recommended, research should be carried out on the ecology and silviculture of this type of vegetation. Sustainable management of natural forests and successful afforestation on poor sites are unlikely to succeed unless natural plant-fungal partnerships are understood and included in such programmes. Mycorrhizal fungal hyphae supplement the root system by equivalent of finer roots than the finest roots that plants use in water and nutrient uptake. Mycorrhizal fungi in the miombo woodlands have not yet been studied in relation to afforestation.

1.5 Outline of this book

This book is based on a number of experiments and observations made during the field work in the areas indicated above. The objectives of this work were the following ones.

- (1) To establish the possible constraints that may hinder natural regeneration of the main miombo tree species, namely those in the genera *Pterocarpus*, *Azelia*,

Julbernardia and *Brachystegia*.

- (2) To assess the symbiotic status of seedlings.
- (3) To make a survey of ectomycorrhizal fungi associated with the dominant tree species in nature and in the nursery.
- (4) To assess the symbiotic status of pine and indigenous tree seedlings in existing nurseries and advise accordingly.
- (5) To develop a package of recommendations as how to raise the species with special reference to seed germination, timing, root pruning or direct sowing, fertilization, climatical range and inoculation.

Chapters 2 to 4 present and discuss results concerning the germination, establishment, early growth and symbiotic status of *Pterocarpus angolensis*, *Julbernardia globiflora* (Benth.) Troupin, *Brachystegia microphylla* Harms, *B. spiciformis* Benth. and *Afzelia quanzensis* in the nursery and in the field. Chapter 5 is concerned with pines, specifically mycorrhizal deficiency and establishment in the nursery and in the field. Chapter 6 is a general discussion. The book concludes with practical hints for nurserymen and foresters. The taxonomy and typology of ectomycorrhizal fungi and ectomycorrhizae is under preparation and will appear later in a separate publication. Appendices of the current book, however, illustrate the main mycorrhizal types encountered in this study and the mycorrhizal fungi used in inoculation trials (pages 181-185).

1.6 Data analysis

Unless stated otherwise, data were entered using the spreadsheet program Lotus 123, version 2.2. They were then analyzed using the relevant test within SPSS/PC+ version 3.1. The relevant tests are indicated in the text.

Chapter 2

PTEROCARPUS ANGOLENSIS (LEGUMINOSAE-PAPILIONOIDEAE)

2.1 General introduction

The genus *Pterocarpus* is pantropical (Rojo, 1977). *P. angolensis* occurs on the African mainland (Boaler, 1966) where it is one of the most valuable timber tree species of the miombo woodlands (Mgeni and Malimbwi, 1990). *P. angolensis* grows naturally in areas with a harsh, long dry season and irregular rainfall and affected almost yearly by wildfires. For the description and details of the geographical distribution of the species see Rojo (1977).

Due to the high value of its timber and the near-absence of information on alternative tree species, *P. angolensis* has been cut back to vulnerable levels. Its exploitation goes back to the 18th century with the arrival of Arabs. Since 1977, the government of Tanzania has banned replacing indigenous forests by exotic ones (Kimariyo, 1990). In addition a decision to specifically protect *P. angolensis* was taken. Today, isolated *P. angolensis* trees can be seen in farms, pastures, abandoned lands, or degraded miombo woodlands. However, illegal cutting is hard to control.

Though the value of the species is known since time immemorial, information on its silviculture is scanty. The ecology and the survey of the species in Tanzania have been studied by Boaler (1966). Nonetheless, no adequate knowledge on raising the species in artificial plantations is available.

Up to this moment the DANIDA/TANZANIA NTSP has tried with very limited success to increase germination of the species and to produce a nursery stock for afforestation and agroforestry programmes.

Artificial afforestation with this species should be encouraged for three reasons.

1. The low levels to which the species has been reduced

demand restoration.

2. Human society values the species, which has a negative outcome mentioned above and a positive one in the governmental decision to protect it.
3. The species has a low regeneration and survival rate.

The objectives of this study were therefore:

- (1) to assess the fate of fallen fruits of the species and germination and survival of seedlings under natural conditions;
- (2) to develop germination techniques for the nursery;
- (3) to assess the symbiotic status of the species;
- (4) to assess the effect of root pruning and fertilization;
- (5) to establish the causes of seedling mortality currently observed in nurseries.

These objectives were pursued in 4 experiments.

It is very important to understand the natural constraints on germination and the problems encountered by the seedlings. There are two reasons:

1. to design proper management systems for the miombo woodlands, with natural regeneration as an objective;
2. to devise an adequate nursery technology for seedling production in afforestation or agroforestry ventures.

2.2 *Pterocarpus angolensis*: its natural survival, fruits and germination

2.2.1 Introduction

P. angolensis produces a huge amount of fruits which ripen during the dry season. Fruits drop at a time when thick layers of dry grass and leaves have accumulated on the ground. The regeneration rate of *P. angolensis* is low. This has been observed in South Africa (Van Daalen, 1991). In Tanzania, Boaler (1966) estimated that only 2% of the fruits produced resulted in seedlings. *P. angolensis* is, however, a species reported to be able to tolerate fires (Celander, 1983). One of

the peculiar features of *P. angolensis* is the nature of its fruit. This is a non-dehiscent pod, some 3 cm in diameter and 2.5 cm thick, surrounded by a wing about 3 cm wide. The central part bears slender bristles, the longest of which are some 2 cm in length. It contains two loculi, one or both of which may be empty (Boaler, 1966). The light weight (4 g) in relation to the surface and the overall flattish shape of the fruit (Rojo, 1977), combined with the presence of the wings and bristles constitute important features in the dispersal and the survival of the species.

However, the real situation is not so simple.

1. The fruit is not attractive to frugivorous mammals and birds, hence liberation of the seed by animals is not likely.
2. It is unlikely that the fruit touches the soil for decomposition and liberation of the seed. The fruit usually lands on top of a thick layer of grass (fig. 3).
3. In the event of fire, such a position makes the fruit vulnerable. Yearly fires in miombo woodlands today are the rule rather than the exception.

The aim of this study was therefore, to assess the fate of fallen fruits, and to monitor the germination and survival of seedlings under natural conditions. This will help establish the cause of low regeneration and survival rate.

2.2.2 Materials and methods

In September 1992, more than 3000 air-dried fruits of *P. angolensis* were picked from one tree at the foot of Uluguru Mountains just above the boundary of the Sokoine University of Agriculture, in Morogoro (fig. 3). The fruits were taken to the laboratory for processing and for seed extraction.

The first task consisted of estimating the number of seeds per 100 pods. Twelve samples of 20 pods each were drawn at random and opened by carefully hammering with a stone to extract the seeds for examination. Extracted seeds were

classified as sound if they had a filled and polished testa. Other seeds with an unfilled testa and/or with fungal growth were categorized as unsound.

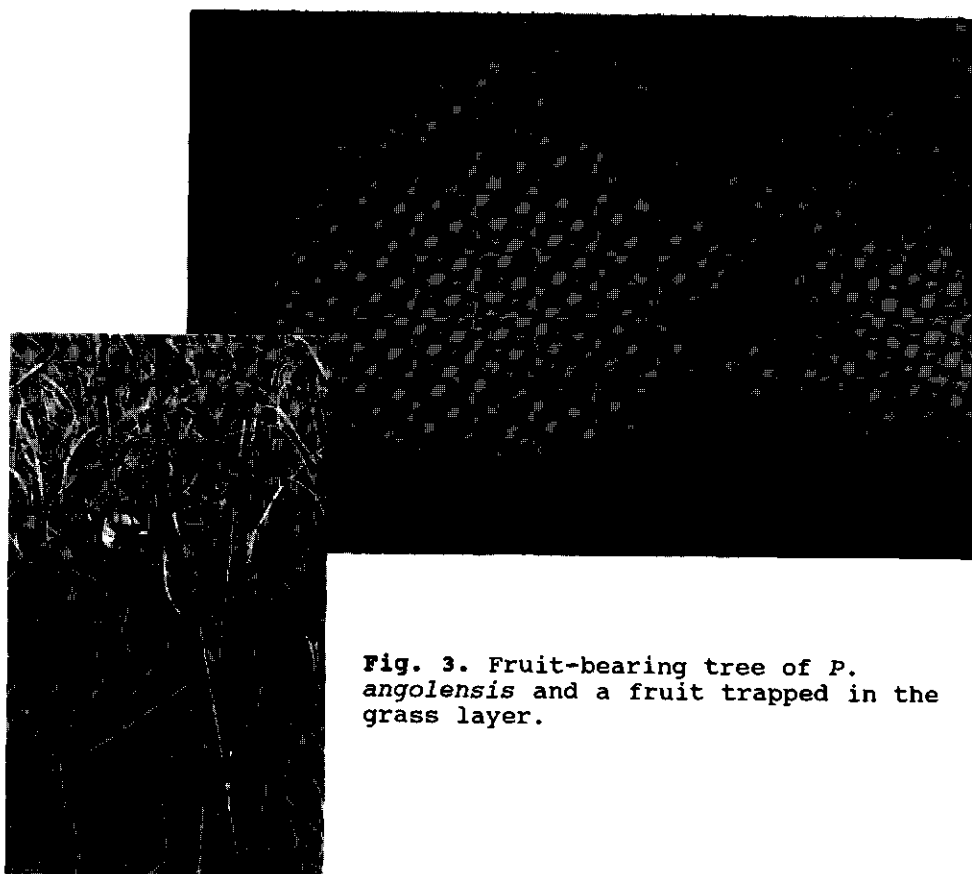


Fig. 3. Fruit-bearing tree of *P. angolensis* and a fruit trapped in the grass layer.

The fruits collected or the seeds extracted were subjected to treatments conceived to imitate the situations that the fallen fruit most commonly encounters. These treatments were applied to fruits and/or seeds laid out in plots of 80 cm X 80 cm. Every treatment covered 80 seeds or 100 pods spread over four replications.

The following treatments were applied.

- (1) Seeds were mechanically extracted and sown within 2 cm depth in a ploughed plot.

- (2) Fruits were laid on a layer of dry grass (80 cm X 80 cm X 40 cm).
- (3) Fruits were laid under the layer of dry grass as thick as (2).
- (4) Fruits were buried in soil within 2 cm depth.
- (5) Fruits were laid on a layer of grass (see 2), the whole pile was set on fire, and recovered fruit material was laid out on a bare plot.
- (6) Fruits were treated with fire as above but the remains of the fruits were buried (see 4).

The above treatments were randomly assigned in four replications.

After each treatment, fruits and seeds were left exposed to the prevailing weather conditions. Germination and disappearance of fruits were assessed. A fence was built around the experimental area to prevent destruction by large animals. The experimental area was very gently sloping.

At the end of the rainy season, i.e. at the beginning of the long dry season (June), an attempt was made to recover the fruits and/or seeds. Recovered seeds were treated with an electric burner (2.3) and sown in pots in the greenhouse. Watering in the greenhouse was plentiful and regular until the end of the experiment.

The experiment took place on the campus of the Sokoine University of Agriculture, on the foot of Uluguru Mountains, and ran from November 9, 1992 to June 23, 1993, so covering both rainy periods.

2.2.3 Results

Extraction of seeds

The number of seeds per pod ranged from 0 to 2. Most of the pods had one seed. The mean number of sound seeds per 20 pods as computed from 12 samples of 20 pods each, was 16 (SD = 2.1). This represents 80 % of the pods processed. But out of

sound seeds only 54 % did not show visible damage caused by extraction. Thirty seeds without visible damage could be extracted per hour.

Burning

Burning reduced 64% of the pods to ashes. The remaining pods lost their wings and bristles. Fire penetrated also inside but the central part of the pod was not reduced to ashes.

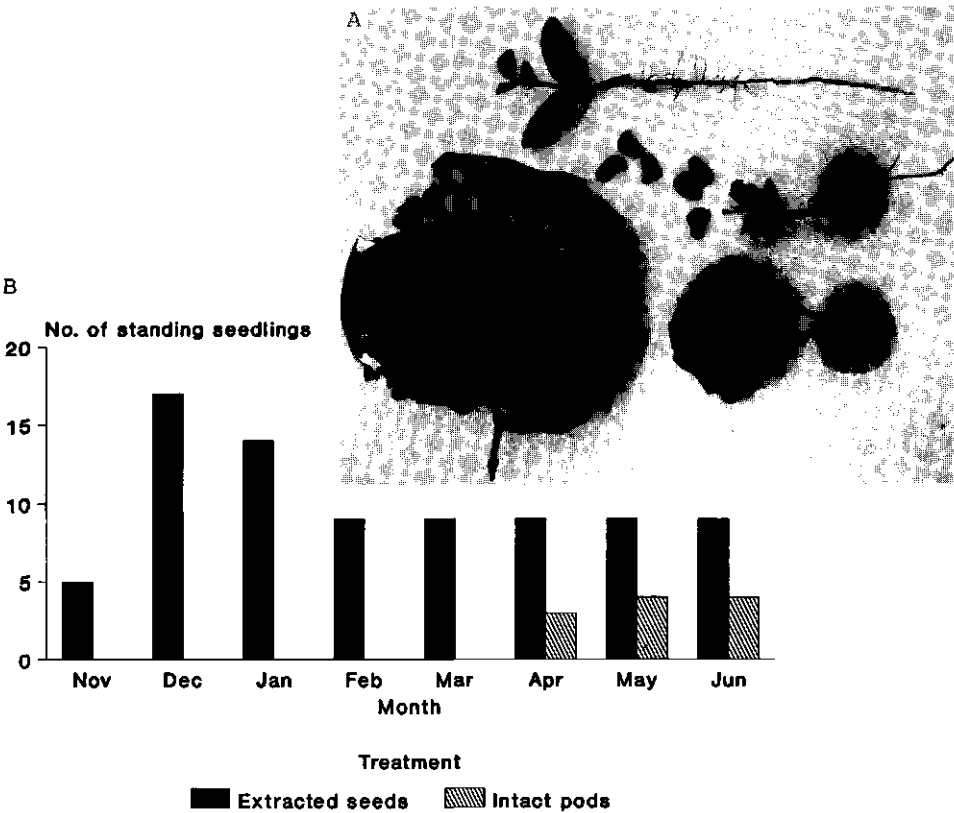


Fig. 4. Germination of *P. angolensis* in the field (A) fruit decomposition and seed germination (B) survival in the field.

Germination

Some of the extracted seeds germinated during the short rainy season (table 1). No seeds germinated during the long rainy season. Burned pods did not germinate at all. No unburned pods germinated during the short rains. Very few germinated during the long rains. Some pods germinated underground, but as the rain stopped and the soil hardened before germination was completed, seedlings failed to emerge above the ground. Such seedlings became deformed and had a whitish colour.

Germination of extracted seeds started on 29/11/92. The germination of intact pods was a process of decomposition of the fruit (fig. 4a). In this treatment, germination started on 18/4/93. The highest cumulative number of surviving seedlings from extracted seeds was reached on 6/1/93. It then dropped to a stable level which survived to the end of the experiment (fig. 4b).

Table 1. Mean number of surviving seedlings per plot from different treatments as at the end of the short and long rains. IP = intact pods; BP = burned pods. Standard deviations (SD) are indicated between parentheses.

Treatment	Mean survival	
	start of January	end of June
Extracted seeds	4.3 (1.6)	2.3 (3.2)
IP above ground	0.0 (0.0)	0.3 (0.5)
IP under organic matter	0.0 (0.0)	0.3 (0.5)
IP in the soil	0.0 (0.0)	0.5 (0.6)
BP above the soil	0.0 (0.0)	0.0 (0.0)
BP in the soil	0.0 (0.0)	0.0 (0.0)

Description of materials recovered at the end of the rainy period

None of the extracted seeds was recovered if they had not germinated. Burned pods remained intact regardless of their having been buried or laid on top of the soil. However, their seeds had turned black as a result of the burning. Unburned pods were broken down by termites together with underlying and overlying grasses. Pods became chopped whatever their previous position. This decomposition process was triggered by rain and was faster in the soil and under the litter than above the litter. However, the difference disappeared with more rain and as the pods became overgrown with grasses. At the end of the experiment, non-germinated pods were reduced to their central core (fig. 4a). From this core, it was easy to extract the seeds by hand or by soft hammering for further experimentation.

The inner part of the pod surrounding the seed and the outer part of the seed were overgrown by a white mycelium. Seeds extracted at the end of the experiment had not expanded. They were still hard. The amount of recovered non-germinated pods was 100 % of the total above the ground, and 96 % below.

Germination at the end of the experiment

When extracted from the pods at the end of the experiment, and scarified by an electric burner, the seeds germinated. They did so in 97% of the cases, 66 out of 68, among the buried pods and in 94% of the cases, 148 out of 158, in non-buried ones, within 11 days and over a period of 6 days since first germination was detected.

2.2.4 Discussion

The shape and the nature of *P. angolensis* pods has advantages and disadvantages in nature.

1. The species can disperse its pods over long distances, increasing the likelihood of pods landing where conditions favour germination.
2. Toughness of the indehiscent pod prevents germination until sufficient rain has mechanically and/or chemically unblocked the seed by triggering pod decomposition.

The shape and the nature of the pod is detrimental in at least two respects.

1. In all likelihood, dispersed pods will land on a pile of fuel. This is a potential hazard for wildfires.

The destructive effect of fire on the propagules of *P. angolensis*, as reported by Van Daalen (1991), was confirmed in the present study.

The amount and type of fuel used in our experiments were far less intense than in natural conditions. However, damage caused by fire to seeds in our study was enormous. Mgeni and Malimbwi (1990) estimated that yearly fires in miombo woodlands can completely destroy even big trees of *Julbernardia* and *Brachystegia* species.

2. Apart from fire hazards, the shape of the pod nearly always prevents the pods from touching the ground, which they must do for germination.

Van Daalen (1991) also pointed out this as a hindrance to germination. Boaler (1966) and Von Breitenbach (ex Van Daalen, 1991) estimated that fires would be beneficial, because they would establish contact between seed and soil. This would be true only if fires were kept at low levels.

This study has shown that burning destroys the pod but also the seed. Burning also eliminates the organic grass layer which, as a "natural mulch", has a beneficial effect in a fire-free environment.

In this study, the liberation of seeds was highly dependent on the biological activity in the litter, of which the fallen pod is a part. Termites were the primary actors in breaking down the pod of *P. angolensis* together with underlying organic matter, thus creating a natural favourable seedbed for germination. Termites and fires in the savanna

woodlands are the main agents responsible for the breakdown of organic matter (Trapnell, 1976; Wormald, 1992). The former are beneficial, the latter destructive. No termites were observed attacking the remains of burned pods. According to Kajembe (pers. comm.), it is an established tradition in Tanzanian rural areas to protect seedlings against termites by surrounding them with ash. The same practice has been shown to be effective in Rwanda. Fungi also may have played a role in the breakdown of the fruits. A white mycelium had extensively colonised the inner part of the pods and the seed surface.

Elsewhere (Beniwal et al., 1990), the positive effect of mulching on tree seed germination has been reported. No doubt mulching or increased organic matter boosts biological activity and increases soil moisture retention. Mulching was found to be vital for the survival of seedlings and it was recommended in afforestation of dry areas (Singh et al., 1988). This recommendation also holds for miombo tree species. In another study (Munyanziza in prep.), sound seeds of *J. globiflora* were laid out on bare plots which were recently burned, or under a layer of dry grasses. Seeds were daily irrigated. It was noted that higher and more rapid germination was achieved with seeds in organic matter than on a bare plot. Germination failure was mainly ascribed to low moisture on the bare plot. In the same experiment, fire treatment of seeds inhibited their germination while non-treated ones germinated readily.

During the short rains of November to December, pods failed to germinate, but extracted seeds germinated. A lot of rain is apparently needed to produce enough moisture to penetrate the pod, reach the seed and be absorbed. In other words, the short rains were insufficient to reach and soften seeds in the pod, although the same rains were enough to initiate germination in at least 21% of extracted seeds. Germination failure or delay in non-extracted seeds allowed the seeds to survive until the onset of the long rain season of April to June. Extracted seeds did not survive. Their germination during the short period of rain exposed them to

mortality due to the drought which followed.

However, seedlings which emerged from extracted seeds during the short rain, and survived through the drought period following, until the long rain, were better equipped to survive up to the next short rain, providing no fire occurred.

In contrast, seedlings which emerged from pods in the middle or end of the long rains were too young to survive even one month of drought. At the beginning of the long dry season (June), the seedlings which had germinated during the short rainy period had a taproot averaging 47.5 cm in length ($n = 9$; $SD = 5$). Taproots of seedlings germinating during the long rainy period averaged 7.6 cm ($n = 4$; $SD = 4.4$). These were primarily seeds inside pods which had been buried.

In addition, seedlings originating from buried pods were bigger and healthier than those which germinated from pods on the surface. The cause is to be sought in moisture deficit on the surface. This is crucial in areas of limited and unreliable rainfall. Boaler (1966) made the same assessment. Court and Mitchell (1988) noted that survival of superficially germinated seeds in nature was very poor. Khan and Tripathi (1987) observed that the survival of *Albizia lebbek* (L.) Benth. seedlings in nature was higher at higher depth. In other words, germination rate and the survival of germinated seedlings are all sensitive to moisture availability. It was very likely that seedlings which germinated on the surface in our study would not have been able to survive the drought. During the dry, season, the upper layers of the soil in the miombo woodlands dry out (Boaler, 1966), and non deep-rooted young seedlings would not be able to withstand this lack of moisture.

The combined effects of non-dehiscence of the pod, difficulty in reaching the ground, predisposition and sensitivity to fire, the irregular and limited amount of rainfall of the area, followed by the long hostile dry season, and finally poor seedling survival on the surface, explain the strikingly low regeneration level of *P. angolensis*. The role of symbiotic microorganisms in the initial establishment of

seedlings of this species is not likely to be crucial. This is supported by the following facts.

- (1) The seed must first overcome germination inhibitors before it can be infected as a seedling.
- (2) The big cotyledons of the seedlings play a role in the initial establishment. In this respect, Ernst (1988) observed that seedlings of *B. spiciformis*, a miombo tree species, fully depend on reserves in the cotyledons during the first weeks after seed germination. This reduces the dependency of seedlings on microorganisms.

Boaler (1966) found in Tanzania that about 2% of the fruits germinate under normal miombo conditions. He further estimated that half of seedlings die during the first year. These low germination rates and low survival rates of seedlings are confirmed by this study. On average, only 4 seeds per plot of 20 extracted seeds emerged in this study. Less than 2 seeds per plot emerged in non-extracted seeds. The survival of the latter was very much doubtful. Chidumayo (1991) working on *J. globiflora* also noted that the survival of seedlings was very low. Drought was thought to be the main cause. *J. globiflora*, however, stands at a much stronger position than *P. angolensis* in that its seeds germinate at the first showers (3.4). This allows the species to get established quickly within a limited period of rain.

The number of seedlings observed resulting from germination of buried propagules was perhaps smaller than the real number germinating. Others may have not been able to complete their germination and so escaped detection, especially if the rain stopped at a critical moment during the germination process.

Dayanand (1988) and Dayanand and Lohidas (1988) reported pod germination rates ranging from 1 to 40% in *P. santalinus* Linn. f. These germination rates were achieved under artificial conditions, i.e. with a steady water supply and elimination of fires. They are hence far higher than those observed under natural conditions.

The persistence of pods laid out on the surface or under

the litter shows that these fruits are not preyed upon by wild animals.

Ungerminated seeds, which were the majority from non-processed pods were still intact at the end of the long rains and at the beginning of the long dry season. This indicates that the nature of the fruit of the species and the dormancy of the seed is indeed a survival strategy. Seeds survived by not germinating, which is one of the functions of dormancy in nature (Willan, 1985).

The exceptionally high germination percentage and the speed of germination of these seeds, once extracted and pretreated after 8 months in nature, suggest that

1. they retained their viability;
2. a time-related natural corrosion of the seedcoat may have taken place and helped to break dormancy;
3. these seeds would be ready to germinate in the next rainy season if abandoned in nature, and thus survive as seedlings to the next long rains.

This is possible only if there is no fire and if the minimum amount of rain required is reached.

2.2.5 Conclusion and recommendations

The presumed rapid loss of seed viability after release, suggested by Van Daalen (1991) to explain the low regeneration of *P. angolensis*, is not supported by this study. The germination rate of seeds 8 months after release was more than 95%. The main causes of low regeneration include pod properties, sensitivity to fire, the seed dormancy, severe droughts, and irregular and intermittent rainfall. These contribute to both the survival and the mortality of seedlings of the species. Given the high amount of fruits produced by *P. angolensis*, this species can efficiently regenerate itself in nature. Every year, seeds from previous years are ready for germination during rainy seasons and some will survive provided the site is not severely disturbed.

It is recommended that yearly fires such as those known today, at least the late ones, be prevented where natural regeneration of *P. angolensis* is desired. The current practice of extraction of seeds by fire is risky since it is not easy to apply the right level of heat. It should therefore be reconsidered.

2.3 Germination studies in *Pterocarpus angolensis*

2.3.1 Introduction

P. angolensis stands high on the agenda of afforestation in East Africa. In Tanzania, it is among the species whose seeds are collected and distributed to various persons and organisations for afforestation. Its regeneration in nature is very low (2.2; Boaler, 1966; Van Daalen, 1991). Information on artificial germination of the species is still incomplete. In the nursery, the DANIDA/TANZANIA NTSP records indicate that only 20% germination has been achieved. Our earlier observations in the nursery (not included in the present work) indicated that the germination varied between 18 and 30%. In addition, the germination was staggered. This is unwelcome in nursery management.

Information on the germination of *P. angolensis* is vital for several reasons.

- (1) The species has been overcut.
- (2) The species has very valuable timber.
- (3) The extraction of seeds from the pods by beating is very laborious (30 seeds per hour; 2.2) and extraction by fire carries a big risk of damaging the seed (Boaler, 1966; 2.3).

These three arguments demonstrate the need to develop a technique of successfully inducing germination of available seeds of this species.

The objective of this study was therefore to develop techniques for improving germination of *P. angolensis* for afforestation and agroforestry purposes.

2.3.2 Materials and methods

Pods of *P. angolensis* were harvested from one tree on the foot of Uluguru Mountains in Morogoro (2.2; fig. 3). Seeds were extracted from the pods by hammering.

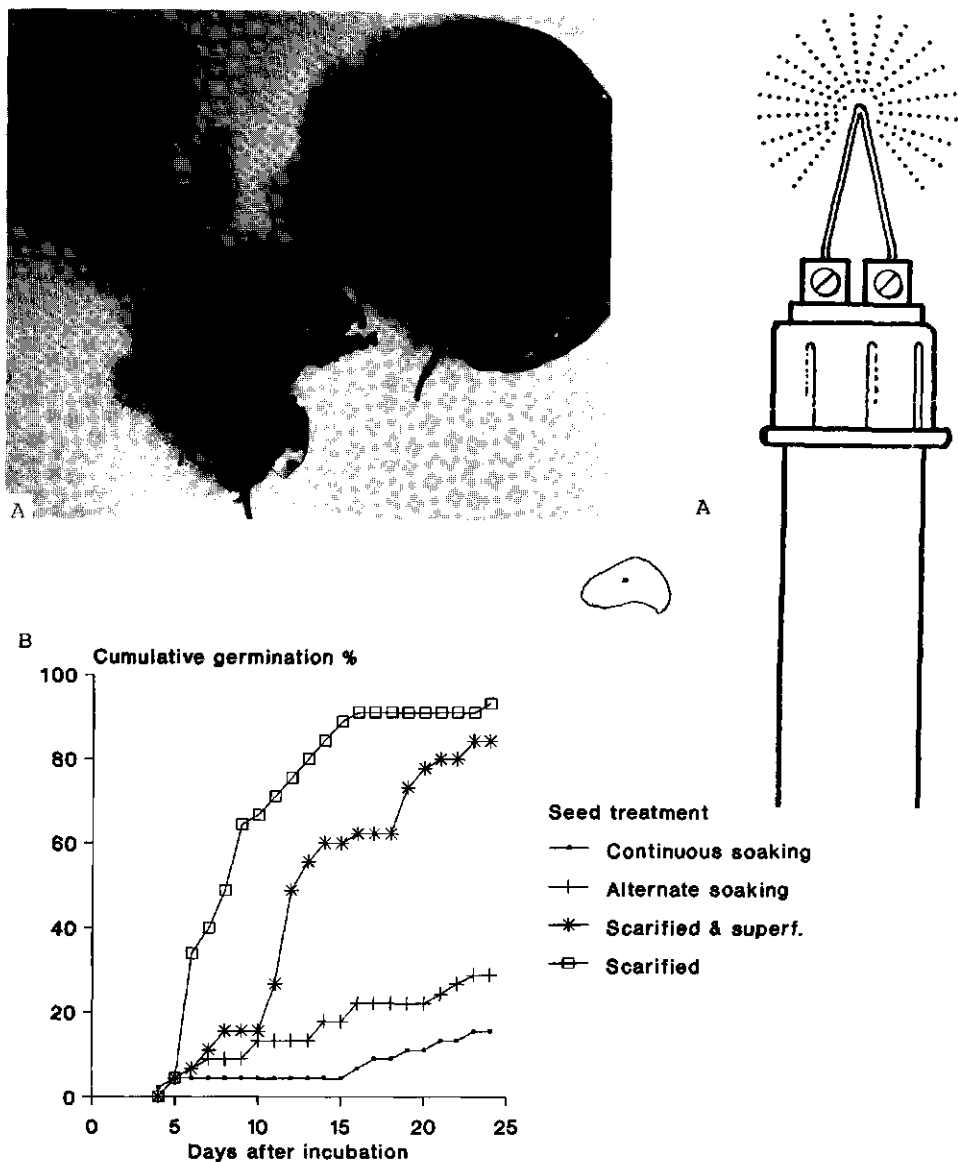


Fig. 5. Germination studies of *P. angolensis* in the greenhouse (A) seed extraction, hot wire and treated seed (B) cumulative germination percentage over 21 days.

Visibly damaged seeds were discarded. The rest of the seeds were used to make 12 piles of 15 seeds each. These piles

represented four treatments, each with 3 replications, which were to be applied to the seeds.

These treatments were the following.

- (a) Seeds were soaked for 16 hours in water, before being sown shallowly: seeds were gently pushed into the germination substrate (sand in square pots) until their upper surface was even with the top of the substrate. Seeds were then covered by a very thin layer of the substrate which still allowed the seed to be partially seen) (S).
- (b) Seeds were soaked for a total of 16 hours: 2 phases of 8 hours soaking, each followed by 24 hours air drying in the greenhouse before sowing shallow (AS).
- (c) Seeds were scarified with a hot wire (fig. 5a) and sown shallowly (ShS), as in (a).
- (d) Seeds were scarified (c) and laid on the surface of the germination substrate. They were then gently pushed into the substrate, until half of the seed (in flat position) was sunk into the substrate. The upper part of the seed was not covered and was kept uncovered till the end of experiment (SpS).

Seeds were watered every day. Germination counts were done daily. Variations in the cumulative germination percentages over 21 days were subjected to analysis of variance, and the significant means sorted out using Duncan New Multiple Range test.

The experiment started on 23/4/1993 and was concluded on 16/5/1993. It took place in the greenhouse of the Sokoine University of Agriculture, Morogoro in Tanzania (fig. 2a).

2.3.3 Results

The 90 soaked seeds revealed that a total of 21% of extracted seeds, 5 per treatment, had one or several injuries. This was visually assessed on soaked seeds before they were sown. At this stage, seeds were not discarded. Table 2 summarizes the results.

During the soaking process, injured seeds expanded in size while non-injured ones remained practically unchanged. Rotting of seeds was visible within 48 hours following sowing. No new seed rotted one week after sowing. Rotting was higher in soaked seeds and none of the superficially sown scarified seeds rotted; scarified, shallowly sown seeds had 7% of their seeds rotten.

Germination started on April 26, 1993 and was observed up to May 16, 1993. It was sustained in scarified shallowly sown seeds, widely spaced and irregular in soaked non-scarified seeds and remarkably influenced by the rain outside for superficially sown scarified seeds. In other words, the germination of seeds in this last treatment, was always initiated after one or two days of rain and no further germination was initiated once the rain had stopped. These patterns of germination are depicted in figure 5b. The total germination percentages were in the following order: ShS = 93% > SpS = 84% > AS = 29% > S = 16%

Scarification significantly enhanced cumulative germination percentages (table 2) compared to other treatments ($P < 0.05$). Alternating soaking and drying enhanced germination compared to one continuous soaking but the difference was not statistically significant.

Table 2. Means of cumulative germination percentages over 21 days for various treatments.

Treatment	Mean	SD
Alternate soaking and drying	16.5a*	8.3
One continuous soaking	7.4a	4.3
Scarified, shallow sowing	70.4c	28.1
Scarified, superficial sowing	47.0b	30.0

* = Means followed by different letters are statistically different at 5% level of significance.

2.3.4 Discussion

Until now, the germination of *P. angolensis* in artificial environments has been a silvicultural bottleneck. In nature, the causes of problems in germination have been linked to the effect of fires, the shape and the toughness of the fruit, limited rainfall and seed dormancy (2.2). As the present study shows, breaking the seed dormancy by scarification of the seedcoat with a hot wire leads to a very high germination percentage.

The reduced or sporadic response of non-scarified seeds to soaking indicates that the seeds of the species have an impermeable seedcoat. This explanation was further supported by the following points.

- (1) swelling of injured non-scarified seeds,
- (2) no swelling of soaked, non-injured non-scarified seeds and
- (3) massive swelling of scarified seeds after the first watering, just after they were sown.

Seed dormancy is characterized by staggered and differential germination (fig. 5b), a survival strategy for species growing in an unpredictable environment (Willan, 1985). *P. angolensis* seeds exhibit a seed dormancy which is mainly attributed to an impermeable seedcoat. In nature, a number of factors contribute to breaking the dormancy. These factors include (1) alternate wetting and drying of the seedcoat resulting from intermittent rain; (2) termites and fungi breaking down the pericarp of the fruit and eating the seedcoat (Albrecht, 1993).

Alternate soaking and drying enhanced cumulative germination percentage compared to one continuous soaking, though the means were not statistically different. Probably longer exposure of seeds to this treatment would have brought significant differences as alternate soaking and drying simulates the real conditions to which the seeds are exposed in the miombo woodlands. Alternating conditions of germination, i.e. moistening and drying or varying temperatures, have been reported to induce germination of

seeds (Khan and Tripathi, 1987). However, in the miombo woodlands, the period between two consecutive rains may be very long. If the rain stops at a critical time in the germination process, germination may abort. For example, the rain may stop when the seed has just swollen and is about to produce the radicle.

Scarification has been used to break the seed dormancy induced by a hard seedcoat or a hard pericarp. In India, Khan and Tripathi (1987) successfully applied it on seeds of *Albizia lebbek* and Dayanand and Lohidas (1988) on pods of *P. santalinus*.

In Malawi, Ngulube (1989) reported successful germination of *Albizia* and *Caesalpinia* species after scarifying the seeds. In Denmark, Stubsgaard (ex Sandiff, 1988) did scarification with a branding tool and reported a very high germination rate for *Acacia* seeds. In our work, scarification achieved a very high germination percentage in another legume tree. The rate could have been even higher had some seeds not suffered mechanical injuries during extraction. The observations made on soaked seeds indicate that at least 5 % of seeds had mechanical injuries. Passos et al. (1988) subjected seeds of *Leucaena leucocephala* Lam. de Wit to various treatments to induce germination. Impact on a solid surface gave the lowest germination percentage, indicating that internal mechanical injuries contributed to lower germination rates.

The hot wire seed scarifier has been evaluated by Sandif (1988) who reported positive effects on the germination of various legume tree seeds including *Acacia*, *Delonix*, *Calliandra* and *Caesalpinia* spp. The scarification done in this manner, with a hot wire or electric burner is more effective than the common scarification methods (Albrecht, 1993) in that seeds are homogeneously treated. Homogeneity in seed treatment implies shorter germination periods. This is important in nursery management.

Shallow and superficial sowing is very important in miombo species. In nature, seeds drop onto the soil surface, where most seeds will germinate (3.2.2) if they are not eaten

by rodents or destroyed by fires. Deep sowing has no equivalent in the wild. In the nursery it leads to increased rotting and germination failure.

Outside rain influenced the germination of scarified, superficially sown seeds. Every rain following a dry spell initiated new germinations in the greenhouse, but only for the seeds treated by scarification and superficial sowing. This dependence on unpredictable factor explains the irregular germination of the species studied here, especially in nature, because it concerns dependence upon an unpredictable factor.

It is no small wonder that *P. angolensis* regenerates sparsely in nature. Neither covered scarified seeds, nor covered non-scarified seeds responded to outside rain. The former had their moisture ensured while the latter were certainly hindered by an impermeable seedcoat.

Superficially sown seeds had a moisture deficit at the upper surface and could react to changes in air moisture which enabled germination. This entails high risk of mortality if long dry periods follow a short period of rain. Germinated seeds do not survive under such conditions. Sharma and Sen (1989) have reported that *Haloxylon* spp. were adapted to germinate during brief periods of water availability. The germination of scarified superficially sown seeds of *P. angolensis* is clearly influenced by the outside rain. Nursery managers should take this into account in planning their activities, for example, sowing during a period of daily rains.

2.3.5 Conclusion and recommendations

Germination tests of *P. angolensis* were carried out. Seeds scarified with a hot wire had the highest germination percentage of all treatments. Mere soaking achieved the lowest results. Alternating soaking and drying resulted in higher but not statistically different germination rates than one continuous soaking. Superficially sown scarified seeds had

synchronized their germination with outside rain. The following circumstances should receive attention for germination of this species.

- (1) Pods should be harvested when mature and while still hanging on the tree. They should not be treated with fire since the right heat level is hard to establish and almost impossible to maintain.
- (2) Seeds should not be extracted by fire but by mechanical or biological process, for example, subjecting fruits to decomposition by termites.
- (3) Extraction by beating should be done very carefully since injured seeds are more prone to rotting.
- (4) Seeds of equal size, with well-filled, polished testa should be selected.
- (5) Seeds should be treated with a hot wire and should be sown very shallowly.
- (6) The prevailing air conditions have an influence on germination. Hence the best period for germination of seeds is a period of continuous rain. This period should be locally ascertained.

2.4 *Pterocarpus angolensis*: symbiotic status, growth, root pruning and fertilization in the nursery

2.4.1 Introduction

P. angolensis forms nodule and VA mycorrhizae in nature. These two attributes make the species attractive for agroforestry and reclamation of degraded sites. *Brachystegia* and *Julbernardia* species do not nodulate (Högberg and Pearce, 1986). Among criteria used for the selection of agroforestry tree species is that the species should be able to improve the soil by fixing nitrogen. Nodulation and nitrogen fixation require high phosphorus status in the host plant. This requirement is easily met in trees which fix nitrogen and form mycorrhizae (Dommergues, 1987). Mycorrhizal formation increases phosphorus uptake by the host plant (Bowen, 1980). VA legumes fix nitrogen and hence are more suitable for agroforestry than ectomycorrhizal legumes, especially when they are grown in nitrogen-deficient soil. Ectomycorrhizal legumes do not fix nitrogen (chapters 3 and 4).

The regeneration of *P. angolensis* in the miombo woodlands is hindered by annual fires and a hostile climate prevailing in the miombo zones. Seedlings of *P. angolensis* produce a shoot of few centimetres every year. This same shoot dies back every year during the dry season for 8-10 years (Celander, 1983). The seedlings expand their taproot during this period. Seedlings then grow a shoot of two to three meters in one year, thereby attaining height by which they may escape fire. This phenomenon of die-back and the development of a strong taproot as observed in nature may be a hindrance when the species is raised in the nursery for later transplanting (Boaler, 1966; Celander, 1983). Like other species of the miombo woodlands, techniques for raising *P. angolensis* in the nursery and then transplanting it for growth in the field are not yet developed.

The objectives of this study are:

- (1) to describe the root architecture of the species at the

seedling stage;

- (2) to assess the effect of root pruning and fertilization on early growth in the nursery;
- (3) to assess the symbiotic status of the species in the nursery.

2.4.2 Materials and methods

Cylindrical black polythene tubes, open at both ends, and 50 X 50 cm seedbed plots were arranged in a completely randomized block design, with 7 treatments and four blocks. Six tubes per replicate were filled with unsterile soil from the rhizosphere of *P. angolensis* growing in the miombo woodlands. For the seedbed plots, existing arable soil was first removed and replaced by the same amount and the same type of soil as that in the tubes. Seeds were then sown in every tube and every seedbed plot to finally achieve one seedling per pot or six seedlings per seedbed per block. Thus, there were a total of 24 seedlings per treatment raised in tubes or seedbeds. Moisture requirements were met by rain or watering. In case watering was done, the amount of water applied per seedling, equal in all treatments, was continuously adjusted depending upon actual weather conditions. This amount varied between 40 and 60 ml.

The various treatments are listed below.

- (1) Direct sowing in the seedbed, no root pruning, no fertilization (A)
- (2) Direct sowing in the tube, no root pruning, no fertilization (B)
- (3) Direct sowing in the tube and root pruning once a month (C)
- (4) As (3) plus iron ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) (D)
- (5) As (3) plus zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (E)
- (6) As (4) plus nitrogen and phosphorus supplied as $\text{NH}_4\text{H}_2\text{PO}_4$ (F)
- (7) As (5) plus nitrogen and phosphorus as $\text{NH}_4\text{H}_2\text{PO}_4$ (G)

Iron and zinc were used in this experiment because seedlings of *P. angolensis* in existing nurseries displayed symptoms which were suspected to be due to a lack of iron or zinc or a combination of both. Iron was supplied every 2 weeks as a solution of 0.5%. Zinc was supplied monthly at a rate of 10 mg per kg of dry soil. $\text{NH}_4\text{H}_2\text{PO}_4$ was supplied monthly at a rate of 0.25 g of phosphorus per seedling in one dosage. Iron was simultaneously applied on leaves and in the pot while the rest of the nutrients were applied only in the pot. The first root pruning was done on 20/1/1993, and the last on 21/3/1993. Root pruning consisted in passing a sharp knife under every pot and cutting horizontally all roots growing out of the pot. The first application of nutrients was made on 26/1/1993 and the last in April. Every application of fertilizers in the pots was followed by an additional light watering. Non-fertilized seedlings were also watered. The experiment took place on the campus of the Sokoine University of Agriculture from November 1992 to April 1993.

Observations of seedling survival and health were made regularly. At the end of the experiment the following parameters were assessed: total height, number of leaflets, the shoot and root dry weights, nutrient content of the foliage, and variation in root architecture. The shoot/root ratio was computed. An analysis of variance was conducted for the total height, number of leaflets, shoot dry weight, the shoot/root ratio and the dry weight of laterals. Furthermore, it was assessed whether or not *P. angolensis* nodulates in the nursery and, if any, what type of mycorrhiza it forms.

Naturally regenerated seedlings were uprooted from the miombo woodlands, and their root architecture and shoot described.

2.4.3 Results

Observation in the miombo woodlands indicated that *P. angolensis* yearly produces many fruits that are dispersed over

a considerable area, around the tree and far away. However, naturally regenerated seedlings taller than 20 cm and shorter than 1.5 m are rare. Those of the smaller seedlings which are present are hard to see since the shoot is very short. They are either more than 1.5 meters high, or only a very few centimetres high and show several dead ends. Most of the seedling biomass is in the taproot (fig. 6a). The taproot is well developed, swollen on its upper part, and tapering with depth, with few laterals.

In the nursery, seedling growth was comparable in all treatments before and at the first root pruning. However, each subsequent root pruning reduced the growth rate of both the shoot and the root systems (table 3). Root-pruned seedlings became paler as time went on. At the end of the experiment, non-pruned seedlings were far taller and greener, had a higher number of leaflets and were heavier, but they had fewer lateral roots. Each root pruning was followed by a period of seedling withering which persisted longer in seedlings which did not receive additional $\text{NH}_4\text{H}_2\text{PO}_4$.

The root architecture of seedlings is remarkable. The bulk of the root system is made of the taproot. Laterals are very thin and look as if they were accessory (fig. 6a). In pruned seedlings, the taproot is nearly cylindrical. In unpruned seedlings, the root system is dominated by a well-developed single taproot, which represents more than 90% of the root dry weight. It has the shape of a carrot, almost cylindrical in the upper 7 cm and then sharply tapering downward. The taproot of non-pruned seedlings had pierced the pot. Most of the nodules were found on the taproot.

The shoot/root ratio was under 2 for treatment A and B, if the whole root system is included. The ratio was above 2 if the root system is considered only within the depth of the pot. Non-fertilized pruned seedlings had a shoot/root ratio below 1, indicating that the shoot was lighter than the root system. Fertilisation changed the balance: the shoot/root ratio shifted to above 2.

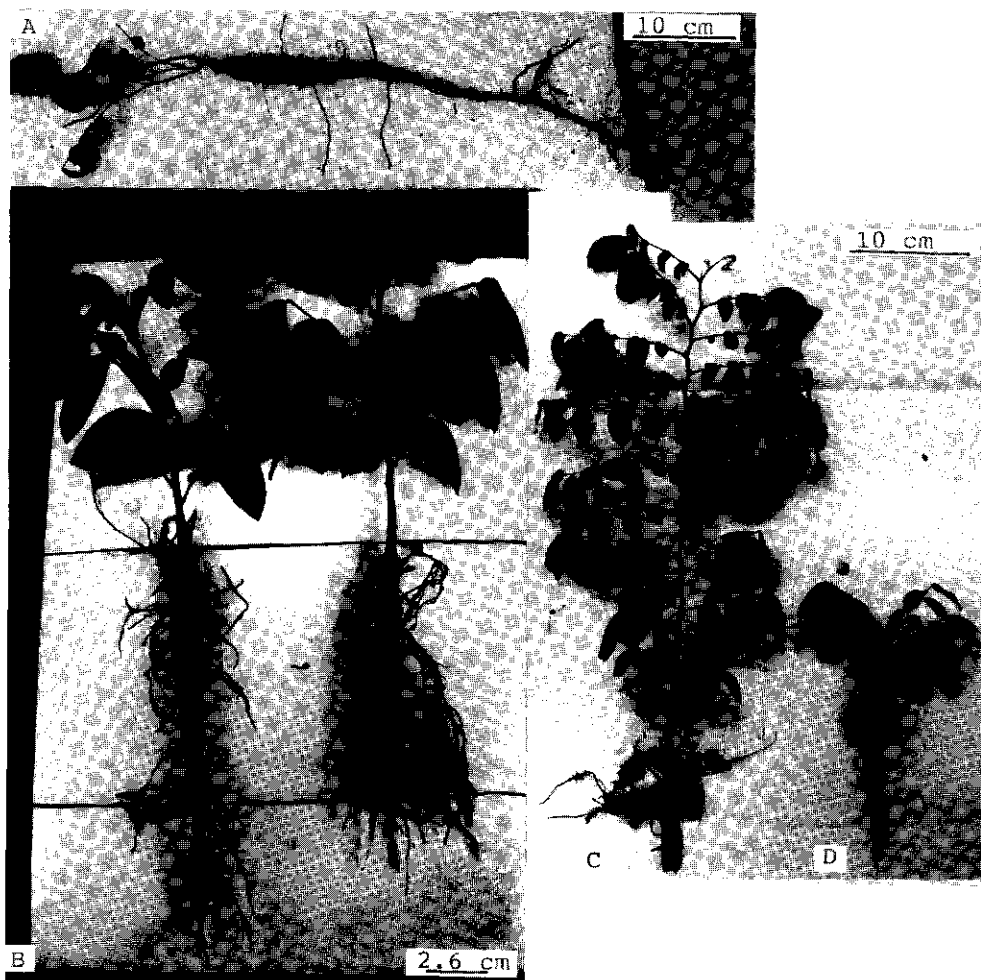


Fig. 6. Regeneration of *P. angolensis* (A) naturally regenerated seedling (B) root reiteration of nursery seedlings after pruning (C) pruned seedling having been fertilized with N and P (D) control.

Seedlings reacted to root pruning by reiterating several taproots just above the level of cut (fig. 6b). This occurred at each intervention. Root pruning stimulated the production of a limited amount of laterals inside the pot, but these did not contribute much weight. The capacity of the root to regenerate by reiteration seemed to decline over time.

Nodulation was visually observed on all seedlings at the

first root pruning. This was done by examining both the point of cut and the part of the root removed by pruning. At the end of the experiment, not all seedlings were nodulated: $\text{NH}_4\text{H}_2\text{PO}_4$ completely eliminated nodules on most of fertilized seedlings (fig. 6c). Attempts to collect and count nodules, were, however, unsuccessful as they were very tightly bound to the sticky soil. The highest mean number of nodules per seedling (31) was found in non-pruned, non-fertilized seedlings. Pruned non-fertilized seedlings had a mean of 4 nodules while seedlings fertilized with nitrogen and phosphorus had a mean of 0.4 nodules.

The fine roots were microscopically examined. Seedlings were found to form VA mycorrhizae. The mycorrhizal infection percentage as affected by fertilization was not established.

Fertilization with iron and zinc did not produce a significant growth increment in the seedlings. $\text{NH}_4\text{H}_2\text{PO}_4$ enhanced seedling growth when compared to the control (fig. 6c), but inhibited nodule formation. Higher growth was attained by seedlings whose taproot was not severed.

Seedlings fertilized with $\text{NH}_4\text{H}_2\text{PO}_4$ showed the highest foliage concentrations of nitrogen and phosphorus. However, unfertilized seedlings had adequate levels of nitrogen in their leaves. Their phosphorus content was inadequate (table 4).

Table 3. *P. angolensis* seedling growth after being treated by fertilization and/or root pruning. Standard deviations are indicated in the parentheses.

ht = total height (cm); lea = number of leaflets; sho = shoot dry weight (g); s/r = shoot/root ratio; lat = biomass of lateral roots (g). For description of treatments A to G see page 32.

	ht	lea	sho	s/r	lat
A	29.58c [*] (9.74)	92c (45)	4.29c (1.59)	2.12d (0.59)	0.021a (0.008)
B	27.62c (4.03)	87c (19)	4.67c (1.12)	2.21d (0.35)	0.023a (0.011)
C	10.64a (1.73)	35a (6)	1.01a (0.27)	0.62a (0.12)	0.093c (0.047)
D	10.65a (2.79)	38a (2)	1.18a (0.41)	0.68a (0.21)	0.076bc (0.026)
E	12.61a (1.10)	40a (10)	1.36a (0.25)	0.88b (0.19)	0.068b (0.025)
F	21.87b (4.26)	79b (18)	3.05b (0.89)	1.11c (0.34)	0.161d (0.068)
G	21.63b (5.79)	84bc (13)	3.09b (1.00)	1.30c (0.23)	0.150d (0.047)

* = values followed by the same letter in the same row are not statistically different (5% level of significance); mean of 24 values.

Table 4. Nutrient analysis of seedling leaf tissue (treatment as above).

Treatment	Total N (%)	Total P (ppm)	N/P
A	1.924	1250.00	15.4
B	2.122	1125.00	18.9
C	1.765	1562.50	11.3
D	2.060	1062.50	19.4
E	1.565	1437.50	10.9
F	2.723	4875.00	5.6
G	2.652	3937.00	6.7

The soil analysis for no fertilizer treatments at the end of the experiment: pH in water (1:2.5): 7.10; total nitrogen: 0.133%; available phosphorus: 3.86 ppm.

2.4.4 Discussion

The reaction of seedlings to various treatments can be explained using the sapstream model (Oldeman, 1974; 1989). In this model, three production compartments in mutual feedback interact via the sapstream. These compartments are the crown, the cambial system and the root system (fig. 7). While important nutrient stocks are mainly in the crown for trees in favourable environments (Gong and Ong, 1983), more is stored in the root system in stressed environments (Oldeman, 1989; Drechsel and Zech, 1993). Thus, while the crown is most important in tropical rainforest trees, the root system plays a leading role in the survival of miombo trees. In the miombo woodlands, the development of a strong taproot is allocated a big share of carbon, and the annual shoots die back for 8 to 10 years until sufficient food and energy reserves have accumulated in the root system (Boaler, 1966; Celandier, 1983).

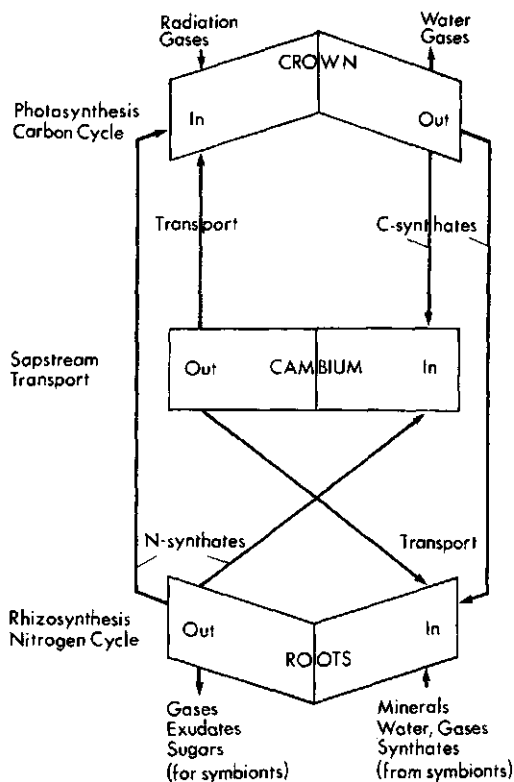


Fig. 7. Sapstream diagram. The tree is considered to contain three production compartments, which are in mutual feedback interaction via the sapstream: the crown (photosynthesis, carbon cycle), the root system (rhizosynthesis, nitrogen cycle) and the cambial system (produces transport tissue for the sapstream and derives its nutrients and energy from the sapstream) (Oldeman, 1989).

In the present study, artificial manipulation of the root and the root environment hampered this natural strategy. Root pruning, although it promoted lateral root production, led to sharp depressions in growth of seedlings. The survival of miombo trees in their natural habitat is not primarily due to their shoots, nor to their lateral roots but to their taproot. Whereas the shoot and most of the lateral roots in seedlings die back at a certain period of the year, the taproot is a perennating organ (Chidumayo, 1991), which ensures the

production of new roots and new shoots when rain resumes.

The response of pruned roots is to reiterate vertical roots (Dyanat-Njad, ex Oldeman, 1990), causing an incomplete regeneration of the severed taproot. This response raises the question of the advisability of pruning in nurseries for miombo species. The main aim of root pruning is to promote the development of a fibrous root system (Sutton, 1967; FAO, 1974) so as to increase uptake of water and nutrients once the seedling is transplanted (Rook, 1971). Root pruning has been successfully applied in pines (Gosch et al., 1974). As personally observed, root pruning is a common practice in countries like Rwanda, Kenya, and Tanzania.

In miombo species, carbon is reallocated for rebuilding the taproot after pruning. The capacity to do so, however, declines as seedling vitality diminishes. Repeated cutting of the taproot is nothing less than periodic removal of carbon stocks from seedlings.

Limited root pruning should be carried out for another practical reason. The taproots of non-pruned *P. angolensis* seedlings in our experiment had pierced the bottom of the pot by the end of the experimental period, growing deep into the soil. This rendered their transplantation impossible. A technical compromise would be to carry out one or two careful root prunings and take the seedlings to the field before the next pruning is due.

Fertilization with nitrogen and phosphorus acted against the natural seedling strategy. Nodulation was inhibited, and the shoot/root ratio was disturbed by fertilization. Seedlings produced more shoot than root biomass. Iron and zinc did not significantly stimulate growth of seedlings (table 3). Indeed the soil was not deficient in these micronutrients. None of the control seedlings exhibited symptom deficiency in these micronutrients.

The architecture of seedling shoots in the miombo woodlands is different from that of nursery seedlings. Nursery seedlings had one shoot while in the miombo woodlands, most of seedlings have more than one shoot. The architecture in the

field is a result of periodic die-back, often triggered by drought. No die-back was observed in the nursery. Die-back was observed in *J. globiflora* seedlings in nature (Chidumayo, 1991) and in *P. angolensis* (this section). Die-back did not take place however, in the nursery for *P. angolensis* and *J. globiflora* (chapter 3), probably because in the experiment described here and in chapter 3, seedlings were daily watered.

A survival strategy of trees in stressed environments (Oldeman, 1989) is their association with symbiotic microorganisms. In this way, *P. angolensis* formed VA mycorrhizae and nodulated. These results agree with field surveys by Högberg and Nylund (1981) and Högberg and Pearce (1986).

All seedlings showed adequate level of nitrogen, while seedlings which did not receive $\text{NH}_4\text{H}_2\text{PO}_4$, showed an inadequate level of phosphorus. The soil used in this study was deficient both in nitrogen and phosphorus. The nitrogen in the leaves for non-fertilized seedlings comes from nitrogen fixed by the plants themselves. This natural process should be encouraged. Seedling growth response to fertilization was most likely stimulated therefore by the artificially applied phosphorus and not by nitrogen.

Similar results in growth and nodulation of seedlings as affected by nitrogen and phosphorus application were achieved by Moloney et al. (1986), Sivasupiramanian et al. (1986) Dutt and Pathania, 1986) and Aziz and Habte (1989).

2.4.5 Conclusion and recommendations

Observations on growth and behaviour of naturally regenerated seedlings of *P. angolensis* were made in the miombo woodlands. In the nursery, trials of root pruning and fertilization were made on seedlings of *P. angolensis*. The seedlings were watered in the nursery. The following was observed. (1) As a natural survival strategy, undisturbed seedlings in the field develop the taproot rather than the shoot or lateral roots. The shoot

dies back yearly while the taproot expands. Shoot die-back did not take place in the nursery. (2) In the nursery, root pruning resulted in depression of seedling growth. (3) Seedlings formed VA mycorrhizal associations and nodules. (4) Artificial application of nitrogen and phosphorus enhanced shoot growth in the nursery but inhibited nodulation and disturbed the shoot/root ratio. More shoot biomass was produced, at the expense of the root. In short, it was observed that manipulation of the root and the root environment had a negative effect on the seedling's natural survival strategy. Direct sowing in the field should be practised where appropriate. (5) All these results can be coherently explained using the model of sapstream feedbacks.

2.5 Iron deficiency in *Pterocarpus angolensis* nursery seedlings: symptoms and cure

2.5.1 Introduction

Attempts by the DANIDA/Tanzania NTSP to raise *P. angolensis* in the nursery have been unsuccessful up till now. Seedlings of this species are often raised in the nursery only to die before transplanting. These seedlings all exhibited the same growth behaviour and the same symptoms, followed by death. This section reports and describes this problem, and its solution.

The architectural flexibility of legume trees helps them survive environmental stresses. Oldeman (1989) described the architectural flexibility of East African *Acacia* grown under natural drought and deficiency stresses. In natural conditions of under limited amounts of rainfall, high temperatures and yearly fires, *P. angolensis* seedling architecture is characterized by underground development of a strong taproot and short above-ground shoots which die back yearly (fig. 6a). Adult trees in their natural habitat have light, spreading crowns with many small branches (fig. 3). Under nursery conditions, irrigated, unpruned seedlings have a single straight shoot with a somewhat curving top. However, the stem remains straight as the seedling grows.

Nursery seedlings, as observed in the present study, go through several repeated cycles of die-back which markedly modify their architecture. Seedlings start with a single shoot but after undergoing several stages of degradation, end up with several small shoots. The sequence of events is as follows: on the shoot the first leaves to be affected are the youngest; the oldest leaves are affected last (APPENDIX 1A, page 181). Leaves turn pale, then yellow, then white, then brown becoming necrotic before dropping. The terminal bud dies, and the seedling produces several short lateral shoots. These have small leaves which sooner or later die back as well. The taproot also dies back, stopping lateral root

production. Degradation may proceed slowly, taking several months before the ultimate death of the seedling. Prior to death, seedling has shorter shoots and a shorter taproot.

At each stage, leaf veins are greener than the rest of the leaf. The symptoms described above were tentatively ascribed to iron deficiency in the plant tissue (cf. Marschner, 1990) after a discussion with Dr Semoka of the Department of Soil Science.

The objectives of the present study were therefore (1) to identify the disease and its cause, (2) to find a cure for the disease, and (3) to suggest preventive measures.

2.5.2 Materials and methods

2.5.2.1 Iron application

Seedlings were not potted especially for this experiment. The seedlings used came from a normally available nursery-stock of potted plants displaying the symptoms described above. First, we grouped seedlings into homogeneous groups of six seedlings each, half of which were treated with 0.5% iron (2.4.2) and the other half untreated. The criteria for grouping were the number of leaves, leaf colour and seedling size. There were 7 paired groups of 6 seedlings. Iron was applied to the leaves of seedlings in the iron treatment. Leafless subjects received 5 drops around the root system until they acquired new leaves. Each seedling and each leaflet were treated individually, but evaluation was according to treatment. Seedlings were placed in the same nursery where they had been growing before for 10 months. They were watered twice a day if there was no rain, and by the same nursery staff. Iron application was applied once every 3 weeks for 3.5 months.

Visits were made at least 3 times a week to record all changes visible on the shoot and to establish mortality rates. At the end of the experiment, the number of surviving seedlings and the number of leaflets on each seedling was

recorded, as well as the total height, the fresh total weight, and the number of nodules. The results were analyzed using the Student t-test.

2.5.2.2 Soil compaction and overwatering experiment

Treated seedlings showed marked response immediately after the first application of iron on leaves, but the cause of the symptom deficiency was not yet proven. The following hypothesis was formulated to verify through experiment:

Failure of the seedlings to absorb or to translocate iron is due to poor aeration resulting from (a) soil compaction and/or (b) overwatering in the pots in which seedlings were raised.

A 2 X 2 factorial experiment involving 2 factors, soil compaction and overwatering, each with 2 levels was set up. The resulting 4 treatments were arranged in a completely randomized block design. Each replicate consisted of 8 seedlings raised in individual open pots filled with fresh soil from the rhizosphere of mature *P. angolensis* growing in the miombo woodlands. The growing medium was treated as follows:

- (a) no compaction, normal watering (control);
- (b) no compaction, double watering;
- (c) compaction, normal watering;
- (d) compaction, double watering.

Watering was considered as normal if water was just enough to moisten the growth medium without running through the bottom of the pot. The amount of water applied was continually adjusted with fluctuating weather conditions. Between 15 and 30 ml were applied per seedling for normal watering. Double watering was a double of normal watering.

Non-compacted soil was a soil which was used to fill the pot without pressing it down by hand or shaking the pot. The soil was neither ground nor sieved.

For compacted soil, the same pot was filled with 1.5

times more soil than that in the non-compacted soil treatment. Non-compacted soil weighed 435 g dry weight while compacted soil weighed 631 g.

2.5.2.3 Observation on non-potted seedlings directly raised in nature

Seedlings of *P. angolensis* were raised under an adult *P. angolensis* tree by direct sowing. They were watered once a day just to keep them alive. Soil used for the compaction experiment came from the rhizosphere of the same tree.

On the Sokoine University campus at Morogoro, seeds of *P. angolensis* were also sown in seedbeds in November 1992. Rainfall was the only source for the moisture required by the seedlings. The experiment lasted 7 months. In all the above experiments, leaf counts were made to assess the development of deficiency symptoms.

2.5.2.4 Soil analysis

Soil N, P and pH of the seedlings of 2.5.2.1 was analyzed in water (1:2.5) at the end of the experiment.

2.5.3 Results

2.5.3.1 Response to iron application

At the beginning of the experiment, 100 % of all seedlings were weak, and, 29 % were leafless. The others displayed yellow, white or necrotic leaves.

Twenty four hours after iron application, the leaves of the treated seedlings had started to change (table 5). The veins were the first to turn green, and the rest of the treated leaflets followed. Non-treated leaflets did not behave

like a neighbouring, treated leaflet belonging to the same leaf (APPENDIX 1A, page 181). One week after application, treated seedlings started to produce new leaves and to grow in height. At the end of the experiment, treated seedlings were significantly taller (APPENDIX 1B, page 181) and had a higher number of leaves and leaflets ($P < 0.05$). There was no significant difference in total fresh weight between treated and non-treated seedlings ($P = 0.065$). Non-treated seedlings did not produce new leaves during the experimental period. Instead, their initial leaves deteriorated, and were sometimes shed. The root systems of treated seedlings had resumed elongation while those of non-treated seedlings had died back, becoming shorter. No seedling was found nodulating. Treated seedlings displayed the normal architecture of nursery seedlings, i.e. they had a single leading shoot and a swollen but tapering taproot with few lateral roots.

Table 5. *P. angolensis* seedling mean total height, fresh weight and number of leaflets as affected by iron treatment in the nursery.

	height (cm)	total frwt (g)	no of leaflets
Treated	9.1b	5.1a	19b
	(2.7)	(1.4)	(6)
Untreated	2.1a	2.01a	2a
	(1.3)	(0.89)	(2)

frwt = total fresh weight

n = 21; SD between parentheses; different letters following a given value denote significant differences (5%).

2.5.3.2 Soil compaction, overwatering and field experiments

Neither the seedlings raised in the field nor those raised on

the campus by direct sowing outside developed any deficiency symptoms. All their leaves were green.

In the compaction experiment, germination started on the 4th day and was completed 9 days later. Initial growth rates were high and homogeneous. During the first months, all seedling leaves and cotyledons were green. However, at the end of 3 months, the cotyledons turned yellow and started to drop. The symptoms described earlier started to develop at the same time: younger leaves in compacted and/or overwatered treatments turned yellow. The percentage of affected seedlings was: 58% of those in compacted soil and double-watered, 29% of the seedlings in compacted soil and watered normally, 24% of those in non-compacted soils and double watered, and 0% in the control group. It was observed under the pot that control seedlings had started to form nodules. The experiment was left to proceed for some more months. Soil pH (H_2O) for seedlings of 2.5.2.1 was 7.1; N = 0.122%; P = 3.47 ppm.

2.5.4 Discussion

The sapstream diagram (Oldeman, 1989) used to discuss the results of the fertilization and pruning experiment (2.4) is again of use here. Plant growth depends upon the extent to which each of the three plant production compartments, crown, cambium and root system, carries out its function. The weakest compartment in the production cycle determines the total output and the intensity or amount of energy and nutrients transferred in sapstream feedbacks. In the present study, both the shoot and the root had problems. The shoot was handicapped photosynthetically, and the root system was apparently unable to absorb enough soil solution since it consisted of the taproot alone. As a result, the whole system was downgraded.

The pattern of degradation from younger leaves to older leaves matches the symptoms of iron deficiency as outlined by Mengel and Kirkby (1987), Marschner (1990) and Drechsel and Zech (1993). Iron, contrary to nitrogen, has a very low

mobility so that it can not be transferred easily from old leaves to younger ones. This was demonstrated by its application on one of the leaflets of the affected leaf, when the recipient leaflet benefited only, while the rest of the leaflets deteriorated.

Iron plays a role in plant chlorophyll formation. Terry and Low (1982) reported that, whenever the supply of iron is deficient, there is a close positive correlation between the total iron content of the leaves and the chlorophyll content. Terry (1980) subjected sugar beet plants to iron stress. Iron stress decreased chlorophyll content, reduced the size of chloroplasts and diminished the amount of all photosynthetic pigments. Iron deficiency therefore results in growth reduction since it directly affects photosynthesis.

Repeated multiple shoot reiteration was a survival mechanism of the plant. Oldeman (1989) discussed the architectural flexibility of legume trees growing in an inhospitable environment. Multiple shoot reiteration was one of the aspects he observed in an East African *Acacia* species grown in a Dutch greenhouse under short day length and low light intensity and in other *Acacia* species growing in the open, dry and hot Kenyan savanna landscape. The reiteration displayed in our *P. angolensis* was sustained by internal energy reserve translocation, since new leaves which were produced at each attempt were smaller and chlorotic. Affected seedlings developed a remarkable crown, somewhat resembling that of adult trees. Moreover, the single-stem architecture of healthy nursery seedlings of the same age had been restored in most of treated seedlings by the end of the experiment. The final death of the plant can scarcely be due to other causes than the exhaustion of its carbon and nutrient reserves.

Iron deficiency is a worldwide problem in crop production in calcareous soils (Marschner, 1990). Iron deficiency in forestry in the tropics, however, is not commonly recognized. Most tropical soils are acidic and hence show a high Fe-solubility. Miombo soils are generally classified as ferruginous or ferralitic (D'Hoore, ex Celander, 1983).

However, nursery soils are often amended. Soils are usually mixed to improve nutrient status. Under normal conditions, the soil pH in Morogoro would be the same as that of tropical soils. These are acid. Soil amendment indeed took place, in the case discussed in these pages as shown by the rather high soil pH.

Iron chlorosis in itself does not prove deficiency or low availability of iron in the soil (Mengel and Kirkby, 1987). Many factors can interfere with absorption. These involve aeration and pH. Aeration depends on soil porosity and soil moisture. Potted seedlings are often overwatered in compacted soils. Before pots are filled, the soil to be used is ground and sieved in some nurseries. This destroys the soil structure. Soil grinding and sieving was presumably carried out in the nursery where this study took place. In addition, as I observed, the soil was compacted when pots were filled. This of course facilitates lifting the pots so as to displace them in the nursery during root pruning or for transplanting them. However, soil compacting also leads to a problem for sensitive species.

Semoka (pers. comm.) observed iron deficiency symptoms on rice growing in swampy areas in Tanzania. For *P. angolensis*, however, the present report is the first.

Deficiency symptoms appeared later in the seedling development. The following facts explain this.

- (1) The seedlings initially use the reserve in the cotyledons.
- (2) Growing seedlings retard upwards translocation of reserves due to low mobility of iron.
- (3) As the plant grows bigger, the demand becomes larger but the supply remains stationary.
- (4) With time, the pots in which seedlings are raised become more depleted, more compacted and more waterlogged.

The problem reported here is not likely to be due to pot size or lack of mycorrhizae. (1) Other seedlings (2.2.2) were raised in smaller pots and did not show the symptoms described above. (2) The seedlings in our experiment in compacted and

overwatered soil as described above were grown exactly in the same soil as those of 2.2.2. (3) Seedlings raised on the university campus by direct sowing in pasture far away from the forest were not inoculated with miombo soil. These seedlings did not show any deficiency symptoms.

Iron deficiency did not affect the aerial part only. Taproot size shortened and fine roots and nodules were absent. Whereas one of the symptoms of iron deficiency in the leaves is inhibition of chloroplast development (Terry and Low, 1982), in the roots, symptoms include inhibition of elongation (Romheld and Marschner, 1981). Taproot reiteration such as often observed in seedlings after root pruning (2.4; 3.3; Dyanat-Nejad, ex Hallé et al., 1978; Oldeman, 1990) was not observed in this study. The most probable cause is that the photosynthetic apparatus had been damaged.

Regarding the absence of nodules on seedlings, it is well known that waterlogging interferes with nodulation (Hong et al., 1977). This explains the absence of nodulation in the subjects. The exclusion of nodulation can also, at least partly, be attributed to the cost that the seedling has to pay for the formation of nodules and the fixation of nitrogen (Minchin and Pate, 1974; Haystead and Sprent, 1981; Ryle et al., 1979) whereas all plants were cut off from external energy supplies by their leafless or chlorotic condition. Before application of iron all seedlings had symptoms.

It was possible to reproduce the same symptoms on initially healthy seedlings and to cure them by iron application. This confirms that indeed iron had been deficient or inactivated in the plant tissues. While the controls remained looking healthy, the reproduction of the same symptoms on seedlings growing in compacted and/or waterlogged soil, firmly established the cause of deficiency symptoms, i.e. poor aeration in the growing medium. This was a combined effect of soil compaction and overwatering.

Poor aeration causes the accumulation of carbonates in the soil. This in its turn can cause chemical and/or biological inactivation of iron after its absorption and

transport to leaves (Sing, ex Mengel and Kirkby, 1987).

None of the seedlings in the field in the places where soil was taken for the compaction experiment, or on Morogoro campus in open air, developed the symptoms. Neither did any seedling of the experiment described under 2.2.2 of this chapter. This is one more proof that deficiency symptoms did not result from lack of iron in the soil but from nursery conditions.

2.5.5 Conclusion and recommendations

Iron deficiency is not commonly reported in tropical forestry. However, with widely applied artificial propagation of lesser known African indigenous tree species, problems and opportunities will certainly emerge. Iron deficiency in plant tissues is one of these problems. This study has diagnosed iron deficiency symptoms, established the cause and cured the problem. The cause was poor aeration resulting from soil overwatering and compaction. *P. angolensis* is sensitive in this respect. It was affected while several other species, e.g. *A. quanzensis*, *Sesbania* spp., *Albizia* spp., *Calliandra* spp., *J. globiflora*, *B. spiciformis*, *B. microphylla*, *Acacia* spp., under the same nursery management or raised in the present study did not suffer this deficiency. The following measures are recommended.

- (1) The structure of the soil for potting should not be lost, in other words, the soil should be neither ground nor sieved.
- (2) The soil should not be compacted during pot filling.
- (3) Watering should be carefully optimized for seedling health. The amounts to administer depend on local conditions, mainly physical soil properties and prevailing weather, etc. Only by local experiments, can one establish the correct water dose for a particular day or a particular period of the year.
- (4) If the soil in the pot is visibly compacted by watering,

the pot should be gently and carefully squeezed to reopen the breathing pores.

Chapter 3

JULBERNARDIA GLOBIFLORA, BRACHYSTEGIA MICROPHYLLA AND B. SPICIFORMIS (LEGUMINOSAE-CAESALPINIOIDEAE)

3.1 General introduction

The genera *Julbernardia* and *Brachystegia* dominate the miombo woodlands (Celander, 1983). Lundgren (1975) estimated that they constitute up to 80% of the standing stems. Lind and Morrison (1974) reported that species in the genus *Julbernardia* make up more than half the trees in any one area. Species of the genera *Brachystegia* and *Julbernardia* do not form nodules. Their mycorrhizal status in nature has been studied by Högborg (1982) who found them to be ectomycorrhizal. Trees of these genera are reported to provide nectar to the bees (Lind and Morrison, 1974), charcoal and timber to people (Bryce, 1967; Abeli, 1990). In addition, edible mushrooms are collected by the local population from the miombo woodlands (Härkönen et al. (1993). Despite their ecological and economical importance, the species are not yet raised in plantations. On the other hand, knowledge about their effective management in nature is very limited (Abeli, 1992).

The objectives of this study were therefore the to assess the following:

- (a) the germination and early mycorrhizal infection of *B. spiciformis* and *J. globiflora* in the nursery or in nature,
- (b) the effect of root pruning of *J. globiflora* seedlings on growth and mycorrhizal infection and
- (c) the effect of inoculation on the growth of *B. microphylla*

These objectives were set in order to contribute to the knowledge needed for raising indigenous species for afforestation purposes.

3.2 *Brachystegia spiciformis* and *B. microphylla*: germination and mycorrhizal formation on seedlings in the nursery and in the field

3.2.1 Introduction

The occurrence of mycorrhizae in nature is nearly omnipresent (Harley and Smith, 1983). The role of mycorrhizae in afforestation on poor sites is well documented (Marx, 1980; Linderman, 1988). Mycorrhizae also play a role in natural regeneration (Bakshi et al., 1972). The occurrence of mycorrhizae depends upon the host and the site factors (Smits et al., 1987). For example, soil disturbance is known to affect mycorrhizal formation. For pine trees, mycorrhizal research in the tropics has brought essential information. However, for indigenous trees available information covers mostly mycorrhizae in natural ecosystems. For the miombo woodlands, Högberg (1982) and Högberg and Pearce (1986) made a list of trees forming mycorrhizae in nature. *Brachystegia* species form ectomycorrhizae. However, it is unknown whether mycorrhizal association between the fungi and seedlings will take place in artificial environments and whether or not it enhances growth and survival of seedlings.

Two studies are reported here on two species, done to investigate the early infection in nature and the effect of mycorrhizal association on seedling growth in the nursery.

3.2.2 Study 1. *Brachystegia spiciformis*: germination and early infection in the field

3.2.2.1 Materials and methods

Study site

The study site is a field which is cropped with maize and beans. In the absence of crops, the farm is grazed by cows.

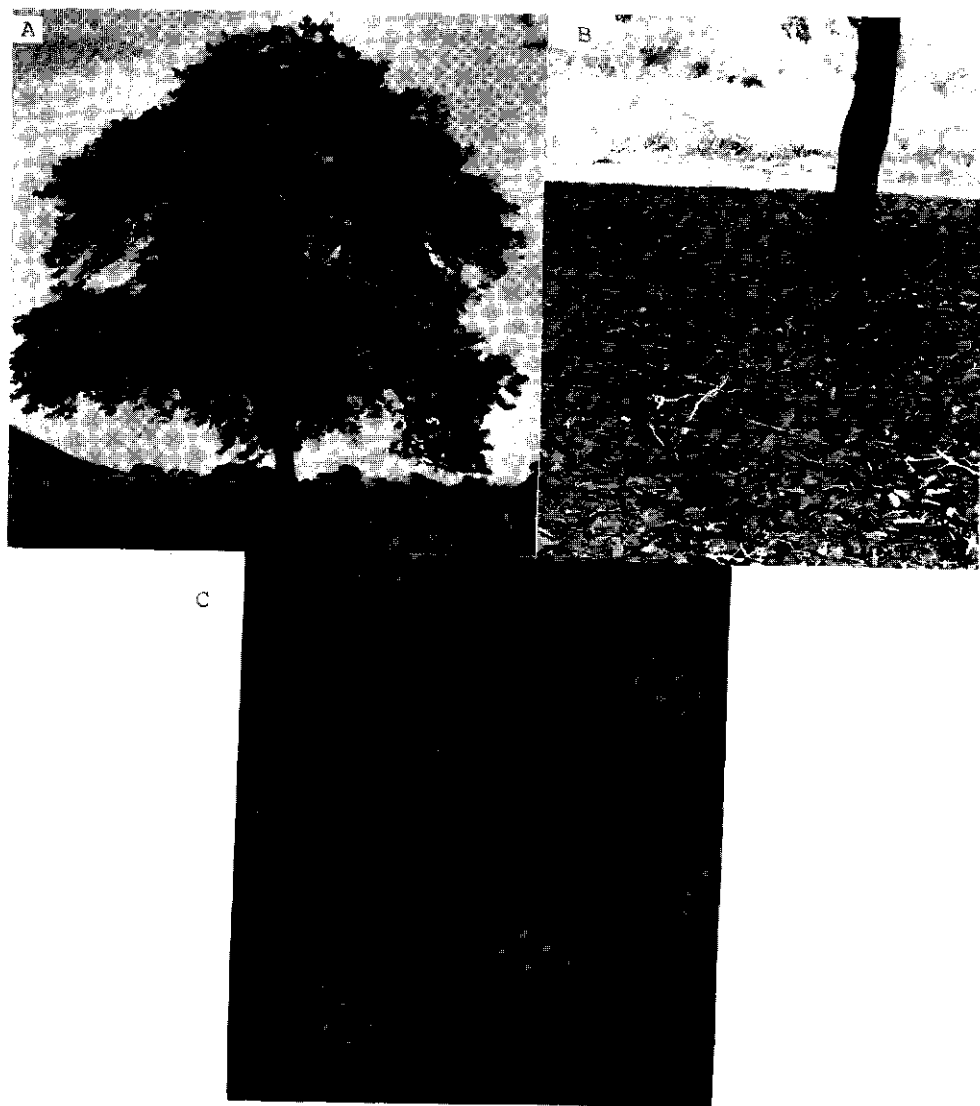


Fig. 8. Seed production, seed germination and early seedling infection in *B. spiciformis* in the field (A) seed-producing trees (B) layer of exploded pods (C) infected seedlings.

The soil is mineral. Lying on it, there are undecomposed pods of *B. spiciformis*. The farm is separated from the miombo woodlands, some 2 km away, by habitations and agricultural fields. In it, two big trees of *B. spiciformis* (fig. 8a) are growing which produce large amounts of fruits and seeds every

year (fig. 8b).

Seed germination and mycorrhizal assessment

The germination characteristics of the species were observed at the beginning of the short rainy period in 1992. At various stages in the early seedling development, seedlings were randomly harvested and their root system examined for mycorrhizal formation. The assessment period started before complete germination and ended some 3 weeks after emergence. About twenty seedlings were uprooted and assessed macroscopically and microscopically. Attempts were made to connect particular mycorrhizae to particular sporophore species.

3.2.2.2 Results

Illustrations and short notes on ectomycorrhizal types found on seedlings of *B. spiciformis*, *B. microphylla* (3.2.3), *J. globiflora* (3.3) and *A. guanzensis* (chapter 4) are in APPENDICES 1C-1G; 2A-2G and 3E, 3F and 4E, pages 181-184.

Seed germination and mycorrhizal development

After two weeks of rain, seeds had germinated and yielded seedlings. Typical ectomycorrhizae were formed before the seedlings emerged (fig. 8c). All seedlings were colonised by one single fungus (APPENDIX 2C and 2E, page 182). Among the fine roots, 25% ($n = 11$, $SD = 7.4$) were colonized before the cotyledons were opened. The infection rate was 76% ($n = 11$; $SD = 6.6$) when the first leaves after the cotyledons became visible. This mycorrhiza was creamy white, had lateral branches and the leading tip itself was mycorrhizal. These mycorrhizae form very big strands resembling those of the indigenous fungus also observed in pines inoculated with miombo soil inoculum (5.3.1.1). These strands are extensive

and were seen creeping along the long roots. The strands emerged from the lower part of the mycorrhizal itself. The surface of the fungal sheath shows short emerging hyphae. Mycorrhizal systems are long and so are individual mycorrhizae.

Mycorrhizae are thick with a well-developed fungal sheath and Hartig net. The same mycorrhizae were observed on the root system of the mother trees and the fungus was widespread. These mycorrhizae were seen connected with creamy white puffballs, probably belonging to *Scleroderma* spp. There were many of such puffballs within a radius of five meters around the mother trees. Puffball base had exactly the same rhizomorphs as those of mycorrhizae and connected different mycorrhizal systems. The same mycorrhizae and the same puffballs were also seen in association with *B. microphylla* in the miombo woodlands.

3.2.2.3 Discussion

Seeds of *B. spiciformis* germinate readily. No pre-treatment is needed. Germination takes place after sustained rain for at least 2 weeks. This period is much shorter than the duration needed by *P. angolensis* (chapter 2). The period could even be shorter if seeds were germinated in the nursery where water is provided at any moment, as desired. In nature the rains are often interrupted. This can extend the germination period. Kariuki (1986) in Kenya observed that *B. spiciformis* seeds do not need pre-treatment. Ernst (1988) made the same observation. So does the present study. The yearly invasion by the many, rapidly germinating seeds of this species may partly explain the high proportion of *B. spiciformis* in the miombo woodlands.

At germination, *B. spiciformis* seedlings have a clear taproot with many laterals. While the taproot grows stronger, the laterals disappear sometimes during the dry season (unpubl. results). The seedling mobilises everything for the

taproot development. This behaviour has survival value in the severe droughts and yearly fires under which the seedling grows.

Graham (1988) reported that mycorrhizal colonization occurs after germination when the radicle is growing rapidly. Early colonisation of seedlings by mycorrhizal fungi was also reported for *Azelaia africana* Sm. and *Uapaca guineensis* Müll. Arg. (Thoen and Bâ, 1989). Colonisation in nature in this case occurs before the emergence of the first leaves. The same is confirmed in the present book for *B. spiciformis*. Symbiosis costs carbon (Bowen, 1980), to be supplied by the seedlings. Early colonisation in this study can not be sponsored by photosynthesis by the seedling since infection was detected even before the epigeal germination was completed. The only possible source lies in the cotyledons. This agrees with Ernst (1988), who proved seedlings of *B. spiciformis* to be fully dependent on their cotyledons for early growth requirements during the first weeks after germination.

In nature the cotyledons are not the only carbon source for early infection of seedlings. In the nursery, infection is almost exclusively by spores. In nature, vegetative mycelium already growing on the roots of mother trees colonises the seedling roots (Harley and Smith 1983) easily and fast. This is partly proven by the same mycorrhizae being present in the fine roots of both the seedlings and the mother tree. The similarity of mycorrhizae in young seedlings and in mother trees as observed in the field in this study, and the difference between mycorrhizae of seedlings of the same age raised either in nature or in the nursery (4.4; 5.3.1.2), raise the question whether there are mycorrhizae occurring exclusively in young seedlings and others in old trees. Mason et al. (1983) noted that seedlings growing in the rhizosphere of the mother tree may form mycorrhizae with adult tree fungi, provided that the hyphae between the seedling and the adult tree remain intact. When the connection between the mother tree and the seedling is maintained through the hyphae, sometimes in rhizomorphs, the fungus finds its energy and

nutrient source in the mother tree. However it has been shown that not all mycorrhizal fungi associated with adult trees will form mycorrhizae when seedlings are raised in artificial environment (Mason et al., 1983).

Primary infection by VAM and dual symbiosis, as reported for *Uapaca guineensis* (Thoen and Bâ, 1989), *Afzelia pachyloba* Harms, *Uapaca staudtii* Pax and *Gilbertiodendron dewevrei* (De Wild.) J. Léonard (Newbery et al., 1988) and *Eucalyptus dumosa* A. Cunn. ex Schau (Lapeyrie and Chilvers, 1985), were not observed in *B. spiciformis*. The *B. spiciformis* we studied were ectomycorrhizal and remained so during the observation period. This agrees with the field observations by Högborg and Nylund (1981).

3.2.2.4 Conclusion

B. spiciformis germinates readily and its seeds need no pre-treatment. The seedlings of the species rapidly form a clear taproot with many laterals. In the field, these laterals interact with the fungal symbiont before germination is completed. Typical ectomycorrhizae were formed before the seedling emerged. These ectomycorrhizae become similar to those on the rootlets of adult trees. Only one mycorrhizal type was observed on the seedling roots. The fungal symbiont involved produced many sporophores. Hyphal connections between plants are hypothesized to be responsible for rapid infection in the field. No nodules were found on the root system.

3.2.3 Study 2. *Brachystegia microphylla*: effect of mycorrhizal inoculation in the nursery

3.2.3.1 Materials and methods

Soil inoculum was collected at various places around *B. microphylla* trees growing in the miombo woodlands. These trees were associated with sporophores of various ectomycorrhizal fungi (taxonomic list under preparation). The soil was characterized by low levels of nitrogen and phosphorus (N = 0.133 %; P = 3.86 ppm).



Fig. 9. Early growth and infection of *B. microphylla* seedlings in the nursery. (A) seedling of two weeks (B) infected and non-infected (middle) seedlings (C) *Inocybe dulcamara* (Pers.) Kumm. sporophore produced in the nursery.

This soil was either sterilized in an oven and directly used, or three parts of sterilized soil were mixed with one part of fresh soil and then used as a substrate to raise *B. microphylla* seedlings. There were only two treatments, i.e. addition or non-addition of mycorrhizal soil. Each treatment was applied to 56 seedlings, distributed over 7 pairs, each pair with 8 seedlings. Seeds of *B. microphylla* were sown directly in litre-pots, one seed per pot. Pots were then placed on a bench in open air. Watering was done daily when there was no rain (2.4.2). At the end of 5 months, the experiment was concluded and the mycorrhizal status and growth in seedling were assessed. A Student t-test was used to analyze the results.

3.2.3.2 Results

Germination

The germination of seeds was completed 13 days after sowing. Figure 9a shows a two-weeks old seedling of *B. microphylla*.

Growth, survival and symbiotic status

No seedling died during the experimental period. From the fourth month onwards, inoculated seedlings became ever bigger than their non-inoculated counterparts (table 6, fig. 9b). Several mushrooms of *Inocybe dulcamara* (Pers.) Kumm. developed around seedlings towards the end of the fifth month (fig. 9c). When the root system was investigated, 63% of the fine roots of inoculated seedlings bore mycorrhizae of one type (APPENDIX 3E, page 183). This was creamy white with very extensive, numerous, long hyphae and rhizomorphs. The mycorrhizae were branched in a flattish structure. There was a complete sheath and the well-developed Hartig net penetrated between the cells of the first layer of the epidermis. No seedling root had nodules.

Table 6. Growth parameters (means) of *B. microphylla* as influenced by inoculation. Each value is a mean of 56 seedlings.

	inoculated	non inoculated
height (cm)	12.35b*	8.85a
	(2.81)	(1.70)
shoot dry weight (g)	0.71b	0.30a
	(0.25)	(0.08)
root dry weight (g)	1.44b	0.93a
	(0.39)	(0.18)

* = Means followed by different letters are statistically significant at 5% level; SD between parentheses.

3.2.3.3 Discussion

Seed dormancy reported in many legume tree seeds was not observed in *B. microphylla*, *J. globiflora* and *B. spiciformis* (Ernst, 1988; Chidumayo, 1991; the present work). In nature, germination failure of these species can only result from insufficient moisture, or seed damage by bush fires, fungi and seed-boring insects (in preparation).

Addition of mycorrhizal soil, or soil inoculum to a growth substrate is the commonest method of inoculation (Mikola, 1973; Trappe, 1977). The strongest advantage of soil inoculum is that it usually contains more than one species of mycorrhizal fungi. As a result it ensures the formation of mycorrhizae and thus meets the premise that any mycorrhizae on tree roots is far better than none (Marx, 1980). However, it has disadvantages, one being that the fungi dealt with are unknown.

Although the soil inoculum used contained a mixture of propagules from various mycorrhizal fungi, only a single

mycorrhizal type developed. It is not clear whether this was an early stage type (Mason et al., 1983). It is well known that a narrow range of fungi characterizes early seedling development in the nursery. The nursery conditions are very artificial and are unfit for many fungi (Marx, 1980). Thus the type formed here fits in with this type of condition and with this age of the seedling. The repeated reproduction of the same mushrooms in the pots in this study suggested that the fungus involved was symbiotically associated with the seedlings of *B. microphylla*. Repeated occurrence of the same mushrooms around *B. microphylla* in nature is an other indication that this fungus is associated with *B. microphylla*.

Trees growing in the very poor miombo woodland sites need mycorrhizae. These sites are burned yearly and as a result are very much eroded (fig. 2b). The present study has shown that the formation of mycorrhizae in the nursery enhanced the growth of *B. microphylla*. This may also apply to other miombo tree species.

The growth rate observed for this species is far higher than that observed for *J. globiflora* in the field (Chidumayo, 1991). The most probable reason is that nursery seedlings are irrigated while those in the field are subjected to the real climatical conditions.

Regarding nodulation, the absence of nodules on the root of *B. spiciformis* in nature (Högberg and Nylund, 1981) was also observed in the nursery in the present study. This is common to ectomycorrhizal legumes (Alexander, 1989).

3.2.3.4 Conclusion and recommendations

Soil inoculum collected from the root zone of adult *B. microphylla* trees growing in the miombo woodlands was used to raise *B. microphylla* in the nursery. Typical ectomycorrhizae were formed within 6 months. The fungal species involved produced sporophores similar to those earlier observed in the miombo woodlands around adult trees of *B. microphylla*.

Inoculated seedlings were far bigger than the controls. It is recommended to undertake further study on the growth of various fungi (cf. Smits et al., 1987) and to find out which are most efficient mycorrhizae under various conditions.

3.3 *Julbernardia globiflora*: mycorrhizal symbiosis and root pruning in the nursery

3.3.1 Introduction

J. globiflora occurs naturally in the miombo woodlands where it is subjected to a harsh climate: low and irregular rainfall, high temperatures and yearly fires. The survival of *J. globiflora* in nature is attributed to its flexible architecture. The architectural flexibility generally displayed by legume trees growing in stressed environments is reflected in many aspects.

These are discussed by Oldeman (1989) for East African *Acacia* species. Trees dominating the miombo woodlands share at least the following features. (1) They have a well-developed root system (Boaler, 1966). (2) They have a shallowly spreading crown (Lind and Morrison, 1974). (3) They shed leaves early in the dry season and flush before the rainy season (Celander, 1983). (4) They produce a huge amount of fruits (personal observation). (5) They are symbiotically associated with fungi and/or bacteria (Högberg and Nylund, 1981; Högberg, 1982; Högberg and Pearce, 1986). (6) Their seedlings yearly die back during the dry season until the year when they have accumulated enough stocks in the root for rapid growth in one season, which will allow them a vertical escape to the canopy (Ernst, 1988; Chidumayo, 1991; this study). (7) They grow on poor sites (FAO, 1974; Högberg, 1992).

These aspects are also found in *J. globiflora* in nature. While enormous amounts of information on the behaviour of miombo trees in nature have been accumulated, there are few reports on their behaviour in artificial environments.

The objectives of this study were therefore:

- (1) to study the symbiotic status of *J. globiflora* in the nursery;
- (2) to describe some aspects of the architecture of seedlings as influenced by root pruning in the nursery.

3.3.2 Materials and methods

Seeds

Seeds of *J. globiflora* were harvested from one tree (fig. 10a) in August 1991 at the foot of Uluguru Mountains in Morogoro, Tanzania. These seeds were used without any treatment.



Fig. 10. Regeneration of *J. globiflora* (A) fruit-bearing tree (B) seedlings of different pruning regimes. left: non-pruned seedling; middle: pruned once a week; right: raised in closed pot.

Soil inoculum

A mature tree of *J. globiflora* which was earlier seen to be surrounded by mushrooms of ectomycorrhizal fungi was selected

in the miombo woodlands.

A circle of 6 meter diameter around the tree base was irrigated for 2 weeks to stimulate the vegetative growth of the fungal mycelium before soil was collected from the root zone. This soil was to constitute one part of a growth medium, the other two parts of which were sand, for a germination experiment of the above seeds. The soil was either used to make a seedbed of 60 X 40 cm or packed in polythene tubes. These tubes were either closed or open at the bottom. The original arable soil was removed from the nursery floor before arranging the tubes or making the seedbeds.

Experimental set-up

Pots and seedbeds were arranged in a randomized block design with 7 treatments (see below) in 3 replications. Two seeds were sown per tube and 16 per seedbed. Each replicate was ultimately thinned back to 8 seedlings. Twice a day seedlings were watered with tap water. The amount of water applied per seedling varied between 40 and 80 ml depending on weather conditions and the period of the year. Seedlings were also provided with a light shade for the initial 4 months.

The various treatments were as follows:

Treatment	Tubes	Root pruning frequency
(1)	open	once a week
(2)	open	every two weeks
(3)	open	every 3 weeks
(4)	open	every 4 weeks
(5)	closed	not pruned
(6)	seedbed	not pruned
(7)	open	not root pruned

At the end of the experiment the following were recorded:

mycorrhizal infection rate; total seedling height; total shoot and total root biomass as well as the biomass of lateral roots. From data, the shoot/root ratio was computed. The root architecture and the various mycorrhizal types were described. The results were subjected to an analysis of variance and significant means were sorted out using the Duncan New Multiple Range.

The experiment started on December 9, 1991. It went on through the 'long rains' and the long dry season and was concluded on September 9, 1992 towards the beginning of short rains.

3.3.3 Results

Germination

After 12 days all seedlings had emerged. At that moment, the taproot was longer than 15 cm while the shoot measured 5 cm. Root coiling had already started in the closed pots and roots had penetrated in the soil beyond the open-bottomed pots.

Nodulation

No single nodule was seen on the root system of any seedling.

Mycorrhizal status

None of the seedlings was found to be associated with VA mycorrhizae. Instead, all seedlings had one or several types of ectomycorrhizae (APPENDIX 1C; 1D; 2G and 4E, pages 181, 182 and 184).

The mycorrhizae were generally elongated. They emerged from long roots and branched sideways building a big system, the leading root of which was itself mycorrhizal. The striking feature of most mycorrhizal types was that their dense hyphae covered not only the mycorrhizal part but also the non-

mycorrhizal part of long roots which bore them. Very thick, hairy and highly branching rhizomorphs emerged mainly from the long root and colonized a big soil volume surrounding the root system. Smooth mycorrhizal types were totally absent. The infection rate was higher in seedlings with pruned roots than in intact seedlings.

The growth parameters of table 7 and observations made allow the following descriptions. All root pruning frequencies strongly reduced height growth, formation of new leaves, shoot and total root biomass (fig. 10b). Unpruned seedlings growing in the seedbeds were more than twice as tall, with higher number of leaves and higher weight, than the ones with pruned roots. Seedlings growing in closed tubes were taller and heavier than seedlings with pruned roots, but had their roots coiled in the pots. The root system of freely growing seedlings as observed in the seedbeds consisted of a well-developed, thick and long single or splitting taproot representing over 95 % of the total root biomass. The mean shoot/root ratio for such seedlings was around 0.25.

The production of laterals was lightly stimulated by root pruning and significantly by mechanical hindrance by a closed pot. Laterals in the closed pots were mainly coarse. Primarily, seedlings react to root pruning by reiterating several vertical roots at the point of cut (Dyanat-Nejad, ex Oldeman, 1990). This tendency decreased with time as the point of cut developed a club-shape.

For non-pruned seedlings, the above ground architecture was characterized by a single, or V-branching stem having a somewhat flattening or curving spreading top. Pruned seedlings had their architecture acquired in the first month after germination, a period after which they did not grow any more in height. This architecture is just a straight non-branched short shoot.

Table 7. Means and standard deviations of various parameters in *J. globiflora* seedlings (n = 24) subjected to different pruning regimes in the nursery. Values between parentheses represent standard deviation.

1 .. 4 wk = pruning once a week to once in 4 weeks

clo = seedlings in closed pots; no pruning

dir = direct sowing in the seedbed; no pruning

dirp = direct sowing in open pots; no pruning

ht = seedling height (cm)

sh = shoot dry weight (g)

sh/r = shoot/root ratio

lat = biomass of lateral roots (g)

inf % = infection percentage of fine roots

	1 wk	2 wk	3 wk	4 wk	clo	dir	dirp
ht	6.61a*	6.86a	6.73a	9.23b	9.00b	16.75d	13.05c
	(1.19)	(1.01)	(0.78)	(2.51)	(2.27)	(4.17)	(0.92)
sh	0.376a	0.462a	0.460a	0.465a	0.594a	1.474c	0.918b
	(0.11)	(0.11)	(0.12)	(0.13)	(0.18)	(1.02)	(0.33)
sh/r	0.505b	0.688c	0.673c	0.618c	0.322a	0.266a	0.234a
	(0.15)	(0.20)	(0.19)	(0.23)	(0.08)	(0.10)	(0.08)
lat**	6.60a	6.03a	6.48a	7.33a	24.73b	4.27a	3.71a
	(0.045)	(0.031)	(0.039)	(0.056)	(0.168)	(0.20)	(0.032)
inf %	95.2d	95.7d	96.0d	96.7d	91.8c	75.1a	81.04b
	(3.1)	(2.7)	(2.4)	(2.6)	(3.6)	(6.10)	(6.5)

* = values followed by the same letter (a to d) in the same column are not statistically different at 5% level of significance.

** = /100

As time went on, pruned seedlings for all treatments did not produce new leaves while at the same time old leaves turned ever paler. None of the seedlings dropped its leaves or

died back during the experimental period.

3.3.4 Discussion

Trees in a stressed environment adopt survival strategies. While trees in wet environments have their nutrient stock mainly above ground, trees in dry areas have their reserve mainly in the root. To discuss the strategy of *J. globiflora*, a sapstream model (Oldeman, 1989) will be our point of reference. Seeds of *J. globiflora* germinated readily. This suggested that they do not display dormancy. The same observation was made in Zambia (Chidumayo, 1991). *B. microphylla*, *B. spiciformis* (3.2) and *B. boehmii* Taub. (observed in the nursery but not included in this work) all germinate readily. Quick germination helps seedlings take advantage of short periods of rain.

J. globiflora seedlings did not nodulate in the nursery. This is in agreement with field surveys carried out in Zambia (Högberg and Pearce, 1986). No ectomycorrhizal legume is known to nodulate so far (Alexander, 1989).

The formation of ectomycorrhizae by *J. globiflora* seedlings in the nursery was expected. *J. globiflora* forms ectomycorrhizae in nature (Högberg, 1982), like other miombo-dominating tree species in the genera *Brachystegia* and *Azelia*. Our field observations indicated that *J. globiflora* is associated with mushrooms of various ectomycorrhizal fungal species producing sporocarps during the short rains and in the long rains. Species observed belong to several genera including *Russula*, *Amanita*, *Lactarius*, *Boletus*, *Scleroderma*. but have not yet been identified to species level.

The main features of mycorrhizal types observed are (1) their dense hairiness (hyphae) and (2) their thick, long and highly branching rhizomorphs. Rhizomorphs as thick as 140 μm have been measured in this study. Dense hairiness in savanna/miombo mycorrhizae has been observed also in Nigeria (Redhead 1968; 1980) and in Senegal (Thoen and Bâ, 1989; Bâ

and Thoen, 1990). These hyphae open to the root an enormously increased soil volume for exploitation and allow a manifold uptake of water and nutrients. Compared to pines (5.2), legume ectomycorrhizae were a bit more slender but with a higher proportion of the fungal sheath. The mean diameter of mycorrhizal roots in *J. globiflora* was 240.6 μm (SD 48.6; $n = 17$). The mean sheath thickness was 26.7 μm (SD = 4.0) and represented 22.9% of the root diameter. This sheath thickness falls in the range of 7 to 78 μm postulated by Alexander and Högberg (1986). Compared to *A. guanzensis* mycorrhizae (4.4), *J. globiflora* has thinner mycorrhizal roots and a lower proportion of the fungal sheath.

The formation of ectomycorrhizae by *J. globiflora* is also part of its survival strategy. Miombo soils are poor in nitrogen and phosphorus. The formation of ectomycorrhizae helps the trees to absorb more efficiently water and nutrients. In addition, the fungal sheath temporary stores nutrients for use in time of need (Harley and Smith, 1983).

The percentage of infection, higher in the tubbed plants with pruned or blocked roots than in the controls, would be explained by reduced root elongation rates in the pot, resulting from regular cutting or from the mechanical barrier caused by the pot wall. The elongation of the fine roots in the pot is mechanically hindered, thus giving an advantage to the slowly growing fungi. In this respect, Marks and Foster (1973) indicated that fast-growing laterals can outgrow the establishing fungi and so escape infection. Contrarily to the tubbed situation, fungi in the seedbed had to deal with freely growing laterals, colonizing some and being outcompeted by others.

Concerning growth variables as influenced by root pruning, all kinds of growth, i.e. in height, in root length, and in producing new leaves were severely depressed by root severing, once a week or once in four weeks. Cutting the taproot and its subsequently regenerated parts, isolated the seedling in a pot where nutrients were steadily being depleted. Cutting the taproot or isolating it in a closed pot

was to attack the very strategy of the seedling. The sapstream model (fig. 7) integrates 3 compartments, crown, cambium and root system. In stressed environments, the energy manufactured in the crown and nutrients absorbed by the roots are used to build a root system with a high storage capacity. The taproot is a perennating organ which ensures regrowth of the tree after periods of droughts and fires (Chidumayo, 1991). By frequent root cutting we interfered with the very survival strategy. The seedling reiterates one or more taproots after pruning, initially using energy manufactured in the shoot but relying on internal reserve translocation later on when the photosynthetic capacity of the leaves gets exhausted. Thus finally in the late stage, the seedling feeds upon itself to maintain itself while waiting for favourable conditions to resume growth. This explains in large part, why the situation only worsened for pruned seedlings as time went on.

Die-back is another characteristic of miombo tree seedlings in nature. During the period of die-back, photosynthesis ceases and the seedling survives modestly on its reserves until conditions for regrowth are favourable. Then its energy reserve is used to produce the photosynthetic organs. Die-back, seen as one of the major hindrances in raising miombo tree species (Boaler, 1966; Celander, 1983) did not occur in the experiment described here. It was not likely to happen, at least in directly raised seedlings, since the experiment was concluded at the time when shoot reiteration had already taken place in nature. Die-back in semi-arid regions is most likely triggered by severe prolonged dry periods. Such dry periods affect the sapstream (fig. 7). Die-back was observed for *P. angolensis* (this study; Boaler, 1966) and *J. globiflora* in nature (Chidumayo, 1991). In the nursery, seedlings were shaded and supplied with water.

Root pruning, however, is an old tradition in plant propagation. Its aim is to promote the growth of a lateral root system in the pot or bed (FAO, 1974), so increasing prospects of successful rapid field establishment (Rook, 1971). It has been successfully applied in the propagation of

various tree species including species from the genera *Eucalyptus*, *Pinus*, and *Picea*.

The promotion of shoot and root growth, increased production of feeder roots and the survival of the seedlings were reported and attributed to root pruning (Stubbings, 1958; Gosh et al., 1974). Root pruning is often a routine in nursery management. So it was in all nurseries visited in Tanzania, Rwanda and Kenya.

Besides enhancing the production of lateral roots, root pruning enables nursery people to lift seedlings easily when these have to be transplanted in the field. For lifting to be possible, an alternative option to root pruning is, to grow seedlings in closed pots. In our experiments, seedlings growing in closed pots presented superior height growth, and more abundant production of mainly coarse laterals as compared with frequently pruned seedlings.

An explanatory and testable hypothesis is that energy and food reserve earlier accumulated in the root, and restricted to the pot during the first months, could be translocated for continuous but limited growth (fig. 7).

Pruned seedlings, on the other hand, were frequently low and poor in roots. This difference in growth pattern was temporary and started to fade away, as the energy and nutrient reserve in the root of previously potted plants became exhausted. Seedlings which grew in closed pots showed root system damage not due to pruning. Root coiling, mainly of the taproot was so pronounced that potted plants are not the best planting stock. It is generally known also (chapter 4) that the effect of root coiling persists permanently after transplanting. Seedlings with roots having suffered in this way have a diminished survival potential in the field. They are vulnerable to wind, fires and drought, since their root system is disabled and shallow.

Closed pots act as a mechanical barrier, a "root floor" (Oldeman, 1990) inhibiting root elongation. This resulted in the production of mainly coarse laterals. Although seedlings with pruned roots had more laterals, i.e. a different biomass

configuration, there was no significant difference in dry weight of laterals between levels of pruning or between pruned and non-pruned plants. However, functional effectiveness of laterals may be high even though they contribute little biomass. Our method of assessment was not designed to detect such functional differences. The survival of miombo trees in their ecological zones in any case is not primarily due to fine roots. Therefore the promotion of these fine roots at the expense of taproot is deleterious.

3.3.5 Conclusion and recommendations

J. globiflora seedlings possess the architectural flexibility displayed generally by legume trees in stressed environments. A study conducted in the nursery showed the following. (1) *J. globiflora* germinates readily. (2) Seedling architecture (above ground) assumed within the first year a somehow curving, flattening or exposed crown built by mixed axes, and for some seedlings, forming V-shaped sympodia like adult trees. (3) For root-pruned seedlings, the total height growth for one year was achieved within the first month after germination. Growth continued for non-pruned seedlings. There was no die-back in any treatment. (4) *J. globiflora* formed ectomycorrhizae with several miombo fungi. (5) It developed more root than shoot biomass. (6) Root pruning resulted in a sharp growth depression of both shoot and root systems. Seedlings with pruned roots primarily reacted by reiterating several vertical roots at the point of cut, a tendency which decreased as the point developed into a club-shape. (7) Lateral roots were promoted to some extent by root hindrance, e.g. root pruning or the pot acting as a mechanical barrier, but no benefits are to be expected after transplantation in miombo zones. It is recommended that further research be carried out to quantify the contribution of various mycorrhizal fungi to seedling growth and survival in the field. Furthermore, it should be studied whether or not one or

two root prunings could be effective, allowing the nursery people to lift the seedlings for transplanting while still conserving taproot reiteration potential at lifting time.

Chapter 4

AFZELIA QUANZENSIS (LEGUMINOSAE-CAESALPINIOIDEAE)

4.1 General introduction

A. quanzensis is a canopy associate of other leguminous tree genera dominating the miombo woodlands, namely *Brachystegia* and *Julbernardia* (Mgeni and Malimbwi, 1990). The high commercial value of the wood of the species (Bryce, 1967) has caused it to be overcut. The same value also won it government protection. The regeneration of *A. quanzensis* is very low where yearly fires occur. Thus, overcutting and low regeneration rates call for artificial regeneration of the species. Like most of the other miombo tree species, information on nursery techniques and later field planting is urgently needed. This chapter discusses seed germination, seedling establishment, survival and mycorrhizal formation in Morogoro and in the highlands of Tanzania.

4.2 Growth behaviour of *Afzelia quanzensis* in the greenhouse and in the field during the first year: practical implications

4.2.1 Introduction

A. quanzensis like *J. globiflora*, occurs naturally in the miombo woodlands where it is subjected, for most of the year, to unfavourable climatical conditions. As a legume tree of semi-arid areas, it also survives in nature by its architectural flexibility (Oldeman, 1989). Features shared by trees dominating the miombo woodlands as outlined for *J. globiflora* (see 3.3.1) also apply to *A. quanzensis*. As for other miombo dominating tree species, most information on the growth behaviour of *A. quanzensis* covers only the natural

habitat. Knowledge of tree survival strategies is important since it can help one elaborate a good nursery technology for afforestation purposes. *A. quanzensis* sheds its leaves earlier in the dry season (June to July) and disperses its seeds by explosive dehiscence in the same period or a little bit later. It has far bigger seeds than those in the genera *Brachystegia* and *Julbernardia*.

The aim of this study is to describe the growth behaviour of the species in the greenhouse and in the field during the first year after sowing in the miombo zone.

4.2.2 Materials and methods

Thirty-six Seeds of *A. quanzensis* were mechanically scarified, their aril was removed and they were soaked for 12 hours before being sown each in closed one-litre plastic pots with 3 drainage holes. The pots were placed in the greenhouse in Morogoro. Watering was done daily, the amount of water applied regularly adjusted to meet changing weather conditions (2.4.2). After 3 months and a half, the seedlings were transferred from the greenhouse to the miombo woodlands in Morogoro. Watering was continued twice a day. Some seedlings were left in the greenhouse. In the course of the experiment, the following features were observed: (a) the duration of germination (b) the period elapsed before the cotyledons drop (c) the growth in height (d) the production of leaves and (e) the root architecture. At the end of the experiment, the total biomass was assessed and the relative contribution of the root to the total biomass was calculated. The experiment started on January 13, 1992 and was concluded on January 13, 1993.

PROPOSITIONS

The survival of miombo seedlings hinges upon their ability to feed upon themselves during periods of drought and to withdraw water and energy from themselves in order to resume growth towards the end of the long dry period.

A forest ecosystem is a living system in which trees can not be claimed to be superior to other organisms, except in biomass.

Deficiency symptoms in biological and socio-economic systems in the tropics do not always indicate the absence of the apparently lacking element.

The majority of Africans have never seen a tropical forest.

Whereas the cold war has apparently finished, the wars seem hotter than ever.

Root pruning of miombo seedlings for the purpose of planting them in the miombo zones, is producing disabled subjects abandoned to the care of nobody.

Although preservation of biological diversity is now high on the agenda in nature management, in human society the extinction of ethnic and cultural diversity is proceeding stronger than ever.

He who takes a hasty decision to cut down a huge canopy tree while living in the neighbourhood of its phyllosphere and rhizosphere is not wise.

All living organisms will once escape life but none will escape death.

The strength of symbiotic bonds in ecosystems is like social ties in human society, in that the amount of food available to each organism plays a crucial role.

In miombo seedlings lateral roots and rhizomorphs are to the taproot, what water streams are to the river passing through a desert.

Survival of just germinated seedlings in tropical forests, apart from physical factors, depends upon the way in which the seedlings fit in an existing network of roots and hyphae.

'Love' is a bottomless pit: millions have fallen inside; it at all anyone reemerges, injuries are severe.

The liberation of man today must seek a way to free him not from external enemies, but from himself.

Seeds of division preserve their viability for a long period and once germinated, they survive periods of drought, floods and frosts.

4.2.3 Results

Germination

The germination of *A. guanzensis* seeds was uniform, and was completed within ten days. All seeds germinated. At emergence, while the seedling is still bending above the soil, the taproot is already on the bottom of the pot.

Growth in the greenhouse

Cotyledons are open within the first week after emergence (fig. 11a). They point upwards, thus supporting the fleshy tender epicotyledonous shoot. They are fleshy and remain green from one to three months, depending upon the prevailing soil moisture and air temperatures. In the field the distance between the root collar and the cotyledons is much larger than in the greenhouse. In the greenhouse, it was 8.3 cm ($n = 13$; $SD = 1.8$). In the wild it was 15.8 cm ($n = 13$; $SD = 1.6$). The epicotyl is also larger in the field compared to the greenhouse. Survival after germination is 100% in the greenhouse. The first leaves after the cotyledons, occurred within the first two to three weeks after sowing and the last within two to three and a half months. Seventy four percent of height growth in one year was achieved in less than 1 month following germination. After the first flush, the apical bud becomes dormant and if this coincides with the dry season it remains so irrespective of irrigation. The leaves age, turning slowly but irreversibly from green to pale green. However, they do not drop immediately. Visual assessment indicated that the size of leaves produced decreases with time within a given growing season. This is more pronounced when there is a stress, such as drought. No die-back was observed. The shoot consists of one unbranched leaf-bearing stem, straight on the lower $3/4$ but slightly curving towards the top.

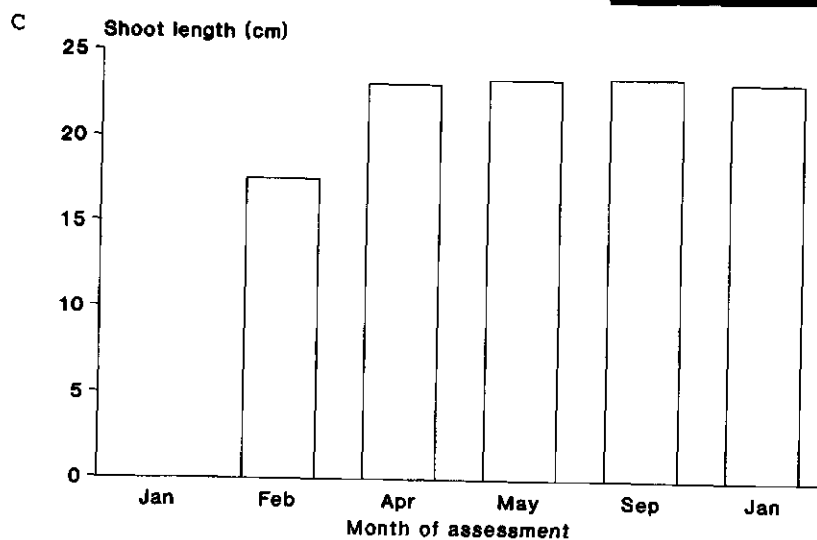


Fig. 11. Early growth of *A. guanzensis* (A) germination (B) root coiling as a result of closed pot (C) total height growth within the first year.

Root system prior to transplanting

The taproot was coiled several times in the pot for almost all seedlings (fig. 11b). It possessed many live laterals. For seedlings whose taproot grew directly out of the pot through the drainage holes, air pruning took place so that the elongation of the taproot was stopped. The end of such taproot developed into a club-like shape. The uppermost 4 laterals are bigger than the rest of the lateral roots.

Growth within the same year after transplanting

Shoot growth

There was a very slight height reduction after transplantation (fig. 11c). The number of leaves remained unchanged (average of 5, $n = 18$; $SD = 1.2$) until they started to drop. Before dropping, leaves turned pale to yellow brown. The slight height reduction was not accompanied by die-back or any seedling mortality. Few days before the rain following the long dry season of June to September, seedling apical buds became active. This was the case also in the greenhouse. New leaves were then produced before the rain came.

The mean total dry weight at the conclusion of the transplantation year was 10.6 g ($n = 18$; $SD = 4.3$) and the mean root dry weight over total dry weight was 0.734 g ($SD = 4.3$, $n = 18$), in other words, 73.4% of the total dry weight was contributed by root biomass.

Root growth

In the root system, deformation persisted. The taproot grew out a little bit, but continued coiling. For the air-pruned seedlings, one or two small taproot reiterations were noticed.

They were, however, thin. No new roots were formed coinciding with the shoot regrowth.

4.2.4 Discussion

The germination percentage of *A. quanzensis* is high as long as sound seeds are used. The seed, however, has a hard coat and a hard big aril which in nature delay germination. The aril was found to be both a mechanical and chemical hindrance (Munyanziza in prep.). Pre-germination scarification of seeds was found to be essential for *P. angolensis* (chapter 2) and most of other legume tree seeds (Khan and Tripathi, 1987; Dayanand and Lohidas, 1988; Sandif, 1988; Ngulube, 1989). Scarification is also useful for *A. quanzensis*. However, untreated seeds of *A. quanzensis* finally germinate provided enough water is available to soften the seedcoat. This was not observed in *P. angolensis*. While most of the seeds of *P. angolensis* do not germinate within one year following seed release, *A. quanzensis* do, provided that there has been sufficient rain (4.3).

Enhanced germination can stimulate seed reaction to the kind of short showers which occur in the miombo woodlands. However, this is detrimental if a dry spell follows germination. Natural germination of *A. quanzensis* in Morogoro takes place during the short rains of October to January. Germination takes two to four weeks depending on the amount of rain and the number of consecutive rainy days.

During a short and strong flush, the seedling reaches a considerable height and forms the final number of leaves. These leaves will sustain the thickening of the stem and the root by providing photosynthates (fig. 7). This timing of processes is essential for survival of the seedlings. The initial flush during the first months is topical. Anything which will hamper growth during this period will affect the whole growing season (4.3.3). Therefore seedlings should be sown at a period when weather circumstances, especially air

and soil humidity and temperatures are hospitable.

Transplantation should be done when growth is possible. Furthermore, because further development, within a given growing season, especially thickening, is relying upon a limited number of leaves, it is important to maximize the numbers and sizes of such leaves by sowing when conditions are optimal for their growth. Timing seed germination so that two months of rain are available is essential. Factors like drought (4.3.3) can strongly hamper initial growth.

The fact that the seedlings do not produce new leaves for a long period means that they are relying more and more on their reserve especially when the older leaves drop while the new shoot still has to develop. Chidumayo (1991) observed that *J. globiflora* seedling leaf production took place within the first few weeks and that it then stopped during that year. The same was confirmed for pruned seedlings of *J. globiflora* (3.3) and for *A. quanzensis*.

Cotyledons play a role in the initial growth, not only as a source of nutrients but also providing energy. Thoen and Bâ (1989) claimed them to play a role in initiating symbiosis, by supplying roots with energy needed. In an ongoing experiment at Wageningen, seedlings of *A. quanzensis* were raised in the greenhouse. After the first pair of leaves was produced, half of seedlings were cut off their cotyledons. Preliminary results clearly indicate that seedlings whose cotyledons were not cut have a higher number of leaves and are far taller than those whose cotyledons were cut. Seedlings which lost their cotyledons stopped growth.

As storing organs, cotyledons support and feed initial establishment of seedlings growing in poor environments. Ernst (1988) observed that seedlings of *B. spiciformis* relied mainly on cotyledon reserves for their growth during the first few weeks after germination. Nutrients are remobilized in the seed tissue and translocated to the developing radicle and epicotyl. As a result, seedlings can grow several days without external supply of mineral nutrients. This partly explains why the initial growth rate of big-seeded *A. quanzensis* is very

high. Its seedlings are far bigger than those of *P. angolensis*, *B. microphylla* and *J. globiflora*, all small-seeded (table 9). Sowing and planting of the miombo species should be carried out in such a season that cotyledons are not shed prematurely.

Continuous irrigation did not induce the production of new leaves, especially during the "wrong period". This shows that growth is not just a result of induced soil moisture. Air humidity and prevailing temperatures play a crucial role at a given period of the year, especially during the dry season. Chapter 2 is an example where germination of *P. angolensis* in the greenhouse was influenced by the prevailing air humidity. In another experiment in the greenhouse, the germination of *A. quanzensis* in the dry and cold period of the year took longer time in Morogoro (Munyanziza, unpubl.). In the highlands of Tanzania germination was totally inhibited. The species is sensitive to low temperatures (4.3.2.2).

How can *A. quanzensis* be artificially propagated? There are two options. (a) direct sowing or (b) sowing in the nursery or in the greenhouse and transplanting. The advantage of direct sowing is that the seedling taproot grows unhindered. The function of such a root in areas of marginal rain is well known (Boaler, 1966). The main disadvantage of direct sowing is that the rain is unpredictable in miombo areas. There is always a risk of drought soon after sowing, which can defeat the whole purpose. In the nursery, one can successfully raise seedlings according to his plan. In that case, other problems arise, especially taproot management. Pruning it is detrimental. Blocking it in a closed pot is to permanently disable the seedling and prevent it from reaching the water table in its final site after transplantation. Seedlings in this experiment certainly ran a high risk of dying in the long dry season if watering had not been carried out. The risk was enhanced by taproot deformation and restriction in the upper 15 cm of the soil as a result of earlier root coiling in closed pots. Soil moisture deficit in the miombo woodlands during the dry period is very high

(Boaler, 1966). This is partly due to yearly burnings which consume all organic matter.

Rhythmic growth in *A. guanzensis* seedlings also presents a problem to managers. It requires matching seedling transplanting with the unpredictable start of the growing season. For example, raising *A. guanzensis* seedlings in June or July is not good in Morogoro. That period is cold and dry and no growing season ahead is liable to be synchronous with seedling rhythms. September or October is not the best period since the rain of this period is generally very short, unreliable and too scarce for transplantation.

The best risk would be to sow the seeds at the end of February, to prune the roots once at the end of March and to transplant in the middle of April when the rains have started. Seedlings are likely to successfully regenerate their taproot after one root pruning. However, seedlings may not produce new leaves until just before the rains of October/November. The best period for direct sowing is April. It may be said in general that the time of transplantation should precede at least two months which favour seedling growth. Depending on the nature of seedling dormancy, artificial watering does not imply growth resumption or continuation.

4.2.5 Conclusion and recommendations

A. guanzensis growth in the first year was followed. Seedlings were raised first in the greenhouse and then transplanted to the miombo woodlands. Watering was done both in the greenhouse and in the field. The following facts were observed. (1) *A. guanzensis* germinated readily. (2) Seedling number of leaves and the height were attained in the first 3 months. (3) Stem thickening continued while the height growth and leaf production had stopped. (4) No die-back occurred during the long dry season. (5) Watering did not induce new leaf production until just prior to the first rains after the long dry season. (6) Seedlings developed a strong taproot which

coiled as a result of using closed pots. Given the rhythmic growth and the development of a strong root system by *A. quanzensis*, it is recommended (1) to match the planting time with the rain of April. (2) To use open pots and carry out one or two root prunings followed by transplanting. (3) Where rainfall is reliable, to do direct sowing in the planting site.

4.3 Effects of drought and temperature on the survival of *Afzelia quanzensis* seedlings and their ectomycorrhizae

4.3.1 Introduction

A. quanzensis naturally occurs on poor and dry sites yearly burned and depleted in organic matter. In such sites, ectomycorrhizal formation is vital. Trees and ectomycorrhizal fungi are known to perform differently under different site conditions (Harley and Smith, 1983). With the increased attention for indigenous plant material in afforestation and agroforestry, there is high likelihood that these species will be introduced in regions beyond their geographical areas. Forest decline today is not limited to a given region of a country. Replanting and conservation of forests therefore are also necessary in many regions. As more attention is given to indigenous trees (C.N.R.F.-I.S.A.R., 1992), it is very important to have an idea as to how plants of warm semi-dry areas behave in cooler and moister areas. Care should be taken when introducing a tree species in a new forest ecosystem. Oldeman (1985) warned that there is always a risk of replacing a forest by another forest, the latter being in general more unstable than the former. Zobel et al. (1987) discussed in details other problems associated with exotic species.

The study reported here was primarily meant to assess the performance of *A. quanzensis* and their mycorrhizae in the highlands of Tanzania and to see whether mycorrhizal fungi native to the rainforest and those associated with pine plantations could not colonise *A. quanzensis*. Failure of the seeds to germinate led to a further study, i.e. to investigate the cause of failure. In this new study, it was postulated that the cause of failure was low temperatures. The methodology used in both cases is reported. The objectives of this extended study were:

- (a) to assess early growth and survival of *A. quanzensis* seedlings and its mycorrhizal fungi introduced in the highlands of Tanzania;

- (b) to assess the effect of induced drought on the survival of seedlings and their mycorrhizae;
- (c) to carry out germination tests of *A. guanzensis* seeds at different temperatures in the laboratory.

These objectives are pursued in two separate experiments, which are discussed separately.

4.3.2 Experiment 1. Effect of temperature on the germination of *Afzelia guanzensis*

4.3.2.1 Materials and methods

Two zones with high rainfall, low temperature and different vegetation types were chosen for this study. In Mazumbai, the experiment was conducted in montane rain forest (Härkönen et al., 1993 for brief description of the vegetation). In Arusha, the experiment took place in exotic *Pinus patula* Schiede & Deppe plantations. In both ecosystems, small germination plots (50 X 50 cm) were prepared. Undecomposed organic matter was removed. Small openings were made in the canopy in order to allow light penetration. Plots were set out before directly sowing *A. guanzensis* seeds, treated as above (4.2.2). In one member of the pair, mycorrhizal soil from Morogoro was added to the plot before sowing. There were 14 plots in each zone (7 with addition of mycorrhizal soil from Morogoro, 7 without addition). In each plot, seven seeds were sown. In Mazumbai, the experiment started in November and ran till January. In Arusha it ran between September and February. Regular observations and germination counts were done.

In the laboratory at Wageningen, germination studies were conducted. Seeds were treated (4.2.2) and germinated at three temperature levels (treatments), namely 10, 15 and 25 °C. Each treatment covered a total of 70 seeds in 7 replications, i.e. 10 seeds per replicate. Daily observation during three weeks ensured germination counts.

4.3.2.2 Results

Germination in Mazumbai, Arusha and laboratory

In Mazumbai, no single seed emerged during the observation period. At the end of the experiment, an attempt was made to recover the seeds. 38 out of 98 seeds were recovered. The recovered seeds had lost the seedcoat. Some seeds showed a very short brown necrotic radicle which had emerged but failed to go further. Cotyledons were tightly closed.

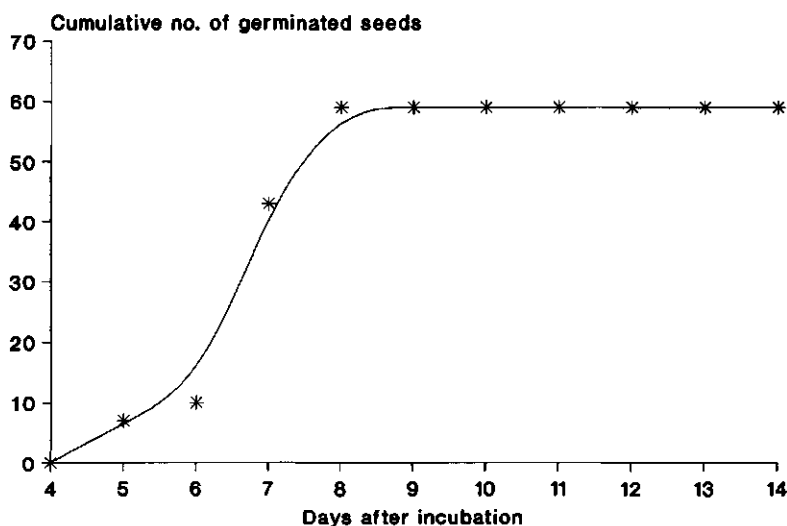


Fig. 12. Germination of *A. guanzensis* in the laboratory at 25 °C, a total of 70 seeds had been sown.

In Arusha, twelve seeds germinated after 45 days. The cotyledons, however, failed to open and the seedlings collapsed. No mycorrhizae were present. The other seeds were unrecoverable.

In the laboratory, only seeds sown at 25 °C germinated. Germination started five days after sowing and was completed 4 days later (fig. 12). No seed germinated at 10 °C and 15 °C. Seeds germinated at 25 °C were overgrown by an unidentified

pathogenic fungus. The fungus could be seen with the naked eye.

4.3.2.3 Discussion

Introduction of plants in alien environments in the past focused on exotic species. Zobel et al. (1987) have discussed the pros and cons of growing exotic species. The success or failure of the introduction of exotic species depended on research and investment. Ecological factors are important determinant aspects in afforestation. There are reports of trees growing faster in an alien environment than in their country of origin (Zobel et al., 1987). Pine species in the tropics provide examples. Whereas exotic trees have been well investigated in the tropics (ex. *Eucalyptus*, *Pinus*, and other fashionable species; see Burley et al., 1989), there is limited information on trials of indigenous trees and their fungal partners in ecologically different parts of one tropical nation. This remains a challenge to researchers and decision makers affecting the fate of forestry and environment in the tropics.

Among the requirements for seed germination (Willan, 1985; Albrecht, 1993) is a proper temperature regime. In Morogoro, seeds of *A. quanzensis* species completed germination within an average of 10 days (4.2). Morogoro is one of the warmest regions of Tanzania.

Miombo trees grow in warm areas (Lind and Morrison, 1974) and germinate in nature any time during the period from October to end of May of the following year. Different species germinate at different periods within this temporal range depending on the level of their dormancy and their moisture requirements. *Azelia*, *Julbernardia* and *Brachystegia* species usually germinate during the short rains of October to January while *P. angolensis* species germinate mostly from April to the end of May (Chapter 2 and 3). On average, this period is the hottest of the year.

In an unpublished experiment (Munyanziza unpubl.), the germination of *A. quanzensis* during the month of July-August took twice as much time. This is the coldest period of the year in Morogoro.

It is during this cold season that *Afzelia*, *Julbernardia*, *Brachystegia* and *Pterocarpus* species disperse their seeds or fruits. Minimum temperatures drop to around 15 °C in July according to the last 5 years of meteorological records by SUA. More cold and lower humidity during this period probably prevent seeds to "commit suicidal" germination in nature. Germination during this period would result in death since this period is dry. Ernst (ex Celander, 1983) reported that the miombo trees were sensitive to low temperatures.

In Arusha and Mazumbai, germination failures and poor survival had the same causes. The highlands of Arusha are subject to cold periods. According to Balmforth (ex Lundgren, 1978, the temperature range is between 7 and 29 °C. The yearly average in the miombo woodlands, days and nights is 20 °C while daily maximum temperatures may exceed 35 °C (Celander, 1983).

The mean annual temperature in Mazumbai lies below 20 °C (Lundgren, 1978). The minimum according to SUA meteorological records drops below 15 °C and approaches 10 °C in the coldest months. There is high likelihood that even if *A. quanzensis* seedlings had been introduced, poor growth and possibly death would have occurred. While the temperature regime was the limiting factor for seed germination and later growth of *A. quanzensis* in Arusha and Mazumbai, rainfall limits growth in the miombo woodlands (Celander, 1983). In warm moist areas, the growth rate of miombo species can probably be increased. For example, the initial growth rate of *P. angolensis* and *J. globiflora* regularly irrigated and unpruned, was far superior than the growth rate of exotic pines in Morogoro (chapter 2 and 3; Maghembe, 1982). The miombo trees can indeed respond positively to moisture supply so long as the temperature is maintained in the right range. There is more rain in Mazumbai and the Arusha highlands. However, as this study showed these

areas are unfit for *A. quanzensis*.

A. quanzensis seeds germinated quickly in the laboratory at 25°C. This temperature lies within the temperature range during the natural germination period in Morogoro. Germination failures of *A. quanzensis* in the laboratory at low temperatures agree with field results of Arusha and Mazumbai.

4.3.2.4 Conclusion and recommendations

An attempt to introduce *A. quanzensis* in the highlands of Tanzania by direct sowing was unsuccessful because of its vulnerability to cold during germination. Field and laboratory results both proved this. In the laboratory seeds failed to germinate at 10 and 15 °C but did at 25 °C. The performance of *A. quanzensis* fungal symbionts and the possibility of *A. quanzensis* forming mycorrhizae with fungi native to the rain forest or occurring in pine plantations of the highlands was not assessed, since the host failed to germinate. It is recommended to grow *A. quanzensis* in the habitat range of their natural area or in moister areas having a similar temperature range.

4.3.3 Experiment 2. Survival of *Afzelia quanzensis* seedlings in the field and in the greenhouse

4.3.3.1 Materials and methods

On the campus of the Sokoine University of Agriculture, seeds of *A. quanzensis* were sown 2 cm deep in November, just at the beginning of the short rains. In the miombo woodlands, spontaneously released seeds of *A. quanzensis* were mapped and their germination monitored. None of the seedlings was watered. Survival rates up to the beginning of the "long" rains were assessed.

In the greenhouse, *A. quanzensis* seeds were sown in 1-litre pots filled with three parts of sterilized soil mixed with one part of fresh *A. quanzensis* soil, for inoculated seedlings. Non-inoculated seedlings were raised exclusively in sterilized soil. One seed was sown per pot. Watering was done daily (2.4.2) during the first month after sowing. Thereafter, seedlings were arranged in a randomized block design in which the following watering treatments were made.

- (1) Inoculated and watering once a day;
- (2) Inoculated and watering every 3 days;
- (3) Inoculated and Watering once every 4 days;
- (4) Non inoculated and watering once a day;
- (5) Non inoculated and watering once every 3 days;
- (6) Non inoculated and watering once every 4 days.

Each treatment consisted of 21 seedlings distributed over 3 blocks. Survival counts were made. At the end of the experiment, the condition of seedlings was described, their mycorrhizal types sorted out and the total seedling biomass was computed. An analysis of variance was carried out for the mean biomass.

4.3.3.2 Results

Seedling survival in the miombo woodlands and on the

University campus: effect of drought

In the miombo woodlands and on the campus none of the seedlings died despite longer periods of drought than in the greenhouse, where seedlings were watered at least once in four days. All seedlings in the field were still alive when the long rains of April started. None lost its leaves.

Survival and growth in the greenhouse

Seedlings in the greenhouse had fully formed the first pair of leaves, besides the cotyledons, when watering regime started. One month after germination, most seedlings had three leaves beyond the cotyledons. At this moment, cotyledons in seedlings watered once every three or once every four days were already shrinking and dropping. Moreover growth in height had ceased. At the second assessment, two months after sowing, moisture deficit in these treatments was extreme. Most of the seedlings watered once every four days had dried out, their stem wrinkled, shrunk and bent back to the ground (fig. 13a). The same situation was developing in seedlings watered once every three days. Mycorrhizal inoculation had no visible effect. At the next assessment, one month later, the leaves of most of the drought-treated seedlings had dropped and many seedlings were apparently dead.

However, one week before the rain season came, seedlings produced leafy shoots. There was a significant difference in the amount of biomass between the control and drought-treated seedlings (table 8). Inoculated seedlings of the same watering regimes were not significantly heavier than non-inoculated ones within a bracket of 5%. There was a significant difference in dry weight between the biomass of daily irrigated seedlings and the rest. There was no significant difference in biomass of seedlings irrigated once in 3 or 4 days, whether inoculated or not. The root system was modified by moisture deficit. Elongation of the taproot stopped and fibrous roots were produced (fig. 13c). However, these were

dry and showed no sign of life and function. Non-inoculated seedlings remained non-mycorrhizal.

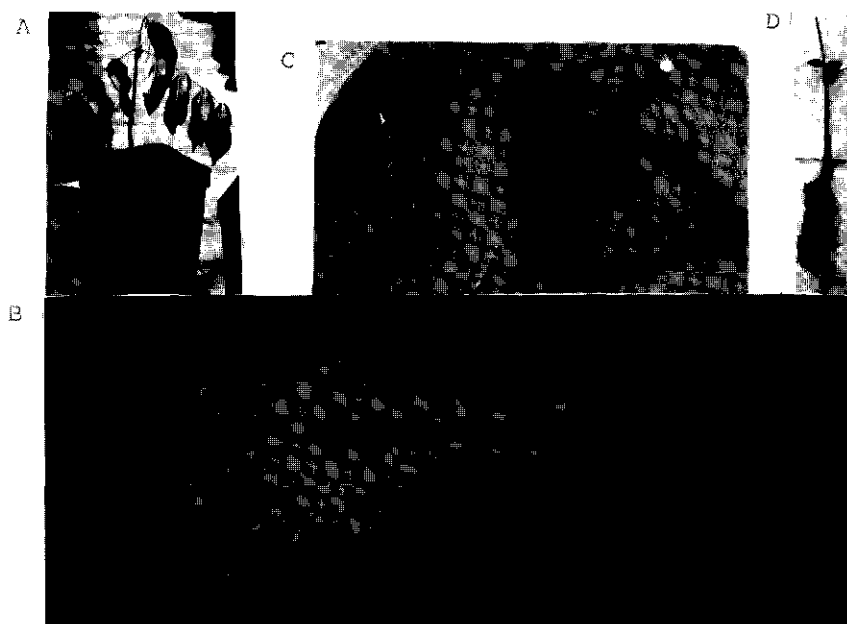


Fig. 13. Effect of induced drought on *A. guanzensis* seedlings and their mycorrhizae (A) seedlings watered once every 4 days, condition one month after the beginning of the treatment (B) mycorrhiza in this same treatment (type 1; see APPENDIX 1D, page 181) (C) root system of a seedling watered once in 4 days (left) and control (right) (D) reshooting seedling watered once in 4 days, in the beginning of the rainy season.

Seedlings irrigated once every three and four days were colonised by one mycorrhizal type (type 1). Mycorrhizae were dark brown, and had very long and stiff hyphae. Mycorrhizae had a very thin, desiccated mantle (fig. 13b). The percentage of fine roots infected by this type was 3% for seedlings watered once every four days and 7% in those watered once every three days. The infection percentage in seedlings daily watered was 54 of which type one made 11% (APPENDIX 1D, page 181). The other mycorrhizal type found in this treatment was yellow brown, healthy and covered by thick hyaline hyphae. The infection rate by this fungus was 43%. For more details on the

mycorrhizal types encountered in this experiment see APPENDIX 1C and D (page 181). In the miombo woodlands, seedlings were colonised by type 1 and all fine roots were mycorrhizal.

Table 8. Mean dry weight of inoculated or non-inoculated *A. quanzensis* seedlings treated with different watering regimes in the nursery. Values are means of 21 observations.

Treatment	Dry weight (g)	SD
inoculated, watered once a day	12.4b*	3.1
non inocu., watered once a day	11.8b	3.1
inoc., watered once every 3 days	2.1a	1.6
non in., watered once every 3 days	2.4a	1.3
inoc., watered once every 4 days	1.2a	0.5
non inoc., watered once every 4 days	1.4a	0.7

* = Means followed by different values are statistically different at the 5% level of probability.

4.3.3.3 Discussion

A. quanzensis germinates some two to three weeks after the first rains, preceding the short dry period. At one moment or another, establishing seedlings face severe drought.

In nature miombo tree species develop first a taproot deep enough to function as a pump and as a stock of water and nutrients (Boaler, 1966). This taproot ensures the survival of seedlings notwithstanding fires and drought. In potted seedlings or regularly root-pruned ones, the development of this taproot is hampered, as proven for *P. angolensis* (2.4) and *J. globiflora* (3.3).

This explains why survival was higher in nature and poorer in the greenhouse. In addition, seedlings in nature

have access to deeper water and nutrients via interhyphal connections and via natural root grafts between the seedlings and the adult trees (Molina et al., 1992). Reduced watering in the greenhouse had quicker and more severe repercussions on the greenhouse seedlings than on wildlings. This was shown by their wilting behaviour. However, the leaves did not drop immediately. The final loss of leaves marked the intensity of the drought but not the death of seedlings. Seedlings survived in a leafless state.

Taproot elongation stopped, and a fibrous root system was formed. Abundant shoot reiteration, often observed on stressed seedlings (2.5) corresponds to multiple root reiteration in seedlings subjected to drought. Feil et al. (1988) also observed that drought reduced root elongation while promoting the development of a fibrous root system. The same is observed here. Fine roots are efficient in water absorption. Whenever the conditions are adequate, the production of a fibrous root system also increases chances of colonization by mycorrhizal fungi. The role of mycorrhizal development in tree nutrition and survival is well known (Bowen, 1980; Mikola, 1980). One of the parts played by mycorrhizal fungi is to help the host thrive in adverse sites. Molina et al. (1992) have reported that several species of *Rhizopogon* had been experimentally demonstrated to provide drought tolerance to seedlings in a region of seasonal drought. However, mycorrhizae can only form if the tree symbiont is able and ready to compensate the carbon balance of the association. In this experiment, drought affected the photosynthetic capacity of seedlings. This indirectly affected the amount of carbon available for the fungal symbiont.

The degree of sensitivity of mycorrhizal fungi to site conditions depends on the species of the fungi. Screening of the fittest fungus is therefore necessary. Marx et al. (1970) observed that *Thelephora terrestris* (Ehr.) Fr. was affected by changes in soil while *Pisolithus tinctorius* (Pers.) Cok. & Couch species was not. Duddridge et al. (1980) demonstrated the role of mycorrhizal fungi with big rhizomorphs in water

transport in dry areas. Theodorou and Bowen (1971) reported a rapid decline in mycorrhizal production resulting from changes in temperature. Smits et al. (1987) reported that regeneration failures in *Dipterocarps* were due to logging methods which increased soil temperature and as a result, affected mycorrhizal formation. Water deficit in the plant stops root growth and root suberisation is accelerated (Marks and Foster, 1973). In this study, fibrous roots formed as a result of drought became non functional as far as could be visually assessed. Such roots are not usually infected. The dark brown mycorrhizal type with extensive rhizomorphs was seen to be more tolerant to drought than the yellow type. The drought was, however, so extreme in treatments irrigated every 3 or 4 days that mycorrhizae had a very thin mantle.

Finally, the total collapse of the photosynthetic organs marked the end of any assimilation, though not the end of the seedling's life. Some few days before the rain in March, meristems became active in apparently dead seedlings. These were apical meristems of the leafless stem or, in case these had died back, axillary meristems of the cotyledons (fig. 13d). Resumption of growth was not the result of extra watering or rain since the frequency of watering was pre-established in the greenhouse. One of the characteristics of miombo trees is their ability to detect favourable (or unfavourable) seasons. Leaf initiation and unfolding starts some days before it rains (Lind and Morrison, 1974). This allows the trees to effectively use the limited rain which would follow, in order to flower and fruit. The production of new meristems and leaves was not clearly synchronized with the phenology of new roots. This corresponds to the findings of Hallé and Martin (ex Hallé et al., 1978) in rhythmically growing *Hevea brasiliensis* Muell. with unsynchronized root dynamics. Water used in new meristem and leaf production hence can only come from either internal reserves or from a structural overcapacity in roots. In view of the enormous water transport in plant production (cf. Oldeman, 1990) and the flexibility in water absorption needed in the highly

varying miombo wetness and in view of miombo root strategies in general (2.4; 3.3), the hypothesis of overcapacity in root absorption best fits the known facts. While in the greenhouse mycorrhizal inoculation did not influence seedling survival, in nature it is more likely that mycorrhizae played a role in this respect. Interaction with the fungus in nature, contrarily to the greenhouse or the nursery, starts even before emergence (Thoen and Bâ, 1989; 3.2.2). This favours seedling establishment within a very limited period of rain. In artificial environments, infection in *Azizelia* spp. starts late (Munyanziza in prep.; Bâ, et al., 1994).

4.3.3.4 Conclusion and recommendations

In this experiment, potted, inoculated or non-inoculated seedlings were assessed as to their growth and survival in the greenhouse under different moisture regimes. In the field, seedlings directly raised were also assessed for their survival and the nature of their mycorrhizae. Both inoculated and non-inoculated seedlings suffered the same fate in the greenhouse. In drought-treated experiments, both shoot and root extension stopped. Some seedlings died. The majority withered and came near death. But most of them flushed anew just before the rain came. In the field all seedlings survived. Only the dark brown mycorrhizal type grew on the shrunk laterals in the root system of drought-treated seedlings. Laterals, however, showed no visible traces of functioning. In the field, the same dark brown mycorrhizal type dominated but it was visibly functional. In daily watered seedlings, a yellow-brown mycorrhizal type dominated. It was concluded that mycorrhizal association did not heighten the chances of seedling survival in extreme stress in the greenhouse. It is recommended to design well-timed calendar of nursery and planting work, in such a way that favourable conditions are available at the time of sowing, inoculation and planting. Studies also should be carried out to screen the

microflora to discover the ecological requirements of its species.

4.4 *Afzelia quanzensis* mycorrhizae: synthesis and specificity

4.4.1 Introduction

Sporophores of a wide range of fungi were found in association with *A. quanzensis* in the miombo woodlands. Most of them are probably ectomycorrhizal. Similarly, *Brachystegia* and *Julbernardia* species were surrounded by the sporophores of several fungal species (Redhead 1968; Härkönen et al., 1993; Munyanziza and Kuyper in prep.). Whether these fungi can live in symbiosis with seedlings of *A. quanzensis* in the nursery is not known. Neither is it known whether fungi around species of other genera like *Eucalyptus* or *Pinus* can form mycorrhizae with *A. quanzensis* in the nursery or in the greenhouse. Understanding the level of specificity in tree-fungal relationship is important especially when one is launching an afforestation on sites where the tree species in question did not previously occur.

The aim of this study was therefore to examine the ability of various fungi associated with adult trees of the above species to form mycorrhizae with young *A. quanzensis* in the greenhouse and in the field.

4.4.2 Materials and methods

Three experiments were set up.

4.4.2.1 Inoculation with spores in the greenhouse

Mushrooms frequently seen in the field in association with species of *Afzelia*, *Brachystegia*, *Pinus* or *Eucalyptus*. were collected, air dried and stored for some 3 weeks before use. They were then mixed with sterile soil at a rate of 10 mg per litre. This mixture was used as a substrate for raising *A. quanzensis* seedlings in the greenhouse. The fungi were *Russula*

sp., *Pisolithus tinctorius*, *Suillus granulatus* (Fr.) O. Kuntze, *Lactarius chromospermus* Pegler & Pearce and *Pisolithus* sp. collected respectively in association with *J. globiflora*, scattered trees of *Eucalyptus* spp., *Pinus caribaea* Mor., *B. microphylla* and *A. quanzensis*. Illustrations of the fungal materials used are united in APPENDIX 5, page 185. The experiment had 5 blocks in which the 5 treatments, different inoculum, were randomly assigned. Watering was done to meet seedling moisture requirements (2.4.2).

At the end of 5 months, the experiment was concluded to compute the infection rate and to sort out and describe the different mycorrhizal types present.

4.4.2.2 Mycorrhizal formation in the greenhouse by seedlings of *A. quanzensis* raised in soil containing mycorrhizal fungi associated with other miombo tree species

Greenhouse inoculation

Soil inoculum from various sources was used in the greenhouse to raise *A. quanzensis* seedlings. The soil came from the rhizosphere of the following tree species growing in the miombo woodlands or in a farm in Morogoro (fig. 2).

- (a) *J. globiflora* (miombo woodlands, Uluguru)
- (b) *B. spiciformis* (isolated trees in a yearly cropped farm (3.2.2))
- (c) *A. quanzensis* (Uluguru)
- (d) *A. quanzensis* (Mikese)

Each soil type was packed fresh in plastic pots and used unamended to raise seedlings of *A. quanzensis*. Watering was done as on 4.4.2.1. At the end of the experiment, the seedling root system was examined and different mycorrhizal types were sorted out and described.

4.4.2.3 Mycorrhizal assessment on *A. quanzensis* seedlings directly raised in the root zone of different miombo tree species and pines

Germination plots were established within a radius of 5 meters away from the stem base of trees belonging to the following species: *A. quanzensis*, *J. globiflora* and *B. microphylla*, all three in the miombo woodlands, Uluguru. *A. quanzensis* seedlings were also directly raised within a radius of 5 meters around *Pinus caribaea* adult trees growing on the edge of the SUA nursery, Morogoro. These pines have been planted to provide mycorrhizal inoculum for pine nursery seedlings. These field trials comprised 18 to 120 seedlings of *A. quanzensis* per tree species. Watering was continually adjusted according to the prevailing weather conditions. During the rainy seasons, mycorrhizal mushrooms growing around the various trees were collected for identification. The list is under preparation. After 8 months, seedlings were carefully uprooted and their mycorrhizal status was assessed.

4.4.3 Results

Inoculation with spores

Out of the available spore materials used, only the *Pisolithus* species associated with adult *A. quanzensis* formed mycorrhizae with *A. quanzensis* seedlings in the greenhouse. All seedlings were colonized but the infection rate varied greatly. The mean infection was 64 % (SD = 13.2; n = 25) of the fine roots. The characteristic mycorrhiza of this fungus is represented in figure 14a. Mycorrhizae are ochre in colour just as the spore mass of the sporophores. They are totally wrapped in extensive hairs covering both the fine roots and the main roots. The hairs collapse on the surface of the roots when immersed in water. The mycorrhizae are long, somehow straight, emerging from the long root and not branching. They have heavy

rhizomorphs escaping from the mass of individual hyphae. The fungus involved has a characteristic smell. Further details are in APPENDIX 2F, page 182.

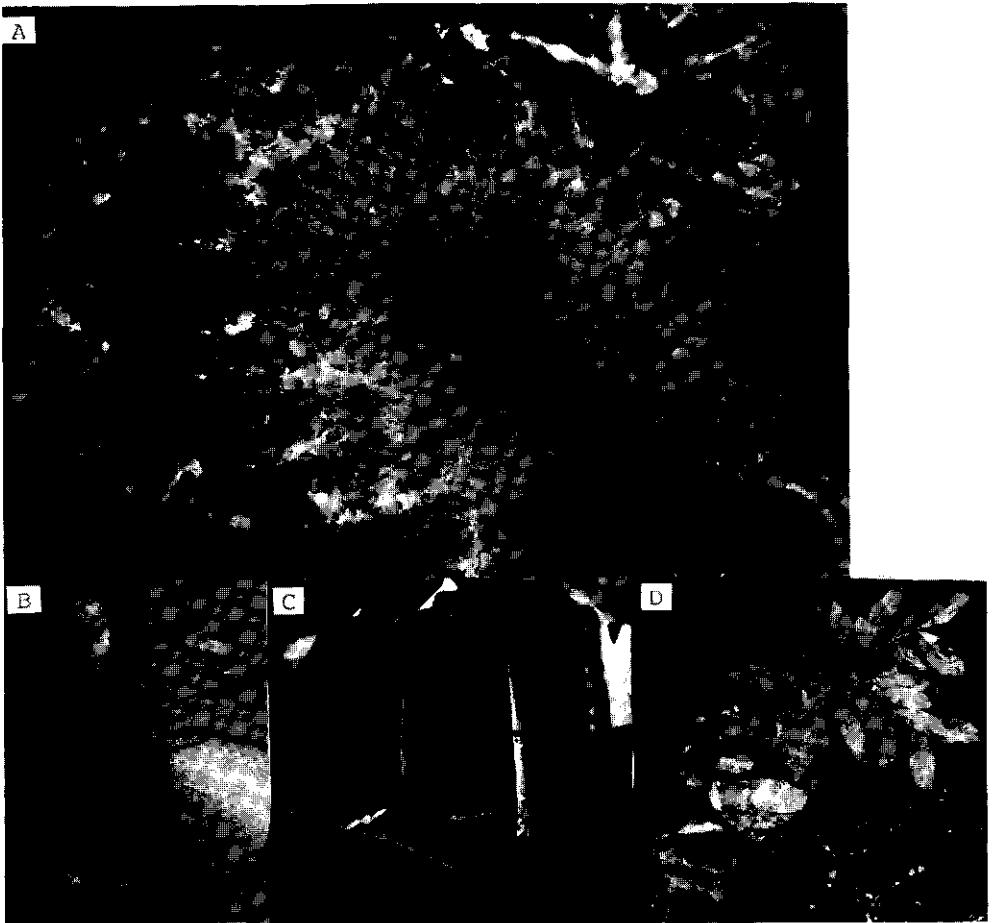


Fig. 14. Mycorrhizal formation on *A. quanzensis* seedlings raised in various inocula (A) mycorrhiza from spore inoculum with a *Pisolithus* sp. (B) mycorrhiza formed by seedlings in the root zone of adult *A. quanzensis* (C) mushroom formed in the greenhouse around seedlings having received fresh soil from adult *A. quanzensis* tree (D) *Suillus granulatus* sporophores around *A. quanzensis* grown in the root zone of adult pine trees.

Soil inoculum

None of the seedlings inoculated with mycorrhizal soil in the greenhouse or directly raised in the field formed VA-mycorrhizae nor did any nodulate. Irrespective of the source of inoculum, all seedlings formed well-developed ectomycorrhizae as assessed by the presence of a well-developed fungal sheath and the Hartig net. The infection rate ranged from 92% (SD = 7.13; n = 43) in the greenhouse to 100% in the field around *A. quanzensis* trees. The mean mycorrhizal root diameter was 282.4 μm (SD = 37.6; n = 19). The mean sheath thickness was 40.3 μm (SD = 6.2; n = 19). This sheath represented 29.0% (SD = 5.4; n = 19) of the mycorrhizal root diameter. Like for *J. globiflora* and *B. microphylla* and *B. spiciformis* mycorrhizae, *A. quanzensis* mycorrhizae are characterized by the presence of massive rhizomorphs. Rhizomorphs as thick as 150 μm have been measured. Regarding the mycorrhizal types present, there was variation according to the tree species from which seedlings received their inoculum. In addition, there were types which developed in the greenhouse but not in the field and vice versa, and the same was observed for mushrooms (fig. 14b, c and d). An example of such mushrooms is given in figure 14c showing mushrooms developing around *A. quanzensis* seedlings in the greenhouse. This mushroom was not observed in the field. Figure 14d shows mushrooms of *Suillus granulatus* around *A. quanzensis* seedlings raised under mature *Pinus caribaea* trees.

4.4.4 Discussion

Spore inoculum has been used to produce specific ectomycorrhizae on tree seedlings (Marx, 1976; Marx et al., 1976; Trappe, 1977). This technique has been successfully applied to pines in Kenya (Ivory and Munga, 1983). The use of mycelium inoculum and the spore inoculum is a way to prove that a given fungal species form ectomycorrhizae with a given

tree species. Spore inoculum, however, gives problems as discussed by Marx and Kenney (1991). One of the commonly reported problems is that the germination of spores is generally very low and erratic (Trappe, 1977; Fries, 1978). The germination of spores is usually induced by root exudates.

In the light of this, failure to form mycorrhizae by most of the spore materials used may have been caused by one or a combination of the following points. (1) The fungus does not form mycorrhizae with *A. guanzensis*; (2) the fungus forms mycorrhizae with *A. guanzensis* at a later stage; or (3) the requirements for the germination of spores or subsequent growth for the test fungi were not met. Failure to produce mycorrhizae using basidiospores does not exclude the possibility of the given fungus to form mycorrhizae with a given host. This may have happened in our experiment. With the *Pisolithus* sp. associated with *A. guanzensis* in the miombo woodlands, we achieved mycorrhizal synthesis using spores. The frequent occurrence of sporophores of this fungus around *A. guanzensis* trees even if the trees were isolated is a sign that the fungus concerned forms mycorrhizae with *A. guanzensis*. Its mycorrhizal formation in this experiment indicates that the spores of the fungus germinate under greenhouse conditions and that the fungus forms mycorrhizae with young seedlings. This is one of the fungi which form mycorrhizae both with adult trees in the field and young seedlings in the greenhouse. However, it remains uncertain whether or not it belongs to the early successional mycorrhizal types of Mason et al. (1983). The fungus did not form sporophores in the greenhouse. The sporophores are rather huge and as observed in this study, occur massively in association with adult trees. Sporophores occurred exclusively in nature and around *A. guanzensis*.

Soil inoculum, another method we used, is the commonest method of inoculation (Mikola, 1973). Its strongest point is that it ensures the formation of mycorrhizae and thus meets the first premise that any mycorrhizae on tree roots are far better than not at all (Marx, 1980). It has, however,

disadvantages, one of which being that the fungi dealt with are unknown.

Brachystegia, *Julbernardia* and *Afzelia* species occur naturally in the same miombo ecosystems (Lind and Morrison, 1974). The likelihood that they may share the same fungal symbionts is therefore high. In Uluguru, where soil inoculum was collected, *A. quanzensis* stems were fewer than ten per hectare and very scattered. A given fungus can colonize different hosts and a given host can be associated with various fungi. This was found in Senegal in *Afzelia africana* and the euphorbiaceous tree *Uapaka kirkiana* (Thoen and Bâ, 1989). The two tree species had many fungal partners in common. They occur in the same ecosystem but belong to different families. An example of an ectomycorrhizal fungus reported in all continents, under various sites and in association with a broad range of hosts is *Pisolithus tinctorius* (Marx, 1977). *A. quanzensis* appears to have a broad taste regarding its mycorrhizal partners. It formed mycorrhizae with fungi associated with other ectomycorrhizal legumes, namely *J. globiflora*, *B. microphylla* and *B. spiciformis*. Which miombo tree species has the highest absolute number of mycorrhizal types can not be stated from this study since *A. quanzensis* stems would be misrepresented. The species has been overcut and only few trees have been surveyed in the present study. Also some of the sporophores (list in preparation) observed in association with *A. quanzensis* were seen in association with *B. spiciformis*, *B. microphylla* and *J. globiflora*. In an ongoing experiment at Wageningen, *A. quanzensis* seedlings also formed mycorrhizae when inoculated with mycorrhizal inoculum from Dutch *Fagus silvatica* L. stands. This generalist temperament of *A. quanzensis* coupled with the high commercial value of its timber makes this species even more attractive for afforestation and/or rehabilitation of degraded sites.

Molina et al. (1992) showed that host specificity in nature is not widespread. Danielson and Visser (1989) emphasized soil specificity to show that soil factors play a

role in mycorrhizal infection.

While *A. quanzensis* seedlings were mycorrhizal irrespective of the type of inoculum, it became clear that types differed depending upon the source of inoculum. In addition some types developed in the field but not in the greenhouse. There are several codeterminants, e.g. (1) the greenhouse is an artificial environment. Not all fungi will grow in it. (2) In the field where adult mycorrhizal trees are present, new infection depends mainly on vegetatively growing fungi (mycelium, rhizomorphs) from the root of mother trees. In the greenhouse or in the nursery infection relies almost exclusively on spore germination. Not all spores germinate under greenhouse or nursery conditions. Even under the best conditions, Fries (1978) demonstrated that spore germination rates of mycorrhizal fungi in an artificial environment are extremely low. Spore germination depends upon the fungal species involved and the nature of the germination substrates. (3) Some mycorrhizal fungi characterize a certain stage of tree development. (4) The production of sporophores depends upon many factors including site factors such as moisture and temperature (Peredo et al., 1983). For example in another study, *Thelephora* species produced sporophores in the greenhouse but not in the field (5.3). Maghembe (1982) found that the fructification of *Thelephora terrestris* was more frequent in cool humid areas than in dry areas of Tanzania.

The occurrence of *S. granulatus* sporophores around *A. quanzensis* seedlings did not mean that *A. quanzensis* formed mycorrhiza with *S. granulatus*. The roots of *A. quanzensis* were not colonised by this fungus, which earlier formed mycorrhizae with young pine seedlings in the same place (5.3). Failure of this fungus to develop mycorrhizae with *A. quanzensis* although the seedlings were grown in the mat of live hyphae connected to pines means that *S. granulatus* and *A. quanzensis* are incompatible, at least at that stage of the tree's seedling development. Failure of spores of *P. tinctorius* associated with *Eucalyptus* spp. to form mycorrhizae with seedlings may have resulted from (1) lack of favourable conditions for spore

germination or (2) time being probably not long enough for mycorrhizal development.

Examination of the root system showed that mycorrhizal types earlier seen on other tree species were present on *A. quanzensis* seedlings. For example, the dark brown mycorrhizae earlier seen on *J. globiflora* seedlings developed on *A. quanzensis* seedlings inoculated with *J. globiflora* soil. The yellow-brown mycorrhiza earlier observed on *J. globiflora* seedlings also developed on seedlings inoculated with *J. globiflora* soil. Similarly, the white mycorrhiza earlier seen on young *B. spiciformis* seedlings also developed on *A. quanzensis* seedlings inoculated with *B. spiciformis* soil (3.2.2).

Very unexpectedly, *A. quanzensis* seedlings raised in the rhizosphere of pines also formed well-developed ectomycorrhizae. The type formed was dark brown with stiff hairs and thick rhizomorphs. This brown hairy type was not seen at any one time on pine roots in this study. Was it there loosely associated with pines but not really active? Is it a saprophyte which can turn to be mycorrhizal once the right host is present? Is it a result of routine nursery soil mixtures? More study is needed in this area.

4.4.5 Conclusion and recommendations

Basidiospores of fungi collected in association with various trees of the miombo woodlands, *Eucalyptus* spp. and *P. caribaea* were used to inoculate *A. quanzensis* seedlings under greenhouse conditions. Only one species associated with *A. quanzensis* formed ectomycorrhizae with *A. quanzensis* seedlings. Greenhouse and field experiments with seedlings showed that *A. quanzensis* is not specific regarding its mycorrhizal association, at least in the area covered by the miombo woodlands. In the greenhouse, mycorrhizal soil inoculum from different tree species of the miombo woodlands led to the development of ectomycorrhizae of potted *A. quanzensis*

seedlings. The mycorrhizae formed were, however, of different types than those resulting from *A. quanzensis* mycorrhizal inoculum. Different mycorrhizal types were observed resulting from the same inoculum depending upon seedlings having been raised either in the greenhouse or directly under the mother tree. The non-specific nature of *A. quanzensis* seedlings regarding the choice of their fungal partners makes this species attractive for afforestation. More work is, however, to be done to reach fungal partner optimalization for a particular site.

Chapter 5

EXOTIC PINE PLANTATIONS

5.1 General introduction

Pines play an important role in Tanzanian wood industries. The first recorded introduction of pines in Tanzania was that of *P. radiata* D. Don in 1900 (Lundgren, 1978). Pines were introduced in Africa for the simple reason that indigenous trees were judged without experimentation, to grow too slowly. The attempt was unsuccessful. In many other countries disappointing results were recorded. Pine seedlings failed to establish themselves after the nursery stage. The first successful establishment of pines in Africa was recorded in Kenya and dates from 1927. This was achieved after the introduction of ectomycorrhizal fungi (Kaumi, 1980; Mikola, 1970) from Europe via South Africa. Since then, inoculum of various types is incorporated in the seedling growing medium, so that seedlings are equipped with mycorrhizae before transplantation. Introduction of mycorrhizal fungi by soil inoculum has the weakness that the fungi introduced are unknown (Trappe, 1977). It is indeed known that various fungi differ in performance and that soil factors play a role in their establishment (Smits et al., 1987). Therefore, observations on the root systems of young seedlings are indispensable to survey the types present. Most pine inoculation work carried out so far in East Africa made use of exotic fungi. However, there are reports that indigenous vegetation types are associated with fungi reported to infect pines in other countries. An example is *P. tinctorius*. This fungus was reported in many parts of the world including Africa (Marx, 1977). In Kenya, Ivory and Munga (1983) successfully used basidiospores of this fungus to inoculate pine seedlings in the nursery. In Senegal, Thoen and Bâ (1989) observed *Scleroderma verrucosum* Pers. and *S. dictyosporum* Pat.

occurring in association with *Afzelia africana*. The same fungi were reported to form mycorrhizae in pine plantations and in the nursery in Tanzania (Maghembe, 1982). Ivory and Munga (1992) also reported that the same *Scleroderma* species occurred in indigenous vegetation.

The objectives of this section are:

- (1) To survey the mycorrhizal status of pine seedlings in existing nurseries in various parts of Tanzania.
- (2) To study the mycorrhizal status of pine seedlings raised in contact with various inocula, including indigenous mycorrhizal fungi, in the nursery and in the field.

5.2 Survey of mycorrhizal status in various nurseries in Tanzania

5.2.1 Materials and methods

Study areas

The survey was conducted in Arusha, Sao Hill in Iringa, Mafiga, on the SUA campus and on the foot of Uluguru Mountains in Morogoro (fig. 2a). Arusha and Iringa have in common higher rainfall and a cooler climate. These areas are used for industrial plantations. Plantations are dominated by *Pinus patula*, *P. caribaea* and *P. radiata*. Iringa has one of the largest pine plantations in Africa. Morogoro, on the other hand as mentioned earlier, has a hostile climate because of higher temperatures and lower, irregular rainfalls. The miombo woodlands form the main vegetation type in Morogoro.

Inoculation techniques and sampling procedures

In Arusha and Iringa, forest soil from established pine plantations is, as a routine operation, incorporated in the potting as a 10% admixture (Forest Division, 1982).



Fig. 15. Mycorrhizal formation on *P. patula* seedlings in various nurseries (A) seedlings growing in earthballs (B) stunted non-inoculated seedlings from Mafiga (C) inoculum site

(D) seedlings of B after inoculation (E) mycorrhizae formed on *P. patula* seedlings inoculated with mycorrhizal soil from the miombo woodlands.

In Iringa, besides the use of pots, a trial was conducted to raise pine seedlings in earthballs instead of pots (fig. 15a). Compared to the soil used in the pots, the earthball soil mixture incorporates 10% clay soil instead of sand. In addition, the mixture is moistened and worked out so that the soil lends itself to the process of making balls. In other words, soil structure is destroyed. In Mafiga, potted seedlings, one per pot, had been raised in the nursery within 100 meters from well-established adult pine trees growing on the lower edge of the nursery. Seedlings had not been artificially inoculated.

The sampling procedure consisted in random selection of 20 to 40 seedlings. The root system of these seedlings was carefully washed, the infection rate was calculated and the mycorrhizal types were sorted out. In other words, a destructive harvest method was used. Macroscopic and microscopic features were used during mycorrhizal assessment.

Seedlings in Mafiga were stunted, turning yellow and brown (fig. 15b). To investigate the state of these Mafiga seedlings, I used them in an inoculation trial. In this trial, seedlings were arranged in groups of 7. Groups were paired and per pair one group was inoculated and the other was not. Nine pairs were treated in this way. Inoculation consisted in removing 1/4 of soil from the top of the pot and replacing it by pine mycorrhizal soil. Pine soil used was colonised by the mycelium of *S. granulatus*. This fungus produced several sporophores around pine trees (fig. 15c). Seedlings were then placed in the same nursery under the care of the same nursery people as before. Height and mycorrhizal assessment were made during and at the end of the experiment. Separate seedlings, inoculated or non-inoculated, sampled from the same nursery stock were used to assess, by a destructive method, the infection progress.

5.2.2 Results

Mycorrhizal status in various nurseries

All mycorrhizal types observed on pine seedlings in the present study are shortly described and illustrated in colour in APPENDIX 3A-3D; 4A-4D; 4F; 4G, pages 183 and 184.

In Arusha, three mycorrhizal types were found associated with the five month old pine seedlings. *Thelephora* sp. (APPENDIX 3A and 4G); a yellow-brown mycorrhiza (APPENDIX 4C), and *S. granulatus* (APPENDIX 4A, 5B) were observed. The infection rate was 63% (SD = 10; n = 21). Root colonization was mostly by *Thelephora* mycorrhizae. The survival rate of seedlings was extremely low. In Mafiga, none of the seedlings were mycorrhizal and none became so even 3 months after the time of sampling unless they had been artificially inoculated. The condition of the seedlings was deteriorating rapidly. In Sao Hill, pine seedlings were associated with five mycorrhizal types. The mean infection rate was 78% (SD = 13; n = 40). Species present were *S. granulatus*, probably *Scleroderma* sp. (APPENDIX 4D), *Cenococcum* sp. (4B), yellow-brown mycorrhizae (APPENDIX 4F) and *Thelephora* sp. (3A, 4G). There was a striking decrease of *S. granulatus* in the balls from outside to inside. *S. granulatus* was just present in the soil between balls or on the ball surface. The boundary between balls disappeared in the course of time. Regarding the root and sheath thickness, pine mycorrhizae were bigger than those of miombo trees but had a relatively thinner sheath. The mean diameter of mycorrhizal roots was 381 μm (SD = 39.0; n = 24). The mean sheath thickness was 30.5 μm (SD = 4.7; n = 24). The sheath represented 16.1% (SD = 1.9; n = 24) of the mycorrhizal root diameter.

Mycorrhizal formation on trial seedlings (Mafiga)

Two months after inoculation, inoculated seedlings started to develop a green shoot apex (fig. 15d) and to produce longer

needles. Two months later, the difference between inoculated and non-inoculated seedlings was evident. Inoculated seedlings were taller and displayed longer and greener needles. The average height of seedlings prior to inoculation was 6.4 cm. When the experiment was concluded, the mean height of inoculated seedlings was 17.2 cm (SD = 2.3; n = 63) and 9.4 cm (SD = 1.6) for non-inoculated ones. The live crown depth at start was 5.3 cm. At the end of the experiment, the mean dropped to 3.3 cm (SD = 0.9; n = 63) for non-inoculated and rose to 11.1 cm (SD = 1.9) for inoculated seedlings. These means at the end of the experiment were significantly different (5% level of significance).

Both inoculated and non-inoculated seedlings were non-mycorrhizal two months after inoculation. However, inoculated seedlings were developing a greener shoot compared to non-inoculated ones. Four months after inoculation, all inoculated seedlings were mycorrhizal. Only two mycorrhizal types were seen. These were *Thelephora* sp. and *S. granulatus*. The rhizomorphs and the hyphae of *S. granulatus* had filled all the pot and colonised even the bottom. They could be seen easily with unaided eye. The infection rate of fine roots by *S. granulatus* was 41%.

5.2.3 Discussion

In Arusha and Iringa, ectomycorrhizae observed on pine seedlings were a result of routine inoculation. The use of soil inoculum had earlier been successfully used in East African nurseries (Forest Division, 1982).

The mycorrhizal types observed were expected since for example *S. granulatus* sporophores were earlier seen in plantations. *S. granulatus* is reported in East Africa (Kalaghe and Redhead, 1989; Ivory and Munga, 1992). For *Thelephora* sp., Maghembe (1982) had reported the occurrence of the fungus in the same highlands of Tanzania. The difference in mycorrhizal types as compared to Sao Hill was probably due to the source

of inoculum used (Mikola, 1970). The Northern Tanzania inoculum is thought to have originated from Kenya, while that used in the South is thought to have come from Zimbabwe.

The use of earthballs in seedling propagation has at least one advantage: local materials are used and there is no waste disposal in the environment. There are, however, several inconveniences. From a practical point of view, an earthball is not easily lifted unless the ball has been enriched in clay and the structure of the soil destroyed and compacted. Making earthballs increases soil density and decreases soil structure. Both properties inhibit biological activity. Marschner (1990) discussed soil compaction in relation to root growth. From the mycorrhizal point of view, Marks and Foster (1973) reported that mycorrhizal formation is impeded in heavy, poorly aerated soils. Increasing soil density disrupts gas exchange and affects fungal growth. Populations of fungi can be altered (Amaranthus, 1992). The use of earthballs is also costly in terms of manufacturing them and transporting them at transplantation. There is also a risk of poor survival, if planting in the field is followed by a dry spell.

Most *Cenococcum mycorrhizae* were found on older parts of the roots or colonised other ageing mycorrhizae. Brand (1992) made similar observations. In Iringa they were observed when overgrowing *Thelephora* and *S. granulatus* mycorrhizae while never being overgrown themselves. This fungus was not seen on newly formed lateral roots.

The presence of mycorrhizal pine trees in the vicinity of a nursery does not necessarily mean that spontaneous infection of nursery seedlings will take place. In Morogoro, potted pine seedlings failed to form mycorrhizae despite the presence of pine trees within 100 meters. Artificial inoculation has to be done. The role of such trees should be to provide mycorrhizal soil in the neighbourhood to nurserymen instead of their having to get it from the forest. As reported by Trappe (1977), free propagation of inoculum produces erratic and delayed infection. This leads to heterogeneous stock. Spontaneous infection can only be homogeneous if the roots of

the mother tree are in direct contact with those of the nursery seedlings. This was observed in another study (5.3.1.2). For bare-root seedlings, the contact is easy whereas it is difficult for potted seedlings.

The cause of pine seedling mortality in nursery stock as observed in Mafiga nursery was the lack of mycorrhizae on the root system. This resulted in nutritional problems. Tropical soils are poor in nitrogen and phosphorus (FAO, 1974; Bowen, 1980; Högborg, 1992). Soil amendment by addition of inorganic fertilizers is costly, unsustainable and in the long run destructive (cf. 2.4). This emphasizes the necessity to ensure that seedlings are equipped with mycorrhizae from the very beginning.

Mycorrhizae have enormously contributed to pine growth and survival in many parts of the world (Harley and Smith, 1983). It has been once again observed here. The benefits of the tree symbionts are well known. They include increased nutrient and water uptake, i.e. in the sense of figure 7. This results in increased growth and survival of the host (Bowen, 1980). Failure to inoculate (Mikola, 1980) always yielded the same results as in our control seedlings. Our seedlings lacked mycorrhizae despite the presence of well-established mycorrhizal pine trees at less than 100 meters distance. The possibility of the roots of mother trees reaching the seedbeds was small due to distance. Had root contact been possible, spontaneous infection of seedlings would have taken place. The position of the seedbeds with respect to the location of the adult pine trees made it difficult for mycorrhizal propagules to be carried by water or nursery workers on their feet while carrying out their duties. Seedlings were placed on the upper slope with reference to the position of adult pine trees.

For well-established nurseries or those surrounded by pine plantations, natural infection of seedlings by mycorrhizal fungi can take place (Marx, 1980) but in many instances, it is very erratic (Trappe, 1977) and hence leading to heterogeneous stock. This is not desired by cost-sensitive nursery managers. Therefore, artificial inoculation is

recommended in all circumstances.

5.2.4 Conclusion and recommendations

Survey of the mycorrhizal status in existing *P. patula* nurseries showed that the inoculation techniques and cultural practices influenced mycorrhizal development in the three locations. The use of soil inoculum was practised in Arusha and Sao Hill nurseries. The mycorrhizal fungi common in nurseries for these areas were *Thelephora* sp. and *S. granulatus*. Sao Hill, in addition, hosted *Cenococcum* sp. and *Scleroderma* sp. The use of earthballs in Sao Hill was found to be inhibitory to mycorrhizal development. The most likely cause was poor aeration in the earthballs. In Mafiga, seedlings had not been artificially inoculated. They did not develop mycorrhizae despite the presence of well-established pine trees growing within 100 meters on the edge of the nursery. As a result, they were dying. Seedlings positively responded to artificial inoculation while the condition of non-inoculated ones steadily deteriorated. It is recommended to make artificial inoculation rule whatever the local circumstances.

5.3 *Pinus patula* seedlings: various inocula and mycorrhizal formation

Two inoculation experiments with *P. patula* seedlings were conducted in the greenhouse and in the field. In the first experiment, inoculum material came from the miombo woodlands. In the second experiment, it came from *P. caribaea* trees.

5.3.1 Materials and methods

5.3.1.1 Greenhouse and field experiments concerning indigenous fungi

(a) greenhouse experiment

In the greenhouse potted *P. patula* seedlings were inoculated with basidiospores of the following fungi (4.4.2.1):

1. *Pisolithus* sp.
2. *Lactarius chromospermus*
3. *Pisolithus tinctorius*
4. mycorrhizal soil from the root zone of *J. globiflora*/A. *quanzensis*
5. no inoculum.

Each of the above treatments was applied to 24 seedlings distributed over 4 blocks. At the end of 5 months, the pots were opened to assess mycorrhizal formation

(b) Field experiment

Non-mycorrhizal *P. patula* seedlings previously grown in the greenhouse were planted in the miombo woodlands in the root zone of *J. globiflora* or *B. microphylla*/A. *quanzensis* after addition or non-addition of unsterile pine soil in the planting hole. Seedlings were watered as needed until the end of the experiment.

The experiments covered 5 months, at the end of which the

root system was examined and such mycorrhizal types as found were described.

5.3.1.2 Greenhouse and field experiments with exotic fungi

During the short rainy season, soil inoculum was collected from adult exotic *P. caribaea* trees growing at the edge of the University nursery in Morogoro (fig. 15c). This inoculum, dominated by white mycelium and white mycorrhizae, was used unamended to raise *P. patula* seedlings in the greenhouse in individual pots. This inoculum was also sterilized in the oven and mixed with air dried sporophores of *S. granulatus* collected from the same place. The mixture was used to raise *P. patula* seedlings in the greenhouse in individual pots. In the root zone of the same trees where inoculum was collected, *P. patula* seedlings were directly raised by sowing.

Seedlings were watered as needed to meet their moisture requirements. After 1 month some seedlings were uprooted in every treatment and their root system was examined for mycorrhizal association.

Assessment by digging out some seedlings was again carried out after 2.5 months following seed germination. Three months later, another inspection was made.

Two months later, final assessment was made. At each assessment, mycorrhiza types were sorted out and described. The occurrence of mushroom production was recorded whenever they appeared. The experiment started on January 9, 1992 and ended on September 13, 1992.

5.3.2 Results

Greenhouse experiment incorporating indigenous fungi

In the greenhouse, seedlings inoculated with spore materials did not develop mycorrhizae nor did the controls. Seedlings

inoculated with miombo soil developed one mycorrhizal type (APPENDIX 3C and 3D, page 183). This mycorrhizal type was creamy white and had short swollen tips, dichotomously branching to form a big structure. The fungus involved formed very big strands connecting the bases of different mycorrhizal systems. The surface of mycorrhizae have short stiff hairs all over. These hairs collapse when in contact with water. All seedlings were infected and 86% (SD = 11; n = 19) of fine roots were colonised in the greenhouse (fig. 15e). In the field, the infection percentage of fine roots was 92% (SD = 10; n = 21). It was not possible to assess the contribution of the fungus to the growth of seedlings, these having been raised in different soils. It appeared however that indigenous fungi may enhance growth over the control seedlings.

In the field, seedlings raised in the miombo woodlands developed the same mycorrhizal type as observed in the greenhouse. Addition of exotic pine soil in the planting hole led to the formation of *Thelephora* mycorrhizae and to the preclusion of mycorrhizae by indigenous fungus. Where non pine soil was added, seedlings developed the mycorrhizal type described above. In that case, *Thelephora* mycorrhizae did not develop.

Greenhouse and field experiment with exotic fungi

The inoculum as it could be seen by naked eye, was heavily infected by white mycelium and white huge strands of the mycorrhizal fungus which later on was seen to be connected to the sporophore of *S. granulatus*. In addition, the inoculum consisted of white mycorrhizae of adult pines connected to each other by the same white strands.

In the field, sporophores of 3 species were observed in/around the experimental plots. The species involved were *S. granulatus* (more than 300 specimens), in January and during the long rain season of March to June; 6 specimens of *Russula* species at a spot where I removed the litter, and more than 50 puffballs along the path outside the canopy of pine trees,

probably *Scleroderma*, during the long rain.

In the greenhouse, the soil mixed with the pieces of *S. granulatus* did not develop any sporophore. The pots with unsterile soil from the root zone of pine trees did not form any of the sporophores observed in the field. Instead, numerous *Thelephora* sporocarps developed after 4.5 to 5.5 months. No further development of *Thelephora* sporocarps was observed afterwards.

Mycorrhizal development

Seedlings raised in soil mixed with pieces of *S. granulatus* remained non-mycorrhizal during the observation period. At the end of one month, none of the seedlings had developed enough laterals to be infected. Seedlings raised in the root zone of pine trees had, however, after 2.5 months, i.e. by 6/4/92, 95% ($n = 19$; $SD = 3$) of their fine roots exclusively colonised by the white mycorrhiza. Careful investigation showed that the mycorrhizae of the young seedlings were similar and connected to those of the mother tree. During the same period, seedlings in the greenhouse had started to develop brown mycorrhizae supposed to be *Thelephora* sp. The infection was, however, lower (30%; $n = 17$, $SD = 3$). By that time, there was no one sporophore yet produced in the greenhouse. By the end of 5.5 months after germination, the infection percentage in the pots was 88% ($n = 22$; $SD = 3$) and consisted still of *Thelephora* mycorrhizae. At the end of the experiment, that is on 13/9/1993, two new types, *S. granulatus*, and the white mycorrhizae with dense strands, probably *Scleroderma* had in addition to *Thelephora* mycorrhizae, invaded the root system of the seedlings in the pots.

Seedlings under pine trees were chopped by animals at the end of 3 months so that it was not possible to follow their infection over a longer period for possible shift in species composition.

5.3.3 Discussion

Mycorrhizae which develop on seedlings usually result from the germination of spores or the contact with live vegetative mycelium. In the greenhouse or in the nursery, mycorrhizal development relies more on the germination of spores than on the vegetative mycelium. In this study, spore inoculum of all test fungi failed to develop into mycorrhizae.

For *S. granulatus*, failure for spores to develop into mycorrhizae may have resulted from the lack of proper conditions for their germination. Fungi differ in their growth requirements. Some require more carbon for growth. Some are more adapted than others in moist or dry environments (Trappe, 1977). In India, Mohan (ex Last et al., 1992) reported success of *Thelephora* mycorrhizal formation on pine seedlings inoculated with basidiospores while *S. granulatus* failed in the same experiment. For spores collected in vegetation types other than pine plantations, the failure of fungi to form mycorrhizae in our study may have resulted from other causes such as the incompatibility between the fungi tested and pine trees. However, there may be a possibility that the fungi used could infect pine seedlings had they been given longer time. Such a delayed infection would be useless since non-mycorrhizal seedlings suffer early in their growth (5.2).

The mycorrhizal type formed on greenhouse pine seedlings inoculated with miombo soil resembled the one formed on pine seedlings directly raised in the miombo woodlands. This type was not formed on pine seedlings inoculated with pine soil. The fungus involved is most likely indigenous in the miombo woodlands. The miombo dominating trees form ectomycorrhizae with their natural fungal partners. An account of the association in nature has been made by Högberg and Nylund (1981) and Högberg and Pearce (1986). The study conducted by Thoen and Bâ (1989) and Bâ and Thoen (1990) showed that *Scleroderma dictyosporum* and *S. verrucosum* formed ectomycorrhizae with *Afzelia africana*. In Kenya, Ivory and Munga (1992) indicated that *Scleroderma verrucosum* and *P.*

tinctorius occurred in indigenous forests. These fungi reported in indigenous African forests form ectomycorrhizae with exotic pines in various countries including Tanzania, Kenya and New Zealand (Marx, 1977; Chu-Chou, 1979; Maghembe, 1982; Ivory and Munga, 1983). It is therefore possible that the mycorrhizal type formed on pine seedlings inoculated with miombo soil in our study was an indigenous one. More research is needed to assess the performance of the indigenous fungus discussed above regarding its ecology and contribution to pine growth and survival.

Thelephora mycorrhizae were observed in this study on roots of potted greenhouse seedling inoculated with pine soil. This mycorrhizal fungus is commonly reported in early stage of seedling development. It was reported in Tanzania by other researchers (Maghembe, 1982; Kalaghe and Redhead, 1989). As observed in this study, addition of mycorrhizal soil in the planting hole led to seedling infection by mycorrhizal fungi present in the inoculum added. Addition of mycorrhizal soil in the planting hole to induce mycorrhizal formation on seedlings has also been done by Amaranthus and Perry (1987).

Thelephora mycorrhizae precluded the formation of the indigenous mycorrhiza (discussed above) on pine seedlings in the field where pine mycorrhizal soil was added in the planting hole. *Thelephora* mycorrhizae were however precluded by *S. granulatus* in the field as observed on pine seedlings directly raised in the root zone of adult pine trees where *S. granulatus* predominated. Fungi differ in competitive ability. The competitive ability depends upon the compatibility with the host, the mycelial size of the fungus, the site condition and the age of the host. For example, when seedlings are grown in the root zone of adult compatible mycorrhizal trees, fungi of adult trees which have the biggest mycelial size have advantage. When seedlings are grown in the greenhouse or in the nursery, fungi adapted to greenhouse or nursery conditions and characteristic of young seedlings will dominate.

Various fungi produced fruitbodies at various times and depending upon where the seedlings were raised. *Thelephora* sp.

produced sporocarps in the greenhouse, while *S. granulatus* mushrooms developed around seedlings raised in the root zone of the mother trees. *Thelephora* sporocarps were associated with young pine seedlings. These had developed *Thelephora* mycorrhizae. In the field *S. granulatus* mushrooms were primarily associated with the mother trees. Being raised in the root zone of the mother trees, seedlings were also in contact with this fungus.

Fructification of mycorrhizal fungi is a reflection of mycorrhizal prosperity on the root system (Jansen, 1991). However, the absence of fructification does not mean necessarily mycorrhizal failure on the root system. In our pots, production of sporocarps may have coincided with the colonization peak of *Thelephora* mycorrhizae. Later on, when sporocarp production stopped there is ground to suggest that other fungal types were colonizing the root system: at the end of the experiment, new types were observed. A sequential root colonization by mycorrhizal fungi as the host ages was also observed elsewhere. Chu-Chou (1979) observed that early colonisers stopped producing sporophores while new species came in. In this respect, Marx and Kenny (1991) warned that there is a risk that some fungi may disappear after transplanting. What would result is low survival rate of the host unless appropriate fungi are available to take over.

The absence of *Thelephora* mycorrhizae on the root system of seedlings planted in the root zone of adult pine trees, and the predominance of *S. granulatus* on the root of the same seedlings may be explained as follows. (1) The early stage *Thelephora* sp. was outcompeted under field conditions by the relatively 'late stage' *S. granulatus*. (2) Competitive ability of mycorrhizal fungi depends on the mycelial size. In the field this gives a general advantage for species associated with mature trees as such species can always outcompete other species on seedlings. Mycorrhizae on the rootlets of the seedlings were initiated by already existing mycorrhizae/live mycelium on the adult pine trees. This gives advantage to *S. granulatus* whose extensive rhizomorphs could be seen

everywhere in the growing medium where seedlings were raised. In other words, previously existing and continually formed rhizomorphs or hyphae on the roots of the mother trees connected easily the adult and the young regeneration. This is in agreement with Last et al. (1987) who reported that most of roots of regenerating tree seedlings seem to be colonized by late stage fungi (those on adult trees). This observation was also made on birch by Fleming (1983). Harley and Smith (1983) have also suggested that the mycelium on the roots of standing trees play a role in the infection of regenerating seedlings.

Other researchers (Brownlee et al., 1983; Finlay et al., 1986; Söderström et al., 1986) working on various tree and fungal species including *S. granulatus*, observed that interplant hyphal connection occurs in nature. Thus in natural communities as opposed to man-made sites, primary mycorrhizal infection may take place from root to root rather than by germination of spores. In that case the infecting hyphae/rhizomorphs are attached to a food base in a living root and are therefore in a better position to spread rapidly on non-infected young seedlings than would do germinating spores which are dependent on competition for carbon in the rhizosphere. In this case the fungus gets most of its carbon from the established tree. This may be an explanation of earlier colonization of seedlings raised in the root zone compared to late colonization observed in the pots in this study.

5.3.4 Conclusion and recommendations

Exotic pine seedlings developed typical ectomycorrhizae in the greenhouse when raised in miombo soil or in the field when raised in the root zone of *J. globiflora*, *B. microphylla* and *A. quanzensis* trees. One mycorrhizal fungus most likely indigenous in the miombo woodlands, was involved. This observation represents an area for further research. Pine seedlings developed different types of ectomycorrhizae

depending upon whether they are raised in contact with the mother trees or whether they are raised in the greenhouse in the mycorrhizal soil from those same mother trees. *Thelephora* sporocarps and mycorrhizae developed in the greenhouse while in the field seedlings in contact with the mother trees formed *S. granulatus* mycorrhizae. At the same time *S. granulatus* mushrooms occurred in great numbers. This difference in mycorrhizae leads to a number of considerations in nursery management.

- (1) Collection of soil inoculum for nursery operations based on the occurrence of mushrooms is not always a good criterion.
- (2) Collection of soil inoculum based on mycorrhizae dominating the root system of adult trees may also be misleading.
- (3) Raising mycorrhizal trees in the seedbeds in order for these to spontaneously provide mycorrhizae to young seedlings raised underneath or adjacent for later field planting may lead to the development of seedlings with mycorrhizae which are not fit for that particular stage or the next stages in seedling development.
- (4) On the other hand, inefficient early stage fungi can be skipped and late stage fungi developed before the seedling is transplanted.
- (5) Quick and high infection can be achieved. There is however a risk that these late stage fungi may disappear after transplanting.

Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

Most forestry research aiming at plantation establishment in Africa dealt with exotic species. Similarly, most of the tropical forest activities geared towards conservation focused on rainforest. However, for a considerable part of Africa, tropical forest means "woodlands" or "scattered trees". The miombo constitutes the most complex vegetation of this kind (Temu, 1979; Celander, 1983). The climate in miombo zones is unfavourable for exotic trees, especially pines. This requires study on the ecology and silviculture of indigenous vegetation and their associated microorganisms. As admitted by various authors (Mgeni and Malimbwi, 1990; Abeli, 1992; Refsdal, 1992) knowledge on silviculture and management of this type of vegetation is imperfect, although it is urgently needed. The present study concerns the natural and/or artificial germination and the nursery or greenhouse and/or miombo environment studies on 5 main tree species of the miombo woodlands, all of them legumes. These species are *A. quanzensis*, *B. microphylla*, *B. spiciformis*, *J. globiflora* and *P. angolensis*. *P. patula* also was investigated. In the nursery or greenhouse studies, the symbiotic status of the tree species was a central issue.

6.1 Natural regeneration

The regeneration of miombo trees and the site in Morogoro are very much affected by yearly fires and long drought. Factors acting on seeds and symbionts in the miombo floor, and seedlings and trees are shown in figure 16. As the figure indicates, the floor is one of the main sites of interaction. The impact is enormous when fire hits late in the dry season. Seeds are killed, seedlings die or die back and the site is

left naked and hence susceptible to erosion.

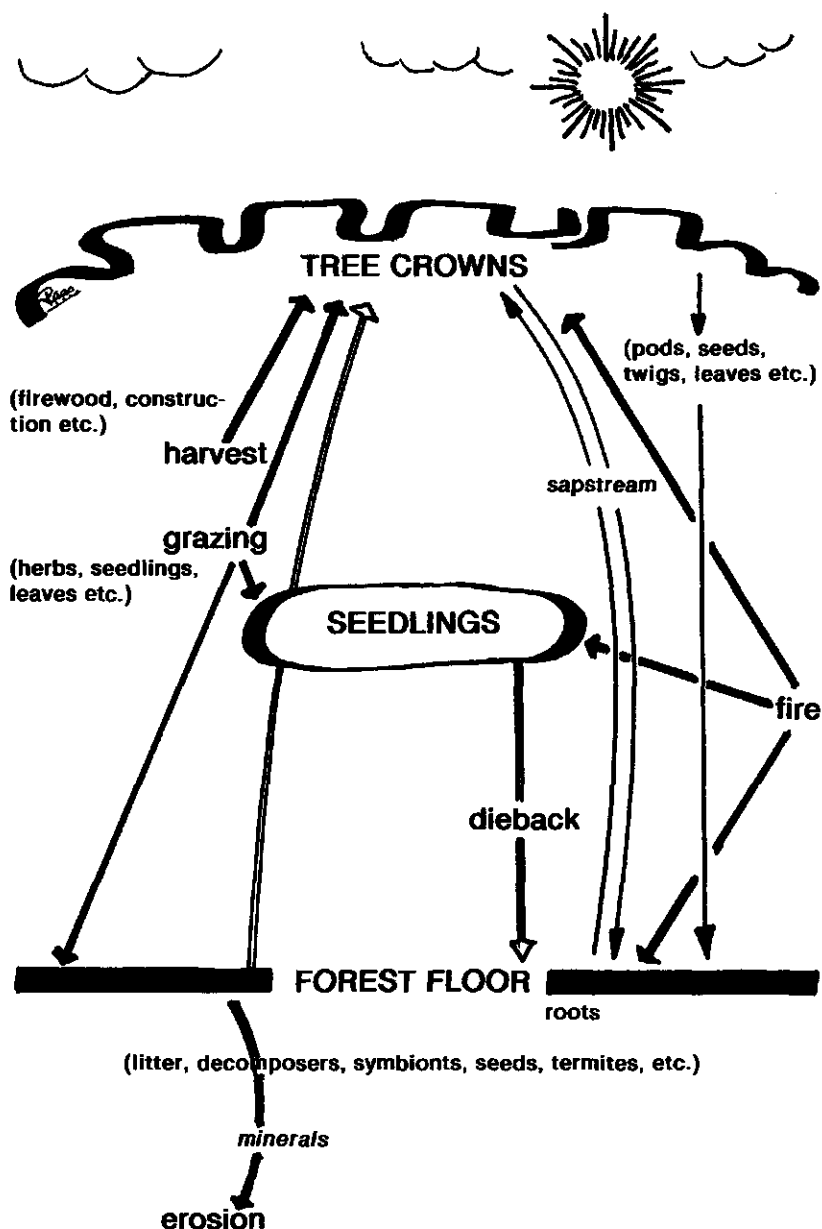


Fig. 16. Diagram showing main factors acting upon the floor, seedlings and trees in the miombo woodlands.

The seeds of all species studied are sensitive to fire (table 9). Experimental fire treatment in this study reduced seeds or fruits to ash. Apart from *P. angolensis*, the seeds of all species if not incinerated, germinate within 3 weeks of rain. *J. globiflora*, *B. microphylla* and *B. spiciformis* germinate within a period of less than two weeks. *A. quanzensis* requires relatively longer period due to its hard seedcoat and aril. Rasping off the seedcoat, soaking the seeds and removal of the aril reduce the germination period to around 10 days. *P. angolensis* has a hard non-dehiscent pericarp and a hard seedcoat which hinder germination. Its natural regeneration was found to be very scarce in South Africa (Van Daalen, 1991). In Tanzania, Boaler (1966) found that only 2% of the seeds of this species germinated under natural conditions. In our study, the cause of scarce regeneration was established to be due to seed dormancy, wildfires and severe drought occurring when the seedlings were not yet established. In the greenhouse, germination was raised to almost 100% after hot wire treatment (fig. 5b). In nature, biological activity in the litter, especially by termites, is essential for the preparation of the seedbed for germination. For *P. angolensis*, termites liberate the seeds by chopping the pericarp. Destruction of the litter by fires increases moisture deficits on the surface and indirectly affects germination. In India, mulching was recommended for the establishment of seedlings on dry sites (Singh et al., 1988).

Burning, besides killing the seeds, consumes organic matter and affects organisms involved in breaking seed dormancy. Organic matter is essential in the miombo woodlands for moisture retention.

6.2 Nursery studies

Three phenomena are feared, often without experimental proof, in raising the species of the miombo woodlands.

- (1) The species are feared to grow slowly;

- (2) the species are feared to die back every year;
- (3) the species are known to produce a vigorous taproot which makes common nursery routine activities, usually applied to pines, difficult.

In reality the growth rates of indigenous trees treated here are far superior to those of pines, at least in the initial stage of growth. For example, in Kenya, Ivory and Munga (1983) found that 4 and 10 months old pine seedlings had an average height of around 15 and 20 cm respectively. In the present study, non-pruned *P. angolensis* seedling populations reached a mean height of almost 30 cm within 5 months. High values were achieved by other species studied. In the nursery, die-back did not occur in any of the species studied. Die-back proves to be the result of long dry period and yearly fires. Seedlings in the experiments discussed here were supplied with water and protected against fires. The taproot of the miombo trees was indeed found to be a hindrance for routine nursery activities. Cutting this root resulted in a sharp growth depression (2.4; 3.3). Leaving it intact was no solution either for nursery-produced seedlings. If the seedlings are grown in closed pots, their taproots coil permanently (3.2.3; 4.2). If the seedlings are raised in open pots and are not root-pruned, lifting the pots for planting becomes difficult since the taproot penetrates through the pot in the soil (2.4; 3.3). The taproot embodies part of a strategy for seedlings to survive dry periods in nature. One or two root pruning interventions carried out within the first two months after sowing and followed by planting, would be a good compromise.

In the nursery other species, like pines in South Africa, are often fertilized (Donald, 1987; Donald et al., 1987). We fertilized *P. angolensis*. Seedling growth was enhanced by nitrogen and phosphorus application. There was no apparent response to zinc and iron. It is well known that miombo soils are deficient in nitrogen and phosphorus (Lind and Morrison, 1974; Högborg, 1992). This was also confirmed by soil analysis in the present study. The seedling growth response observed was mostly due to phosphorus and not to nitrogen. The reason

is, that even non-fertilized seedlings had an adequate nitrogen level in their tissues. This resulted from biological nitrogen fixation by *P. angolensis*. For this tree species, it is not nitrogen but phosphorus which is limiting. Other legumes discussed in the present study do not fix nitrogen. For these species and other ectomycorrhizal legumes, both nitrogen and phosphorus are limiting. This underscores the need for mycorrhizal inoculation in silviculture in the miombo zones.

The symbiotic status of the above indigenous species and that of pines was assessed. *A. quanzensis*, *B. microphylla*, *B. boehmii*, *B. spiciformis* and *J. globiflora* formed ectomycorrhizae in the nursery. Infection in nature started before the germination of seeds was completed. Early infection was also observed by Thoen and Bâ (1989) in *A. africana*.

The main feature of the ectomycorrhizae observed is that they are very hairy. Similar observations were made by Redhead (1968) on mycorrhizae in the genera *Afzelia* and *Brachystegia*. Rhizomorphs constitute a characteristic feature of miombo mycorrhizae. Duddridge et al. (1980) demonstrated that rhizomorphs are effective in water transport under dry conditions. This function is vital in the miombo woodlands. As demonstrated for *A. quanzensis*, the fungal species have a broad host range. For example *A. quanzensis* formed ectomycorrhizae when inoculated with inoculum from *B. microphylla*, *B. spiciformis* and *J. globiflora*. None of the species nodulated. The symbiotic status of the species as observed in this study is in agreement with the findings of Högberg and Nylund (1981). However, these authors reported that *A. quanzensis* formed nodules in nature. In this study *A. quanzensis* did not nodulate.

P. angolensis formed arbuscular mycorrhizae and nodulated. The formation of nodules by this species, together with its excellent timber make the species apt to be used for agroforestry and timber plantation establishment. Seedlings of *P. angolensis* positively responded to fertilization in terms of growth but stopped nodulation. It is important to encourage

natural nitrogen fixation by proper cultural management. Fertilization is expensive, unsustainable and destructive in the long run (2.4).

Nursery practices affect seedling development and interaction with symbiotic microorganisms. These practices include soil mixing, soil compaction and watering. For example, nursery stock of pine seedlings was dying as a result of lack of mycorrhizae despite the presence of well-established adult pine trees within less than 100 meters (5.2). The condition of seedlings was very much improved by artificially inoculating. Inoculation should be made a rule.

P. angolensis seedlings were also dying in the nursery. When seedlings were raised in the field where they relied on prevailing conditions, none developed the symptoms observed in the nursery. The cause was later identified as iron deficiency in the plant tissue. This in its turn was due to soil compaction and overwatering of seedlings. The subject seedlings were cured by application of an iron solution. Iron deficiency is not a common problem in tropical silviculture. Iron deficiency, however, as established by this study can occur in manipulated environments such as pots.

The use of earthballs in nurseries is another aspect which can hamper seedlings and microbes interacting. Making of earthballs requires increased clay content in the soil to be used, added water, a broken soil structure and a compacted mixture. Otherwise the ball is likely to collapse. This change of soil properties has a negative impact on mycorrhizal fungal populations. Mycorrhizal formation is affected by soil structure (Marks and Foster, 1973). The earthball practice has other disadvantages, namely, the cost involved in making the balls and their transport to the field for transplanting.

The miombo woodlands have various mycorrhizal fungi. This is reflected in the amount of sporophores produced. Inoculation of young *A. quanzensis* with spores of various fungi was done. Only basidiospores of a *Pisolithus* species associated with *A. quanzensis* in nature led to the formation of mycorrhizae in the greenhouse. It is well known that the

germination of spores of mycorrhizal fungi is very low. Thus, some of the fungi used as spore sources in this study may well form mycorrhizae in nature although they have failed to do so in artificial environments.

The contribution of inoculation to seedling growth was assessed for *B. microphylla*. In the nursery, inoculated seedlings grew bigger than non-inoculated ones. Mushrooms of *Inocybe dulcamara* were produced around the seedlings in the nursery. The same mushrooms were earlier seen in the field around mother trees of the same species.

Pine seedlings were inoculated with mycorrhizal inoculum from the miombo woodlands, or directly raised in the miombo woodlands. In both cases, the same type of ectomycorrhiza was formed. This makes it plausible that there are indigenous fungi able to colonise exotic pines. Reports by Thoen and Bâ (1989), Maghembe (1982) and Ivory and Munga (1992) indicate that *Scleroderma verrucosum*, *S. dictyosporum* or *P. tinctorius* occur in association with both indigenous savanna or miombo trees such as *A. africana* and exotic pines in plantations. This confirms the hypothesis of pine seedlings forming mycorrhizae with indigenous fungi.

The ecological strategies of miombo trees can be summarized in the following points. (1) The strategies focus on the underground compartment. (2) They are related to the architecture and dynamics of the root system with its deep and thick taproot. While the shoot in seedlings yearly dies back due to severe dry seasons, the taproot yearly expands. (3) The architecture with thin lateral roots lends itself to mycorrhizal formation and/or nodulation. (4) Mycorrhizal formation increases the soil volume exploited by the tree, participating in the building up of the taproot and hence in increasing chances of seedling survival in dry and unpredictable environments. (5) The ability of seedlings and trees to develop new shoots using reserves drawn from the taproot towards the end of the dry season, allows them to efficiently use limited showers (6) Recognition of these facts is a basis for a successful afforestation with miombo trees.

This research showed indeed that good nursery practices are beneficial for seedling growth and survival in the nursery and later in the field. The following points deserve attention in nursery management. These are (1) seed treatment (for *P. angolensis*), (2) timing of activities, (3) soil physical conditions, (4) inoculation and (5) restricted pruning of the taproot.

6.3 Final recommendations

The following subjects are recommended for further study.

- (1) Permanent plots should be established in various warm regions of Tanzania in order to assess the performance of various miombo trees over longer periods. These trials should include inoculation with various symbionts. Comparison should be made between the performance of VA mycorrhizal and ectomycorrhizal legumes.
- (2) There is an urgent need to produce a comprehensive guide to the mushrooms occurring in the miombo woodlands.
- (3) Research in agroforestry in Africa should focus on indigenous legume trees. These constitute the bulk of indigenous trees and the main genetical plant inheritance of this vast continent.
- (4) Given the cost and labour required for mechanical extraction of seeds of *P. angolensis* and the high risk for seeds to incur mechanical injury as observed in this study, it is suggested to experiment incubating the pods of the species in the soil, watering them over different periods, before seed extraction and pre-sowing treatment. This can facilitate extraction and probably boost the germination rate and speed.
- (5) There is a possibility that shoot die-back in miombo seedlings coincides or comes just before or after lateral root die-back. If fine roots die back during the dry season, mycorrhizae also die. It could be interesting to investigate whether the same mycorrhizae as before would

develop at the end of the dry season when new fine roots reappear when the rain resumes.

Table 9. Comparative table for the studied indigenous miombo tree species.

Species	Parameter																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>A. quanzensis</i>	a	a	a	d	a	a	b	b	a	a	a	a	a	a	a	b	a	-
<i>B. boehmii</i>	a	a	a	c	a	a	a	a	a	a	a	a	a	a	-	a	a	b
<i>B. microphylla</i>	a	a	a	c	a	a	a	a	a	a	a	a	a	a	a	a	a	-
<i>B. spiciformis</i>	a	a	a	c	a	a	a	a	a	a	a	a	a	a	a	a	a	c
<i>J. globiflora</i>	a	a	a	b	a	a	a	a	a	a	a	a	a	a	a	a	a	a
<i>P. angolensis</i>	b	a	b	a	a	a	c	c	b	a	b	a	a	b	b	a	b	d

1. Family: a = Leguminosae-Caesalpinioideae; b = Leguminosae-Papilionoideae
2. Fruits maturation: a = long dry season
3. Seeds release: a = by explosion, fruit still hanging
 b = mainly by fire or by decomposers
4. Seed size: a = rather small; b = small (Chidumayo (1991) estimated the seed of *J. globiflora* to be 0.26-0.27 g air dry weight); c = medium (0.530 g for *B. spiciformis* (Malaise ex Ernst, 1988)); d = big (>3 g; the present work)
5. Seed sensitivity to wild fires: a = high
6. Sensitivity to low temperatures/frosts: a = yes
7. Germination in nature: a = less than two weeks; mostly during the short rains following seed release
 b = 2 to 3 weeks; mostly during the short rains following seed release; c = several months; long rain and subsequent rain seasons
8. Germination in the nursery: a = less than 2 weeks; b = 2 - 3 weeks; mechanical scarification makes it < 2 weeks
 c = several weeks; hot wire treatment makes it < 2 weeks

9. Fire tolerance as sapling (Celandier, 1983). a = the species is sensitive; b = the species is tolerant
10. Survival in the nursery after germination: a = 100%
11. Iron deficiency symptoms: a = not observed; b = only in the nursery
12. Die-back in the nursery: a = none
13. Taproot: a = robust; pruning it depresses growth
14. Symbiotic status: a = ectomycorrhizal; b = VA mycorrhizal and nitrogen fixer
15. Have at least one common ectomycorrhizal type: a = at least one type in common; b = not at all; - = not checked
16. Height growth in the first month in the nursery: a = lower than 10 cm; b = around 15 cm
17. Height increment 2-4 months after germination. a = very low; b = very high
18. mature tree height (Celandier, 1983). Height increasing from 15 m for *J. globiflora* (a) to above 25 m for *P. angolensis* (d) *A. quanzensis* mean height, information not available but likely to be equal or bigger than the biggest. *B. microphylla* in the same group as *B. spiciformis*.

Chapter 7

PRACTICAL HINTS FOR NURSERYMEN

7.1 Germination of the miombo dominating tree species

Not all seeds can germinate. Well-polished seeds with filled testa are usually sound and germinate once other conditions are met. Seeds should be examined before sowing. Empty or aborted seeds and seeds with fungal mould should be disqualified. For various tree species the following is advised.

7.1.1 *Afzelia quanzensis*

The species can germinate satisfactorily without any treatment provided water requirements are met. Germination will take up to three weeks. Quicker germination can be achieved by (1) making two carvings on the seedcoat near the aril using a sharp blade and (2) by soaking some 12 hours in cold water. The aril should be removed after soaking and before sowing. Germination can then be expected in a period shorter than 2 weeks.

7.1.2 *Brachystegia boehmii* , *B. microphylla* and *B. spiciformis*

Seeds germinate readily. What is needed is only to water the germination substrate. Germination is expected in less than 10 days.

7.1.3 *Julbernardia globiflora*

Seeds germinate without any treatment. Only watering the

germination substrate is needed. Germination period is as short as in *Brachystegia* species.

7.1.4 *Pterocarpus angolensis*

Seeds are tied up in tough, winged fruits. Seeds should be extracted mechanically by hammering until a better method is available. This is very laborious; 30 seeds per hour! A suggestion can also be to try to extract seeds after incubating fruits under an organic layer. The mixture organic layer and fruits should be maintained moist. Regularly one can check if the fruits are ready for extraction. There is a possibility that seeds extracted in this way can not be stored any longer!

Injury to the seeds leads to rotting. Fire is deleterious! Extracted seeds should be treated. With a hot wire treatment (fig. 5a), germination is achieved within 10 days. Without this, germination will take more than a month. In addition it will be very scarce and very irregular. Soaking in water is no solution. Sow seeds just on the surface and cover them with a very thin layer of soil. Deeply sown seeds rot.

7.2 Survival and early growth

All or almost all seedlings survive, provided proper cultural measures are ensured. The measures in question are caring for optimal soil physical properties, e.g. by sparing the soil structures and watering. Early growth exceeds that of pines by far.

7.3 Potting and seedling growth behaviour

Seedlings develop a tough taproot which grows far longer than

the shoot. Direct sowing in the field is a good option in areas where rainfall is predictable and regular. In areas with unreliable rainfall preparing the regeneration by raising seedlings in the nursery is recommended. In that case, only one or two root prunings (both in the first 2 months) followed by transplantation are advised. Soil grinding and sieving should be avoided at least for *P. angolensis*. When combined with overwatering, this practice caused iron deficiency symptoms in the plants.

7.4 Inoculation

A. quanzensis, *B. micropylla*, *B. spiciformis*, and *J. globiflora* formed ectomycorrhizae at least with their natural fungal partners. Inoculation promoted growth for *B. micropylla*, as proven in this study. It is however not known which fungi are more effective. Until further information is available, nurserymen are advised to mix potting soil with mycorrhizal soil collected from the mother trees. This mycorrhizal soil can be 10% of the potting mixture. *P. angolensis* forms VA mycorrhizae and nodulates in the nursery. Similarly, fresh soil from mother trees should be added to the growing medium.

7.5 Ecological range

Species should be grown in warm areas. Cold areas where the temperatures drop below 18 °C regularly are not fit.

7.6 Duration in the nursery, in case direct sowing is not appropriate

It is best to take seedlings to the field after 2 to 2.5 months. At this age, seedlings are still able to regenerate

their taproot. Seedling survival in areas with long dry periods depends upon the regeneration of this taproot. Transplanting to the field should be done when there is at least 2 months of rain ahead.

7.7 Pines

7.7.1 Soil mixture

Incorporation of mycorrhizal soil in the growing medium is a must. Assumption of natural infection is risky and should be avoided.

7.7.2 Be careful with the use of earthballs

The use of earthballs should be reconsidered since they may do harm to seedling development in the nursery and survival in the field. Instead, as practised at Sao Hill Project, whenever possible, seedlings should be removed carefully from the polythene tube at planting so that the same tube is re-used. This practice is environmentally friendly and economical. However, precautions should be taken in areas of marginal rainfall since the practice can result in poor seedling survival. Removing seedlings in this way disturbs the seedling root system compared to cutting the tube when removing.

POSTSCRIPT

AFRICA, THE EVER YOUNG CONTINENT

Your youth, wealth and hospitality make your identity. Africa, you are often called an old continent. You are not. You are often characterized as one of the poorest blocks on the earth. You are not.

You are not old because you embody rejuvenating agents whose capacity is immense. Though some of these agents can only be seen under high magnification, their effect is often assessed with naked eyes.

You are not an old continent because your children exceed by far in number the elderly. Your children, however, find in this elderly minority, a source of joy, inspiration and wisdom.

Those who call you one of the poorest continents use inappropriate parameters. You have what money can not purchase.

Your nature exceeds human comprehension. The beauty of your nature will remain undescrivable. Your rivers, your lakes and their lives, your mountains and valleys, your savannas, habitat of wild animals and host of beautiful mushrooms, your forests and their dwellers, your deserts, your sky and your climate, your cultural and ethnic diversity, these constitute your identity.

Your hospitality will always amaze visitors. Within your limited material means, you receive unquestioningly ever expanding number of refugees because your non-material wealth is ever elastic.

Your humanity is your very natural immunity against modern diseases. Your immense fertility is your very security against traditional diseases. To share and to be content with what you have is your very preventive medicine.

The high level of emancipation of your daughters has preserved the stability of the family and made of it a place

not to flee but a place to stay, a place to spend holidays. Those who prepare and dispense emancipation courses should better get curriculum, didactic materials, and part of practical training from you. Then, they will address the right clients, the right problem and using the right materials.

Your prospects primarily lie in the hands of your sons and daughters once they realize, appreciate and cherish the enormous richness and possibilities they have inherited.

GENERAL SUMMARY

This project has covered one or several aspects of the life cycle of the main miombo tree species, namely *Afzelia quanzensis*, *Brachystegia microphylla*, *Brachystegia spiciformis*, *Julbernardia globiflora* and *Pterocarpus angolensis*. These aspects included natural and artificial regeneration, fertilization, artificial inoculation of seedlings and natural occurrence of mycorrhizae on field-grown seedlings. Mycorrhizal survey of pine seedlings in various nurseries and inoculation trials on pines with exotic and indigenous fungi had also been conducted. The ultimate goal was to contribute to the development of techniques needed for the silviculture of miombo tree species.

Seeds of *B. spiciformis*, *J. globiflora* and *A. quanzensis* germinated within 3 weeks in nature provided there was regular rain. Seeds of *P. angolensis* possessed dormancy. Providing water was not enough to induce germination. In the greenhouse a hot wire scarifier boosted their germination to more than 90% within less than two weeks. Outside rain had a clear positive influence on the germination of scarified surface-sown seeds in the greenhouse. Germination was always initiated by outside rain. All species tested were sensitive to fires. For *A. quanzensis* germination tests were carried out at 10, 15 and 25°C. Seedlings germinated only at 25°C. Germination failures were also encountered in the field in the cool highlands of Tanzania.

Seedlings of the above named indigenous species were raised in the nursery or observed in the miombo woodlands. A number of facts emerged.

A big share of carbon is allocated to the development of the taproot. In the nursery, pruning the taproot interfered with the natural survival strategy of seedlings and caused a sharp depression in seedling growth. This was observed on *J. globiflora* and *P. angolensis*. The initial growth rate of the miombo tree seedlings covered here is far higher than that

achieved by pines. As observed in Morogoro on seedlings of *A. quanzensis*, more than 70 percent of the height achieved in one year is attained in the first 2 months. Miombo species with big seeds such as *A. quanzensis*, had an initial higher growth rate than small-seeded species.

The effect of drought on *A. quanzensis* seedling growth and survival during early stage of growth was studied. Inoculated or non-inoculated potted nursery seedlings were irrigated once a day, once every three days or once every four days. Induced drought had a negative effect on biomass accumulation. Non-drought treated seedlings were more than 4 times as heavy than treated ones. Inoculation did not counteract the effect of drought. Inoculated, drought-treated seedlings developed only one mycorrhizal type. The mycorrhizae in this treatment were shrunk and had a very thin sheath. Infection percentage was lower than 10%. Non-drought treated inoculated seedlings developed more than one type including that in treated seedlings. Infection rate was above 50%.

A. quanzensis seedlings were observed under natural dry circumstances in the miombo woodlands for survival and mycorrhizal development. All seedlings survived and all formed one single mycorrhizal type similar to that in seedlings watered once every 4 days. All feeder roots were infected. Survival was 100%.

Seedlings in the miombo woodlands yearly die back during the long dry season. There was no die-back in nursery raised irrigated seedlings.

In the nursery *A. quanzensis*, *B. microphylla*, and *J. globiflora* seedlings were inoculated with mycorrhizal soil and basidiospores of fungi associated with adult trees. As a result of contact with soil inoculum, seedlings of these species formed ectomycorrhizae. Only basidiospores of one fungus were able to form mycorrhizae with *A. quanzensis* in the nursery. In the field observations were made on seedlings of *B. spiciformis* and *A. quanzensis* naturally growing or artificially grown in the rhizosphere of their mother trees or other ectomycorrhizal miombo trees. Seedlings got colonised by

fungi associated with adult trees. The infection started before germination was completed.

Specificity of *Afzelia* was studied by inoculating seedlings with inoculum from other miombo tree species. *Afzelia* was found to have a broad host range.

P. angolensis seedlings were raised in the nursery in the soil from the rhizosphere of adult trees of the same species. Seedlings of *P. angolensis* formed arbuscular mycorrhizae and nodulated. None of the ectomycorrhizal legumes treated in this study nodulated.

In the nursery the contribution of the mycorrhizal formation to the biomass accumulation was assessed for *B. microphylla*. Inoculated seedlings were far taller and had higher biomass than non-inoculated ones.

The main features of miombo ectomycorrhizae is that they have many long and thick hyphae and big rhizomorphs. Rhizomorphs as big as 150 μm diameter were recorded.

Fertilization in the nursery was done for *P. angolensis* seedlings. Simultaneous application of nitrogen and phosphorus boosted the growth. Nodulation was, however, eliminated on fertilized seedlings. Iron and zinc did not have any effect. In a nearby nursery, *P. angolensis* seedlings had iron deficiency symptoms and were dying. Application of iron solution on leaves had a visible effect within 2 days. At the end of the experiment, iron-treated seedlings had significantly grown in height, had produced new leaves and had developed their root system. Non-treated seedlings died or lost their leaves and had their root system very much shortened. In the greenhouse the same symptoms were experimentally reproduced by growing seedlings in compacted and overwatered soil.

Mycorrhizal status of *P. patula* seedlings in various nurseries was assessed in Arusha, Mafiga and Sao Hill, Tanzania. Not all nurseries had mycorrhizae. As a result of lack of mycorrhizae, seedlings in Mafiga nursery were dying. After artificial inoculation, seedlings turned green and grew significantly. The control seedlings deteriorated. Inoculated

seedlings formed mycorrhizae. More mycorrhizal types were found in Sao Hill compared to the other 2 regions. *Suillus granulatus* and *Thelephora cf. terrestris* were the most common mycorrhizae. The use of earthballs in nurseries instead of pots as practised on trial basis in one nursery, was found inhibitory to mycorrhizal development. *Suillus* was found sensitive to this practice. Earthballs have a high clay content and are poorly aerated.

In Morogoro inoculation trials were conducted. Seedlings were grown in the pots in the greenhouse or directly raised under adult pine trees growing within 100 m from the greenhouse. Seedlings of the greenhouse received inoculum from those same adult trees. Seedlings under adult trees formed *Suillus* mycorrhizae of adult trees and were associated with sporophores of this fungi while those in the nursery formed *Thelephora* mycorrhizae and produced *Thelephora* sporocarps within 5 months. *Thelephora* sporocarp production stopped from the 6th month onwards while *Suillus* sporophores production continued under adult trees during the rain seasons. Infection was earlier and higher in the field than in the nursery.

In the nursery pine seedlings were raised in fresh soil from the root zone of *B. microphylla* and *Julbernardia globiflora*. In the field seedlings were directly raised in the root zone of these miombo trees after addition or non-addition of pine mycorrhizal soil. A similar mycorrhizal type was formed in the nursery and in the field where no pine soil was added. Where pine soil addition was made, seedlings formed *Thelephora* mycorrhizae earlier observed in the nursery.

The ecological strategies of miombo trees can be summarized in the following points. (1) The strategies focus on the underground compartment. (2) They are related to the architecture and dynamics of the root system with its deep and thick taproot. While the shoot in seedlings yearly dies back due to severe dry seasons, the taproot yearly expands. (3) The architecture with thin lateral roots lends itself to mycorrhizal formation and/or nodulation. Ectomycorrhizal legumes are of a broad host range. (4) Mycorrhizal lateral

roots participate efficiently in building up the taproot, thus increasing the chances of seedlings to survive in dry and unpredictable environments. (5) The ability of seedlings and trees to develop new shoots using reserves drawn from the taproot towards the end of the dry season, allows them to efficiently use limited showers (6) Recognition of these facts is a basis for a successful afforestation with miombo trees.

This research showed indeed that good nursery practices are beneficial for seedling growth and survival in the nursery and later in the field. The following points deserve attention in nursery management. These are (1) seed treatment (for *P. angolensis*); (2) nutrient and moisture requirements; (3) (ecto)mycorrhiza; (4) restricted pruning of the taproot and (5) timing of activities.

INDUNDURO

Intego y'uyu mushinga kwari ugutanga umusanzu mu bumenyi bukenewe mu migambi yo gusubiza agaciro umutungo kamere w'Abanyafrika cyane cyane mu rwego rwo kwambika imisozi yanamye. Byamaze kugaragara neza ko ibiti biturutse hanze bidashobora gukemura ibibazo dufite birebana n'ibidukikije. Ntabwo dukeneye ibiti gusa byo gucana no kubaka. Dukeneye n'ibiti byo kugarura uburumbuke mu butaka bwacu. Kubera ko amasambu menshi yamaze kuba rubiha, dukeneye ibiti bishobora gukurira mu butaka bukenye. Muri uyu mushinga twize ibiti kimeza (mu magambo ya kizungu ari byo) *Azalia guanzensis*, *Brachystegia microphylla*, *B. spiciformis*, *Julbernardia globiflora*, na *Pterocarpus angolensis*. Ibi biti biboneka mu mugabane munini w'Afrika, cyane cyane ahantu hagwa imvura nkeya hakaba n'ubutaka bwacuyutse. Ibi biti bibaho bite? (1) Uyu mushinga werekanye ko ibi biti bikorana n'udukoko tubifasha kuvoma amazi n'ibyokurya mu butaka bukenye. (2) Kubera gufatanya n'utwo dukoko, ibi biti bifite ubushobozi bwo kugarura uburumbuke mu butaka. (3) Umugabane munini w'ibi biti wera imbuto zifite ubushobozi bwo kumera mu gihe habonetse akito k'akavura gake. *P. angolensis* yo ifite ubushobozi bwo kutamera mu gike imvura idahagije. Ibyo bituma yirinda ibishuko by'imvura idahagije. (4) Ibi biti bibanza gukuza umuzi cyane kugirango bibashe gushyikira amazi ari ikuzimu. Imizi y'ibi biti ni ikigega cy'ibyo kurya bikoreshwa mu gihe cy'amapfa. Akamenyero ko gukata imizi (élagage des racines) gakoreshwa kuri za pinusi muri pikinnyeri kagira ingaruka mbi ku mikurire y'ibi biti. Uburyo bwo gutera no gukorera ibi biti nabyerekanye muri ubu bushakashatsi. Ndizera ndashidikanya ko intambwe (ntoya nyamara y'ingirakamaro) mu kumenya no guteza imbere ibiti byacu itewe muri uyu mushinga. Mu gihe abashakashatsi bazarushaho gufatanya n'abaturage mu gukomeza gushakira umuti ibibazo byugarije Afrika mu bidukikije, ndizera ko ibyakozwe muri uyu mushinga bizababera urufatiro. Mwibuke ko ak'imuhana kaza imvura ihise.

SAMENVATTING

MIOMBO-BOMEN: ECOLOGISCHE STRATEGIEËN ALS BASIS VOOR BOSTEELT

Het in dit proefschrift gepresenteerde onderzoek behandelt verscheidene aspecten van de belangrijkste leguminosen-boomsoorten in de Tanzaniaanse boomsavanne, miombo genaamd, namelijk *Azelia quanzensis*, *Brachystegia microphylla*, *B. spiciformis*, *Julbernardia globiflora*, alle behorend tot de Caesalpinioideae, en *Pterocarpus angolensis*, behorend tot de Papilionoideae. Deze soorten zijn van groot belang voor de bosteelt in Tanzania op basis van inheemse boomsoorten in plaats van op exotische bomen. Ten grondslag aan dit onderzoek lagen problemen in de opkweek van materiaal in de boomkwekerij en problemen met de overleving van uitgeplant of uitgezaaid materiaal in het veld. Hierbij zijn de specifieke omstandigheden op de kwekerij van belang, waarbij kiemfysiologie, bemesting, mycorrhizae (vormen van mutualistische symbiose tussen schimmels en wortels van hogere planten) en stikstofbindende bacteriën, die wortelknolletjes vormen, gedacht worden de grootste rol te spelen.

Bestudeerd zijn de verjonging in het veld en in de kwekerij, effecten van bemesting en vochtregime, effecten van inoculatie met mycorrhizaschimmels en het vóórkomen in het veld van mycorrhizae op kiemplanten. Tevens zijn de ectomycorrhizae van exotische dennen onderzocht. Hierbij is het effect van denne-mycorrhizaschimmels, die grotendeels eveneens exotisch zijn, vergeleken met die ectomycorrhizaschimmels, die van nature aanwezig zijn in de inheemse vegetaties. Het effect van inoculatie met ectomycorrhizaschimmels op dennekiemplanten is eveneens onderzocht. Het uiteindelijke doel was een bijdrage te leveren aan de ontwikkeling van technieken ten behoeve van de bosteelt van miombo-boomsoorten.

De omstandigheden waaronder kieming van zaad optrad, werd onderzocht voor enkele boomsoorten. Zaden van *B. spiciformis*,

J. globiflora en *A. quanzensis* kiemden onder veldomstandigheden binnen drie weken alleen onder regelmatige, doch niet noodzakelijkerwijs voortdurende regenval. Zaad van *P. angolensis* bezit kiemrust. Aanbod van water induceerde hier geen kieming. In de kas kon 90% kieming worden geïnduceerd binnen twee weken wanneer de zaadhuid werd ingeboord. De weersomstandigheden bleken van invloed te zijn op de kieming van ingeboorde zaden in de kas. Alle zaden waren gevoelig voor vuur. Zaad van *A. quanzensis* kiemde wel bij 25°C, maar niet bij 10°C en 15°C. Het mislukken van kieming van deze soort in de hooglanden van Tanzania werd daarom toegeschreven aan de lage temperatuur.

Kiemplanten van bovengenoemde boomsoorten werden in de kwekerij en in het veld geobserveerd. In de kwekerij bleek snoei van de penwortel van *J. globiflora* en *P. angolensis* de groei en overleving negatief te beïnvloeden. De eerste twee maanden was de hoogtegroei van miombo-kiemplanten 70% van die van het gehele eerste jaar. Soorten met grote zaden vertoonden een sterkere initiële groei dan soorten met kleine zaden. De initiële groei van miombo-kiemplanten bleek die van de exotische dennen voor alle parameters te overtreffen.

In de kwekerij werden *A. quanzensis*, *B. microphylla* en *J. globiflora* geïnoculeerd met bosgrond waarin de aanwezigheid van ectomycorrhiza was vastgesteld en met basidiosporen van vruchtlichamen van mycorrhizaschimmels. Inoculatie met bosgrond resulteerde in ectomycorrhizae bij de kiemplanten. Inoculatie met basidiosporen resulteerde alleen bij *A. quanzensis* in mycorrhizae. In het veld werden waarnemingen verricht omtrent mycorrhiza-infectie bij spontaan gevestigde zaailingen van *B. spiciformis* en *A. quanzensis* evenals bij zaailingen die dichtbij volwassen planten van dezelfde of van andere boomsoorten aangeplant waren. Bij zaailingen nabij volwassen bomen vond mycorrhiza-infectie van de kiemplanten in een zeer vroeg stadium, nog voordat de kieming was voltooid, plaats.

Kiemplanten van *A. quanzensis* bleken mycorrhizae te vormen met rhizosfeergrond van verscheidene andere miombo-

boomsoorten. Geconcludeerd werd dat de ectomycorrhizaschimmels van miombo-boomsoorten een lage specificiteit ten opzichte van hun gastheer hebben. Ectomycorrhizae van miombo-boomsoorten hebben in het algemeen vele en dikke hyfen en massieve rhizomorfen. Verscheidene mycorrhizatypen zijn beschreven en gekarakteriseerd in de appendix van dit proefschrift. Kiemplanten van *P. angolensis* vormden (vesiculair-)arbusculaire mycorrhizae en *Rhizobium*-wortelknolletjes in rhizosfeergrond die afkomstig was van volwassen bomen van dezelfde soort. De ectomycorrhiza-vormende miombo-boomsoorten vormden geen *Rhizobium*-wortelknolletjes.

In de kwekerij werd het effect van ectomycorrhizae op de biomassaproduktie van *B. microphylla* onderzocht. Geïnoculeerde kiemplanten waren veel hoger en hadden een grotere biomassa dan niet-geïnoculeerde kiemplanten.

Het effect van droogte op wel en niet met ectomycorrhizaschimmels geïnoculeerde kiemplanten van *A. guanzensis* werd onderzocht in de kwekerij. De behandelingen bestonden uit een dagelijkse watergift, of watergiften iedere drie of vier dagen. De kiemplanten met dagelijkse watergift bleken gemiddeld viermaal zwaarder te zijn dan die met een droogtebehandeling. De kiemplanten met droogtebehandeling bleken slechts één mycorrhizatype te hebben met een infectiepercentage van maximaal 10%. De kiemplanten met dagelijkse watergift hadden minimaal twee mycorrhizatypen en een infectiepercentage van minimaal 50%. In het veld bleken kiemplanten van *A. guanzensis* droge omstandigheden te kunnen overleven en bleek de mycorrhiza-infectie volledig te zijn. Net als in het experiment in de kwekerij werd maar één mycorrhizatype onder deze droge veldomstandigheden aangetroffen. In de praktijk blijken kiemplanten in de miombo vaak af te sterven door droogte. Sterfte bleek niet op te treden bij kiemplanten die in de kwekerij onder vochtige condities waren opgegroeid.

Het effect van bemesting op de groei van *P. angolensis* werd onderzocht. Gelijktijdige toepassing van stikstof en fosfor had een positief effect op de groei. De bemesting zette

echter ook de vorming van wortelknolletjes stop. IJzer en zink bleken geen effect te hebben. In een andere kwekerij werd ontdekt dat *P. angolensis* symptomen van ijzergebrek vertoonde. Deze waren zo ernstig dat sterfte optrad. Toepassing van ijzer had al na twee dagen een zichtbaar effect. Na 100 dagen hadden de met ijzer behandelde planten nieuwe bladeren gevormd en vertoonden wortelgroei. De onbehandelde kiemplanten daarentegen verloren hun bladeren, hadden een onderontwikkeld wortelsysteem en stierven tenslotte. Dezelfde symptomen konden in de kas worden gereproduceerd in compacte en zeer natte grond.

De mycorrhizabezetting van kiemplanten van *Pinus patula* werd onderzocht in verscheidene kwekerijen in Tanzania. Niet in alle kwekerijen werden mycorrhizae aangetroffen op de dennesoorten. In één kwekerij werd geconcludeerd dat kiemplanten stierven ten gevolge van de afwezigheid van ectomycorrhizae. Na kunstmatige inoculatie werden de kiemplanten weer groen en vertoonden ze groei. De niet-geïnoculeerde kiemplanten kwijnden weg. De kunstmatige inoculatie werd bevestigd door directe observatie. De mycorrhizae gevormd met *Suillus granulatus* en *Thelephora cf. terrestris* werden het meest aangetroffen. Het gebruik van earthballs (met de hand gemaakte compacte ronde ballen van kleirijke grond) in kwekerijen in plaats van potten bleek vanwege hun gebrek aan structuur en doorluchting ongunstig te zijn voor mycorrhiza-ontwikkeling, in het bijzonder voor *S. granulatus*.

In Morogoro werden inoculatie-experimenten met mycorrhizae uitgevoerd. Kiemplanten werden opgekweekt in de kas in potten of onder volwassen dennebomen (*Pinus patula*) op 100 m afstand van de kas. De kiemplanten in de kas ontvingen grond-inoculum van dezelfde dennebomen. De kiemplanten die opgroeiden onder de volwassen dennebomen bleken dezelfde *Suillus*-mycorrhizae te vormen als die aan de dennebomen. In de kas daarentegen werden alleen *Thelephora*-mycorrhizae waargenomen. De produktie van vruchtlichamen van *Thelephora cf. terrestris* hield 5 maanden na de start van het experiment

op, terwijl de produktie van vruchtlichamen van *Suillus granulatus* gedurende de gehele periode van het experiment voort ging. Mycorrhiza-infectie trad in het veld eerder en sterker op dan in de kas.

In de kwekerij werd het effect van aanwezigheid van rhizosfeergrond van *B. microphylla* en *J. globiflora* op de ontwikkeling van ectomycorrhizae bij kiemplanten van *Pinus patula* onderzocht. Dit werd ook in het veld gedaan, waarbij gezaaid werd nabij volwassen bomen van bovengenoemde soorten. In het veld werd bovendien wel of geen rhizosfeergrond van dennenplantages toegevoegd. In alle gevallen ontwikkelden zich ectomycorrhizae. De ontwikkeling van *Thelephora*-mycorrhizae trad het eerst op bij de kiemplanten die buiten opgroeiden onder toevoeging van rhizosfeergrond van dennenbos.

De ecologische strategie van miombo soorten kan als volgt worden samengevat.

1. De strategie richt zich voornamelijk op ondergrondse delen.
 2. Zij is gebonden aan de architectuur en groeidynamiek van het wortelstelsel, in het bijzonder de diepe, dikke penwortel met veel reserves. Terwijl de bovengrondse delen in het droge seizoen jaarlijks afsterven, groeit de penwortel verder uit.
 3. De architectuur met dunne zijwortels leent zich in het bijzonder voor vorming van mycorrhiza en/of biologische stikstofbinding. De ectomycorrhiza-vormende miombo-bomen zijn weinig specifiek.
 4. Vorming van mycorrhiza maakt mogelijk en versterkt de benutting van grote grondvolumes, nodig om in zulke droge, onvoorspelbare milieus te overleven.
 5. Het vermogen van miombo-planten om nieuwe bovengrondse delen te produceren op basis van in de penwortel aanwezige reserves aan het eind van het droge seizoen, maakt een efficiënte benutting van beperkte regen mogelijk.
 6. Als deze ecologische strategie niet wordt geïmiteerd in de kwekerij gaat de kweek en teelt van deze boomsoorten mis.
- Uit het onderzoek blijkt dan ook dat goed beheer van de boomkwekerij het succes bij de opkweek van kiemplanten en bij de bosaanplant in hoge mate kan bevorderen. De volgende

maatregelen zijn daarbij in het bijzonder van belang: (1) zaadbehandelingen (voor *P. angolensis*); (2) nutriënten- en vochtvoorziening; (3) (ectomycorrhiza); (4) beperkte snoei van de penwortel en (5) tijdsplanning.

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APPENDIX 1

Abbreviations used in tables of different appendices: AQ = *A. quanzensis*; JG = *J. globiflora*; SI = Soil inoculum; DS = Direct sowing; BS = *B. spiciformis*; BM = *B. microphylla*; PP = *P. patula*; nurs. = nursery

1A and 1B concern iron deficiency symptoms and cure. While 1C to 1F indicate some of the ectomycorrhizal types found on the root system of miombo trees.

Iron deficiency

1A Iron deficiency, symptoms and changes brought on one leaflet just by one application of iron solution on the leaves; 1 cm = 2.17 cm (bar of 1.38 cm = 3 cm)

1B Iron deficiency, conditions of treated and non-treated seedlings at the end of the experiment; 1 cm = 4.65 cm (bar of 1.29 cm = 6 cm)

Ectomycorrhizal types

----- Type Host Location and inoculation techniques -----

1C	AQ	nursery; soil inoculum from AQ and JG trees,
	JG	nurs.; soil inocu. (SI) from JG trees, Uluguru
1D	AQ; JG	nurs.; field; SI from JG and AQ; direct sowing
		(DS) under AQ and <i>P. caribaea</i>
1E	AQ	field; DS under JG
1F	AQ	nurs.; SI from AQ of Mikese
1G	AQ	nurs.; SI from <i>B. spiciformis</i> trees in a farm

Short notes on various types

1C Mycorrhizae are yellow brown, with laterals emerging from the main root and branching in one plane, making somehow a flat structure. Individual tips are crooked. The surface of mycorrhizae have loosely attached intermixed thick hyphae and rhizomorphs. The mantle bears scars marked by stones or other coarse materials in the growing substrate. Main roots are also mycorrhizal; 1 cm = 242.7 μ m (bar of 1.24 cm = 300 μ m)

1D Dark brown mycorrhizae with stiff stoutly attached long and thick hyphae all over the surface and the main root which is itself mycorrhizal. Very thick rhizomorphs emerge from mainly the main root. Rhizomorphs are also very hairy. *A. guanzensis* seedlings become infected by this fungus as soon as they start germination. This type has many clamp connections readily visible; * same scale as 1G

1E Creamy white mycorrhizae, branching laterally to make a flat structure. Leading tip and branches are all mycorrhizal. Tips are relatively long. They are covered by creamy white hyphae all over. These were intermixed and not arranged in any specific direction. Creamy white big rhizomorphs were present. They are big tubes with limited surface hyphae. They remarkably branch in V. With age, mycorrhizae and rhizomorphs turn brown and loose most of their surface hyphae; * same scale as 1G

1F Mycorrhizae are yellow brown medium-sized and do not branch often. They have associated hyphae and big rhizomorphs. The same hyphae cover both mycorrhizae and rhizomorphs; * same scale as 1G

1G Light brown mycorrhizae with long forks, directly emerging from the main root. The overall structure is flat. The tips and both the long root are covered by fine hyphae binding the soil. Mycorrhizae have big light brown rhizomorphs covered by

fine relatively long hyphae all over. Rhizomorphs are attached to the main root or the base of mycorrhizae. The clamps were seen but not so often; * 1 cm = 214.1 μm (bar of 1.40 cm = 300 μm)

APPENDIX 2

Ectomycorrhizal types

Type	Host	Location and inoculation techniques
2A and B	AQ	field; direct sowing under AQ trees. A is a part detached from B, a mycorrhizal system
2C	BS	farm; natural regeneration
2D	AQ	field; direct sowing under BM trees
2E	BS	farm; natural regeneration, mycorrhizal type of the fungus of 2C; also formed by AQ seedlings raised in BS soil in the nursery
2F	AQ	greenhouse; basidiospores of APPENDIX 5D
2G	JG	nurs.; soil inoculum from JG

Short notes on various types

2A Mycorrhizae are visible with the naked eye as white balls giving an impression of underground puffballs. In the infection process, the mycelium of the fungus grows and hardens around a given lateral. Lateral elongation is then hindered. Infected part of the root produces laterals which grow in spiral inside the mycelium. The lateral in the centre of the spiral is thicker than those of the periphery. It is mainly the inner laterals which are mycorrhizal. The mycelium grows not only around the laterals but also on and between laterals. To see the mycorrhizal laterals one has to tear apart the surrounding mycelium with a hard needle. The mycelium of the fungus involved is white and very thin. Clamp connections were not seen. Big white loose rhizomorphs run through the mycorrhizal system. This mycorrhizal type was seen connected to the mushroom of *Leccinum foetidum* growing around adult *A. quanzensis* trees. Roots of nearby *B. microphylla* adult trees were also infected; * same scale as 2G

2B White ball, a mycorrhizal system of 2A; 1 cm = 1 cm

2C Puffballs associated with adult and young seedlings of *B. spiciformis* in the farm; 1 cm = 1 cm

2D Creamy white mycorrhizae with, long tips emerging from non-mycorrhizal main root. Hyaline fine hyphae, pointing to the same direction, emerge from the surface and cover mycorrhizae all over. Associated medium sized brown rhizomorphs were present but not so many. They were sparsely located; * same scale as 2G

2E Creamy white mycorrhizae, branching often and resulting in big structure. Laterals and leading tips are all mycorrhizal. Tips are crooked and medium sized. Mycorrhizae are covered by longer associated hyphae all over. These hyphae have clamp connections. Creamy white, compact rhizomorphs run from the base of mycorrhizae and connect mycorrhizal systems. This mycorrhizal type colonized young seedlings of *B. spiciformis* in the field and seedlings of *A. quanzensis* raised in *B. spiciformis* soil in the nursery. Main roots were not infected; * same scale as 2G

2F Ochre brown, long, mycorrhizae emerging from the main root. They are completely sunk in ochre long hyphae through which run ochre brown big rhizomorphs connected mainly to mycorrhizae. Long roots are also covered by hyphae. Hyphae appear relatively easily removable. They are often stripped off or bent backwards when dipped in running water. In still water mycorrhizae float probably due to their extensive hyphae. When undisturbed, hyphae point forward or outwards. Mycorrhizae have characteristic smell. Younger mycorrhizae are lighter than old ones. Clamp connections were seen but not so obvious. Sporophores of the fungus involved occurred in great numbers around *A. quanzensis* trees even if trees were isolated; 1 cm = 242.7 μm (bar of 1.24 cm = 300 μm)

2G Light brown mycorrhizae, branching laterally making big flat structure. Mycorrhizae are covered by short stiff and long hyphae. The short hyphae have swollen ends. Long hyphae have clamp connections. Surface hyphae can be many to the extent of enveloping the whole mycorrhizal system. Thick light brown rhizomorphs emerge from mycorrhizae and the main root. They have the same types of hyphae as mycorrhizae; * 1 cm = 214.1 μ m (bar of 1.40 cm = 300 μ m)

APPENDIX 3

Ectomycorrhizal types

Type	Host	Location and inoculation techniques
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3A	PP	greenhouse and all nurseries; For Mafiga, mycorrhizae formed after inoculation, soil inoculum from adult pine stands was used in all nurseries. We thought it was early form of APPENDIX 4G.
3B	PP	nursery, Sao Hill; soil inoculum as on A
3C,D	PP	nursery, field; direct sowing in the miombo woodlands; soil inoculum from the miombo woodlands.
3E	BM	nursery; soil inoculum from adult BM
3F	AQ	field; direct sowing under AQ trees

Short notes on various types

3A Young form of *Thelephora* sp. described in APPENDIX 4G; 1 cm = 214.1 μ m (bar of 1.40 cm = 300 μ m)

3B Creamy white mycorrhizae, branching dichotomously. The surface bears short spiny hairs. The colour of mycorrhizae, the presence of spiny short hyphae, and thick and dense rhizomorphs suggest that this type resembles 4D. The main morphological difference is that this type has longer tips; same scale as 3A

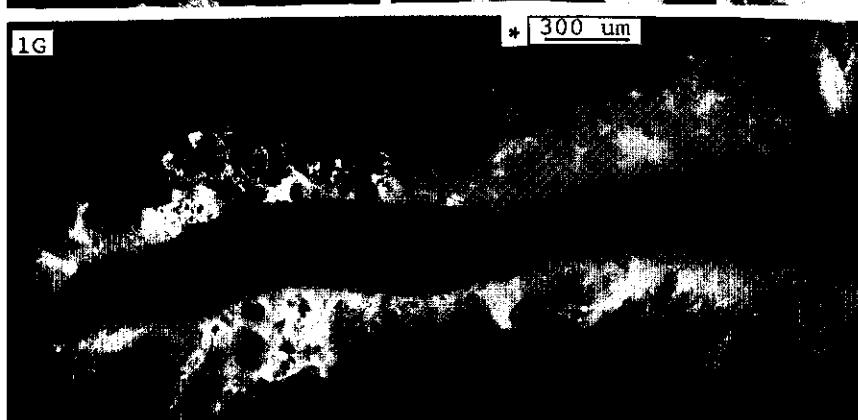
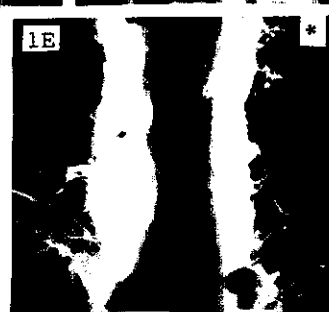
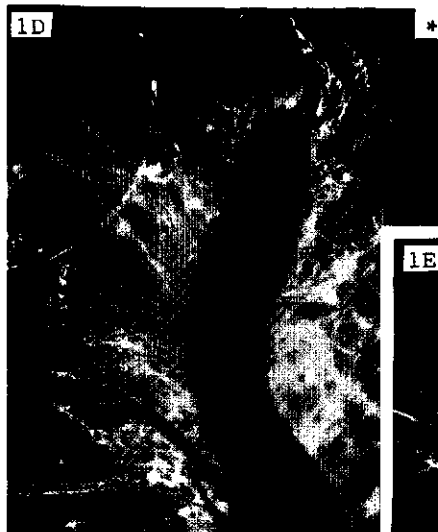
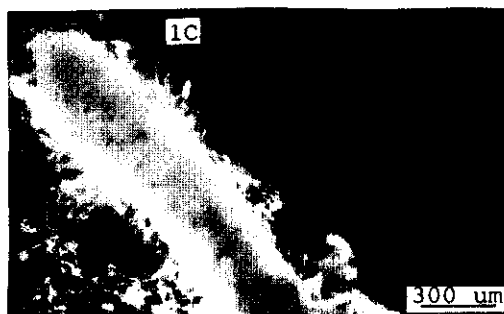
3C This type of mycorrhiza is thought to have originated from the miombo woodlands. It developed on *P. patula* seedlings raised in the root zone of *B. microphylla*, *J. globiflora* or *A. guanzensis* or in the nursery in the soil collected from the root zone of these trees. Mycorrhizae are creamy white but turn to brown/yellowish some days after extraction. Mycorrhizal types branch dichotomously and repeatedly making

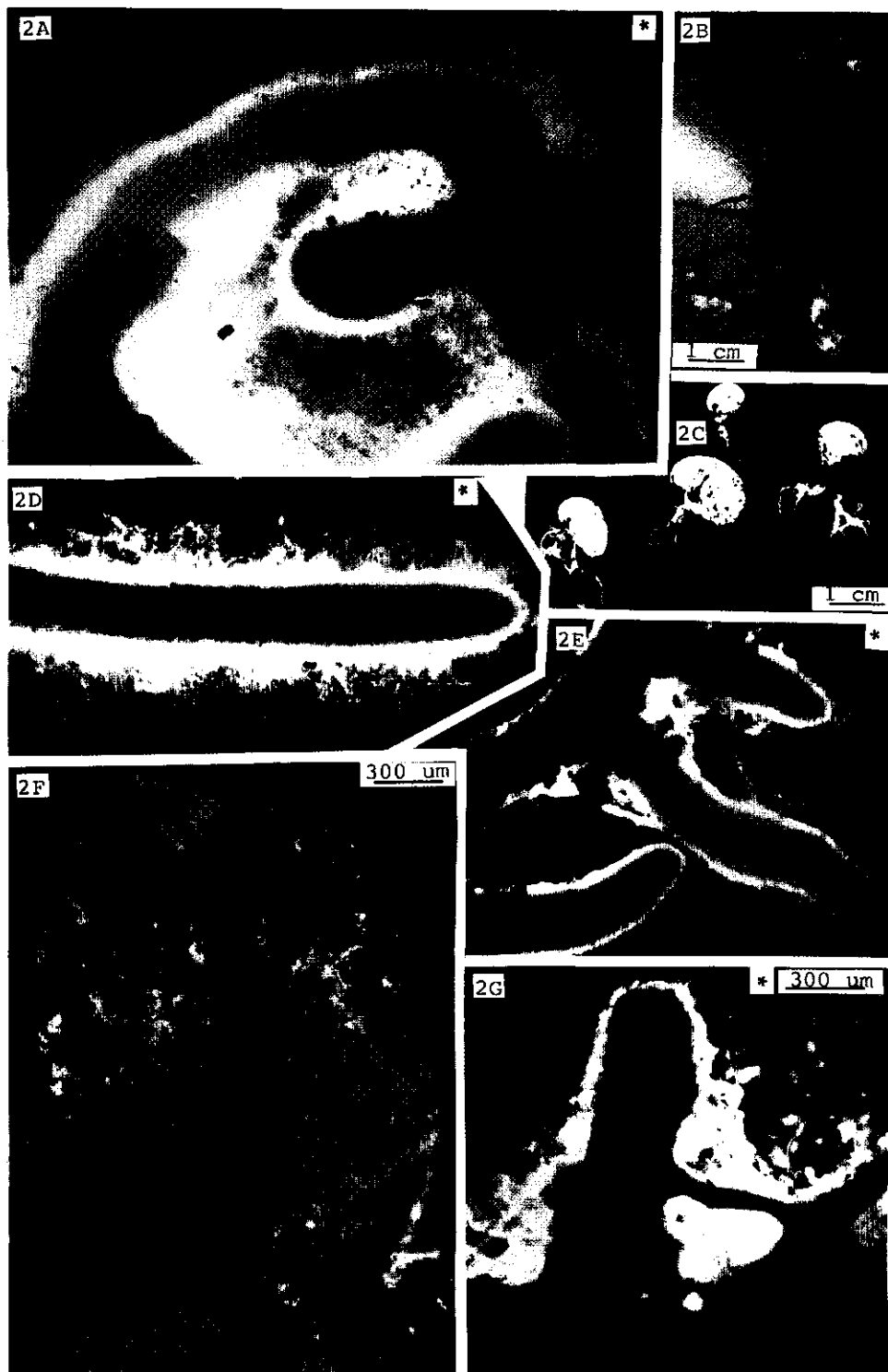
some loose coralloids or big systems. Mycorrhizae have on their surface spiny short hairs which collapse in contact with water. Thick dense rhizomorphs connect the base of individual mycorrhizae or systems. Rhizomorphs are creamy white to brown. They branch dichotomously. Mycorrhizae are not covered by a weft of mycelium but are easily recognizable by their bright colour. The features of this type are also displayed by type 3D. It was thought that the two are just one type; same scale as 3A

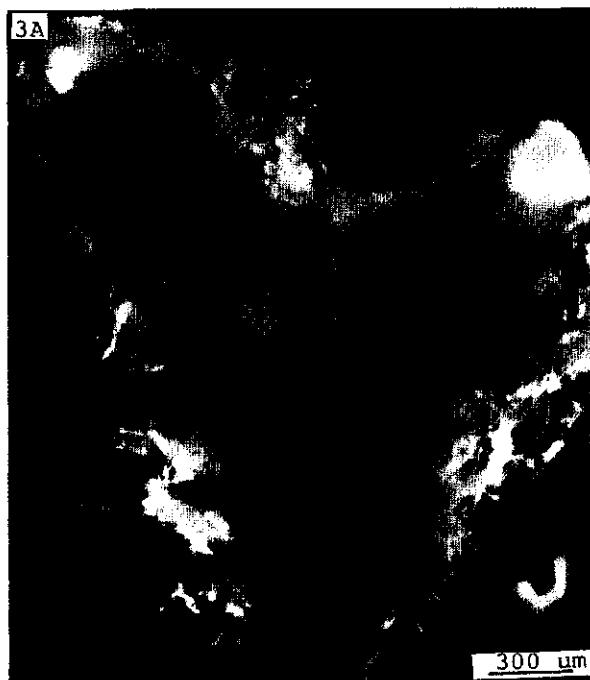
3D cf. 3C; same scale as 3A

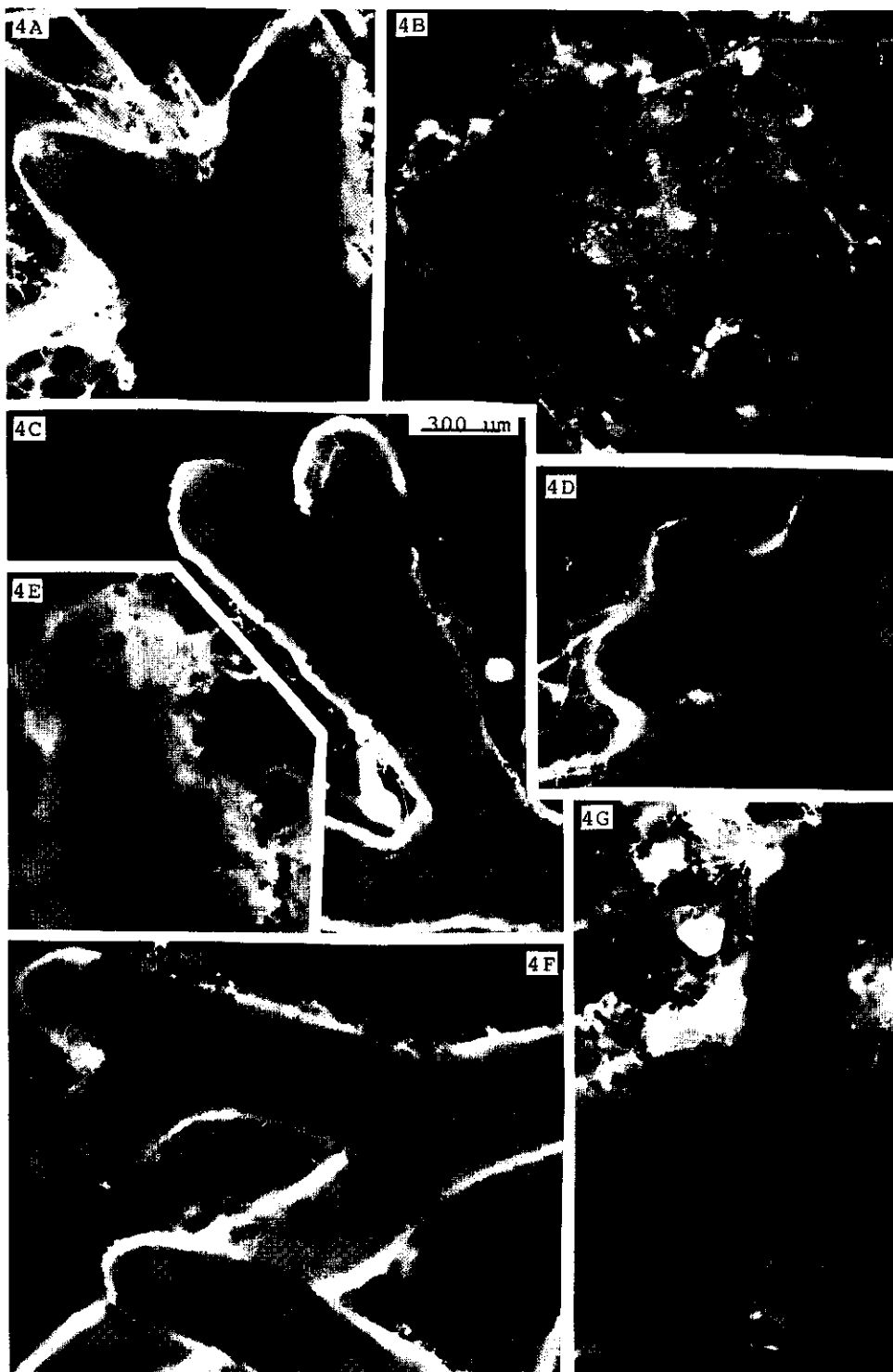
3E Yellow brown small to medium sized mycorrhizae. Branching in three dimensions. Mycorrhiza are wrapped in a mat of creamy white mycelium. Loosely formed rhizomorphs are visible and run through the system. Clamp connections are present; same scale as 3A

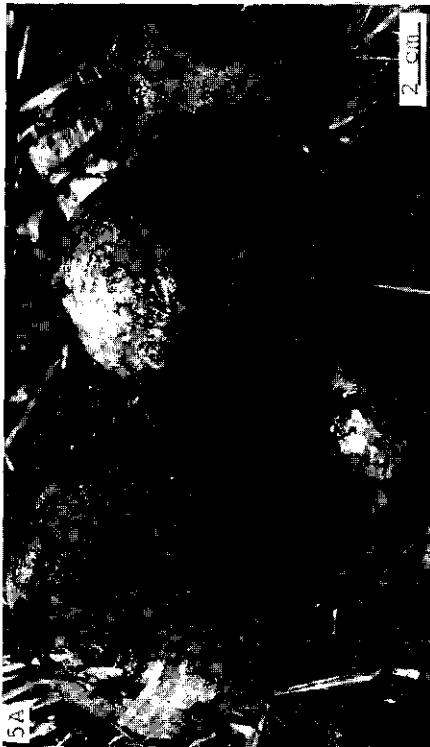
3F Mycorrhizae are very light brown. Branching is not so frequent. The chief feature of this mycorrhizal type is the presence of many surface short hyphae having swollen end. These hyphae are present on both mycorrhizae and rhizomorphs. The fungus involved form very thick rhizomorphs. The few long associated hyphae have clamp connections. This mycorrhizal type was found on natural regeneration of *A. quanzensis* younger than 2 weeks. It formed also on adult *B. microphylla* trees growing in the same surrounding; same scale as 3A











APPENDIX 4

Ectomycorrhizal types

Type	Host	Location and inoculation techniques

4A		all nurseries (for Mafiga after inoculation); soil inoculum
4B	PP	Sao Hill; soil inoculum
4C	PP	nursery Arusha; soil inoculum
4D	PP	nursery Sao Hill; soil inoculum
4E	JG	nursery, Morogoro; soil inoculum from <i>J. globiflora</i> adult trees
4F	PP	nursery Sao Hill; soil inoculum
4G	PP	all nurseries (Mafiga only after inoculation); soil inoculum

Short notes on various types

4A White mycorrhizae, dichotomously branching, becoming a big system but without forming coralloids. Mycorrhizae are totally wrapped in white fine mycelium through which run big loose white rhizomorphs. These features are easily seen under 10 X magnification. Individual hyphae are thin. Clamp connections were not seen. During the rain period, the mycelium of the fungus involved colonized upper soil layers and needles of adult *P. caribaea* trees. Many mushrooms of *S. granulatus* were produced around adult pine trees. Connection between the mycorrhizae and the mushrooms was established; same scale as 4C

4B Brown mycorrhiza (4G) being overgrown by a black mycorrhizal fungus. Individual hyphae in this fungus are thick, long and visible under 20 X. Between the hyphae were brown balls probably sclerotia. These balls have a germinating

tube as observed under 100 X. This mycorrhizal fungus is always overgrowing other types, namely *S. granulatus* and brown mycorrhizae (probably *Thelephora* sp). The fungi involved is most likely a *Cenococcum* species; same scale as 4C

4C Orange brown mycorrhizae, having limited surface hyphae and rhizomorphs. Mycorrhizae branch dichotomously but not so much and as a result, the mycorrhizal system is small. Mycorrhizae have limited, very short surface hyphae. Associated hyphae had clamp connections; 1 cm = 214.1 μ m (bar of 1.40 cm = 300 μ m)

4D Creamy white mycorrhizae branching dichotomously. The tips are very short and stuck to the main root. The ultimate result of branching of such short tips is the formation of coralloids. Mycorrhizae have on their surface spiny short hyphae. These often collapse when the mycorrhiza is dipped in water. Thick dense creamy white to brown rhizomorphs run from one mycorrhizal system to the other. Rhizomorphs often branch in V. The colour often turn to brown when preserved; same scale as 4C

4E Yellowish mycorrhizae with medium to long tips branching or not branching. Tips are totally covered by extensive yellowish hyphae. The mat of hyphae appear yellow but somehow hyaline or so, when considering individual hyphae. Hyphae appear coarse and are not arranged in any specific organization but appear intermixed. Big rhizomorphs emerge from mainly the long roots and run through the system. The long roots are also mycorrhizal; same scale as 4C

4F Yellow/orange brown mycorrhizae, dichotomously branching repeatedly forming very big loose coralloids. Mycorrhizae are characterized by the presence of spiny short hyphae growing all over the surface and the nearly absence of associated long hyphae. This type is probably the same as 4C; same scale as 4C

4G Brown mycorrhizae, dichotomously branching several times

but without forming coralloids. The spiny hyphae observed above were absent. Instead the mycorrhizae had many associated hyphae and rhizomorphs. Young specimen are lighter than old ones. Clamp connections are present; same scale as 4C

APPENDIX 5

Mushrooms used in inoculation trials (4.4.2.1; 5.3.1.1)

Species	associated tree species
5A <i>Pisolithus tinctorius</i>	scattered trees of <i>Eucalyptus</i> spp.
5B <i>Suillus granulatus</i>	<i>Pinus caribaea</i>
5C <i>Lactarius chromospermus</i>	<i>Brachystegia microphylla</i>
5D <i>Pisolithus</i> sp.	<i>Azelia quanzensis</i>
5E <i>Russula</i> sp.	<i>Julbernardia globiflora</i>

All species occurred in Morogoro

5A 1 cm = 2 cm; 5B 1 cm = 4.5 cm; 5C 1 cm = 1.83 cm (bar of 1.09 cm = 2 cm); 5D 1 cm = 1.30 cm (bar of 1.54 cm = 2 cm); 5E 1 cm = 1.46 cm (bar of 1.37 cm = 2 cm)

GLOSSARY

Source: Hallé et al. (1978); Willan (1985); Hale and Margham (1988) and Oldeman (1990).

apical meristem, a region of dividing tissue at the growing tips of roots and shoots that is responsible for increase in length of the plant body.

architecture, the visible morphological expression of the genetic blueprint of a tree at any one time.

aril, an additional integument or appendage formed on some seeds after fertilization.

biome, a major regional ecological community of organisms usually defined by the botanical habitat in which they occur and determined by the interaction of their substrate, climate, fauna and flora. The term is often limited to denote terrestrial habitats, e.g. tundras, coniferous forests and grasslands.

bud, an unextended, partly developed shoot having at its summit the apical meristem which produced it.

cambium, or vascular cambium or lateral meristem, a group of actively dividing cells found in the vascular bundles of roots and stems, whose function is to produce new plant tissue for lateral growth.

cotyledon, a part of the plant embryo in the form of specialized seed leaf that acts as storage organ, absorbing food from the endosperm and functioning as a leaf after epigeal germination.

dehiscent, (of plant structures) spontaneously bursting open to liberate the enclosed contents. Fruits of legume trees split open to release seeds.

die-back, shoot necrosis starting at the plant apex and moving downwards.

epicotyl the axis (or stem) of the shoot in a young germinating seedling located above the cotyledons.

epigeal, of or relating to seed germination in which the

cotyledons are carried above ground and form the first green foliage leaves of the plant.

indehiscent, refers to dry fruits that normally do not split open at maturity.

inoculation, introduction of biological material (the inoculum) into a medium such as living organism, synthetic substrate or soil.

pericarp, the wall of a fruit, developing from the ovary wall after fertilization has occurred.

propagules, any part of an organism that is liberated from the adult form and which can give rise to a new individual, such as fertilized egg or spore.

radicle, the basal part of the embryo in a seed, developing into the primary root of the seedling.

reiteration, any modification of the tree's architecture not inherent in the definition of its model and which is occasioned by damage, environmental stress or supraoptimal conditions.

rhizomorphs, a densely packed strand of fungal tissue, having the appearance of a root which is produced by some higher fungi. In some cases hyphae can be distinguished in the tissue. Rhizomorphs enable the fungi to spread.

rhizosphere, the particular area immediately surrounding the roots of plants in which their exudates affect the surrounding microbial flora.

rhythmic growth, after a period of dormancy, the flushing of tropical trees.

root coiling, root spiralling resulting from mechanical hindrance, e.g. for potted seedlings, by a closed pot.

root floor, the term coined by Oldeman (ex Oldeman, 1990) to indicate all horizontally structured subterranean features that prevent deeper development of root systems.

root pruning, a technique, mainly in nurseries whereby roots growing beyond a given length (normally beyond the tubes) are regularly severed. The aim of such operation is twofold: to stimulate the formation of lateral roots (2) to facilitate lifting at the time of transplantation.

scarification, disruption of seedcoats, normally by mechanical abrasion or by brief chemical treatment in a strong acid, to increase their permeability to water and gases or to lower their mechanical resistance.

sclerotium, a resting stage in fungi. It takes the form of a ball of hyphae varying in size.

seed dormancy, a state in which viable seeds fail to germinate while germination requirements, water, light, temperature are present.

sporophores, any fungal structures that produces spores.

taproot, a primary root structure in which one main root forms the major part of the underground system. Taproots are often swollen, serving as storage organs of perennation.

wildling, seedlings collected from the forest.

CURRICULUM VITAE

Esron Munyanziza was born on 5 December 1958 in The Republic of Rwanda. In 1975 Esron got access to secondary school studies in a missionary college (Collège Adventiste de Gitwe) in Rwanda. The programme was successfully ended in July 1981. He was sponsored by the family of Pastor Jordan. After teaching experience in secondary school in Gitwe, Esron got a chance to follow the BSc Forestry programme at the Sokoine University of Agriculture (SUA), Tanzania. The programme started in January 1984 and was successfully concluded in November 1986. This programme was sponsored through the "Intercoopération Suisse". Esron went back to his country at the end of 1986 and graduated in 1987. Early 1987, he worked for a while in Kigali at the "Appui à la Direction Générale des Forêts". His duty was to elaborate the plan for the conservation and the management of the Rwandese eastern savanna woodlands. He was then nominated as a teacher in the school of agriculture and forestry in the west of the country at Kibuye, the capital of his province. Among other things, he taught forest ecology, silviculture and nature conservation. Late in summer 1988, Esron got the chance to follow the MSc programme in Tropical Forestry at Wageningen Agricultural University. He was sponsored by the Dutch Government. After 1 year of lectures, he went back to Africa for his MSc research. The MSc research was conducted in Kenya in collaboration with the Kenya Forestry Research Institute. The theme was mycorrhizal succession in exotic pine plantations. Esron obtained his MSc Degree on July 12, 1990. Preparation for his PhD studies had already begun. In September 1991, he went back to Africa for his PhD research. He was attached to the Department of Forest Biology (SUA) as a research associate until end of June 1993. He married Yvonne Claassen on September 7, 1993. The author fluently speaks Kinyarwanda, French, Swahili, English and reasonable Dutch.