The Ecology of Endomycorrhizas in some Cameroon forests with respect to species of Terminalia

by

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ABSTRACT

Tropical forests are fast decreasing as they are being exploited to meet demands made on them for fuel, wood and wood products by increasing domestic consumption and increasing requirements of developed countries for tropical hardwoods. Countries such as Cameroon are satisfying such demands partly through the conservation of existing forests but mainly through increased reafforestation. The success of reafforestation schemes depends largely on the silvicultural systems employed. An indication of the ecological sensitivity of such practices can be gauged from the extent to which the soils physical, chemical and microbiological properties are altered. Of particular interest is the impact on the vesicular-arbuscular mycorrhizal (VAM) fungi as they play an important role in ensuring that forest trees especially those growing on nutrient deficient soils acquire sufficient mineral nutrients for growth.

This study therefore set out to examine the effects of different methods of site preparation (Manual 'recru', mechanical 'recru' and complete clearance) and the subsequent outplanting with *Terminalia ivorensis* on the VAM population dynamics in the Mbalmayo Forest in Cameroon.

Observations made prior to site preparation indicated the presence of an array of 17 VAM fungi belonging to the generas *Acaulospora, Glomus, Sclerocystis* and *Scutellispora*. Of the 17 fungi, one, *Glomus etunicatum* always represented more than 50% pf the spore population.

The importance of the tree component of the forest vegetation as reservoirs of VAM inocula was evident from,

a) the higher numbers of spores in association with *T. superba* compared with shrubs and
b) the peak in spore density close to *T. superba* trees (2.5 m).

Seasonal effects were suspected as many VAM fungi sporulated more profusely in the dry season (February, 1987) compared to the two rainy seasons (August 1987 and August 1988).

Site preparation led to a dramatic reduction in spore number with the completely cleared plot losing 65% of its initial spore population.

One year after planting, however, mean spore numbers had increased dramatically in all cleared plots. A major cause of the increase being the increase in root densities from the planted *T. ivorensis* and the invasive ruderal *Eupatorium odoratum* and pioneer tree *Musanga cecropioides*. In the mechanically and completely cleared plots the sharp rise in spore numbers was mainly by the fungal aggregate *G. occultum/A. scrobiculata* which sporulated profusely in the presence of the invasive ruderal *Eupatorium odoratum*.

The amounts of infection within the roots of *T. ivorensis* 1½ years after planting in the manual and mechanical 'recru' plots were significantly greater than observed within *T. ivorensis* roots in the completely cleared plot. These differences may be related to the initial drop in spore numbers following site preparation, the disruption of the VAM hyphal networks in soil and/or the increasing dominance of *G. occultum/A. scrobiculata*, a type believed to be more asociated with *Eupatorium odoratum* and hence possibly less effective on *T. ivorensis*. The value of the silvicultural procedures that were less destructive appeared to be reflected in tree survival.

DECLARATION

I hereby declare that this thesis has been composed by me and the results are my own, except where otherwise stated and no part of it had earlier been presented for any degree.

Mbangu Musoko
May 1991

DEDICATION:

To Mboti and Ikomi,
for their unwavering love and support

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CHAPTER 1

INTRODUCTION

1.1 THE STATUS OF TROPICAL RAINFORESTS

Tropical rainforests constitute 60% of the world's total forest growing stock and 69% of global forest productivity (Sutlive *et al.*, 1981). These forests are found in America (south of the United States), Africa (south of the Sahara excluding South Africa), Asia and the Far East (excluding part of the Union of Soviet Socialist Republics, China and the Democratic Peoples Republic of Korea and Mongolia), lying in a belt centred on the equator and extending $23\frac{1}{2}^{\circ}$ north and south to the Tropics of Cancer and Capricorn (Grainger, 1980). At present tropical forests are extensively exploited and cleared to meet demands for forest products (wood, fibre, energy) for agriculture, urban and industrial expansion (Evans, 1982), all of which have accelerated as populations and their rates of consumption have increased. Although forecasts vary, a crude approximation of the rate of annual loss of tropical forests, stands at 245,000 km^2 (Myers, 1980). Many factors, as succinctly documented by Evans (1982) have accounted for such losses; of these the major factors are:

a) the increase in wood exports (as a means of earning foreign exchange by developing countries) which grew at an annual rate of 7.1% between 1970 and 1980 (US Interagency Task Force on Tropical Forests, 1980).

b) a corresponding increase in wood imports by developed countries, from 5.2 million m^3 to 52 million m^3 (Grainger, 1980).

c) shifting agriculture by local farmers (Nye and Greenland, 1960; Leakey, 1985) and

c) an increased domestic use of forest products (Grainger, 1980).

Although some authors (Evans, 1982; Lugo and Brown, 1982) see no immediate worldwide shortage of wood and wood products, the effects of over-exploiting tropical forests are already manifest regionally.

In tropical W. Africa for instance, Ivory Coast has lost more than 70% of its rainforest (Caufield, 1982) and it is feared that continued deforestation may make it an importer of timber before the end of the

decade (Bourke, 1987). In Nigeria, population pressure has led to continuous conversion of forests to agricultural lands (Spears, 1980). Of an estimated five million hectares of forest in 1950, only two million have been protected as future sources of timber and other forest products (Spears, 1980). It is feared that in the 1990's Nigeria will require at least 100,000 hectares of fast growing timber plantations to restore its domestic self-sufficiency. For this reason all timber exports from the country were banned in 1975 to meet this target (Spears, 1980).

Similarly, Sierra-Leone may be devoid of tropical rainforest in 20 years from now (Myers, 1985).

In Cameroon, 423,720 hectares of forests are cut down annually (6th 5-year National Development Plan of Cameroon, 1986-1991); this being so extensive areas will be depleted of forests by the 1990's if adequate reafforestation programmes are not initiated.

Apart from the legitimate fears of shortages of forest products, which widespread depletion and mismanagement could foster, other undesirable consequences could result, such as the degradation of highly productive lands, increased occurrences and intensity of floods, accelerated desertification, reduced soil fertility, the extinction of valuable genetic resources and the loss of wildlife (Spears, 1980).

Clearly better management strategies must be devised through more rigorous conservation of existing forests and reafforestation, with a clear understanding of objectives whether for the rapid re-instatement of supplies of fuelwood or the longterm re-establishment of distinctive timber supplies likely to re-establish commercial advantage in international markets. Steps like these if, and when, taken will directly and/or indirectly improve living standards in most developing countries.

1.2 FOREST ECOTYPES IN CAMEROON

In Africa, varying climates (Nwoboshi, 1982) and diverse soil types (Sanchez, 1981) give rise to a range of vegetation types; from deserts

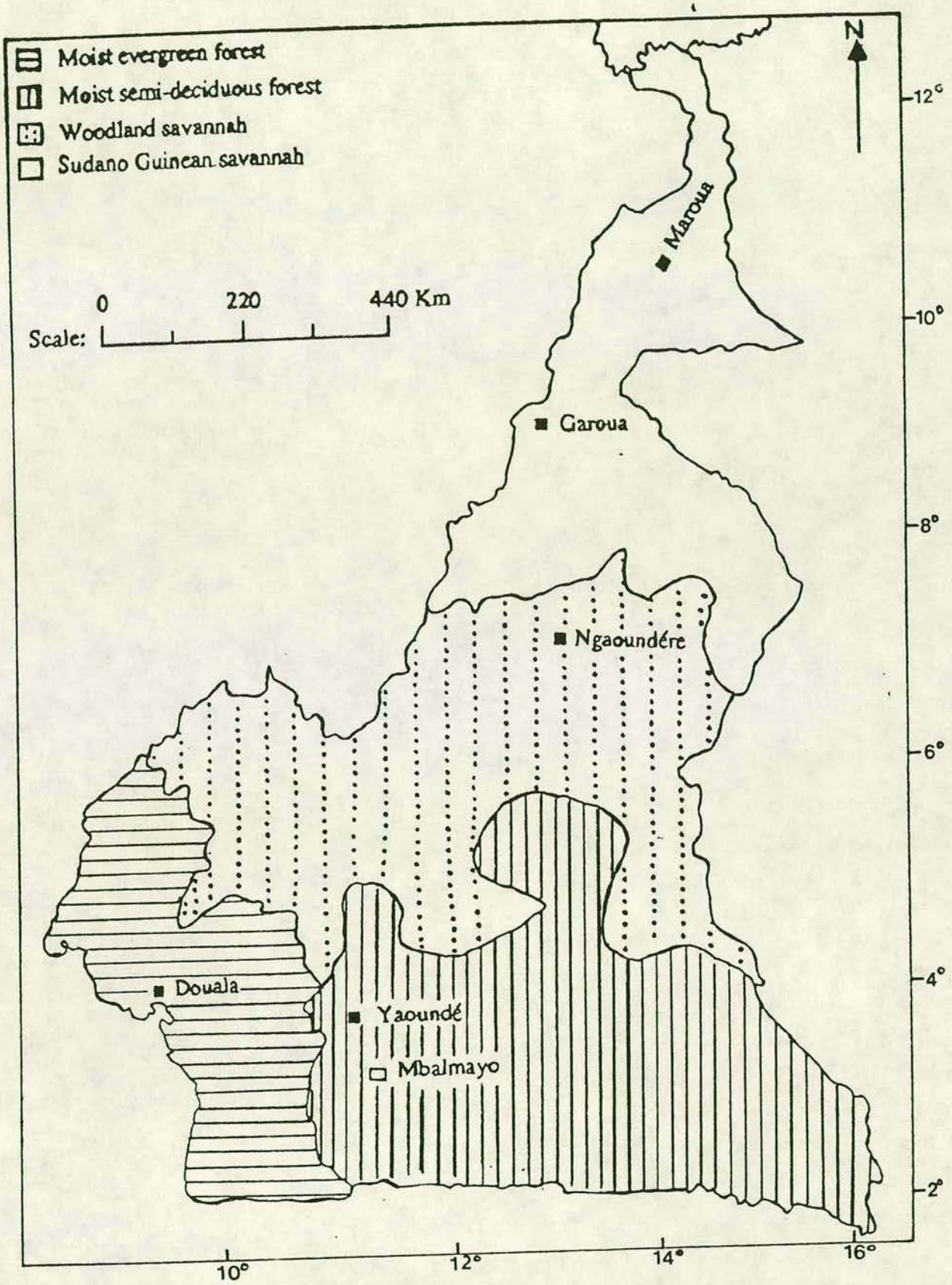


Figure 1.1 Map of Cameroon showing vegetation distribution and research area (modified after F.A.O., 1988)

to lush rainforests.

Cameroon situated at the heart of Africa is rightly described as a microcosm of tropical Africa, having a rich blend of the many vegetation assemblages found in Africa (Letouzey, 1968); closed moist evergreen and semi deciduous rainforests in the south, savanna in the centre, steppes in the north, forest and grasslands on the mountains.

These vegetation types broadly speaking delimit three ecological zones; the Congo-Guinean region in the south, the Sudano-Zambezian region in the north and the montane regions associated with local physiography (Fig. 1.1) (Letouzey, 1968).

Mbalmayo Forest Reserve, the focus of this study is located in the Congo-Guinean region. Its forest is moist and semi-deciduous occurring at medium altitudes (see chapter 2) immediately to the north of the moist evergreen forest. It has an abundance of trees some of major economic importance belonging to the families of Sterculiaceae (*Triplochiton scleroxylon, Mansonia altissima, Cola* spp., *Sterculia* spp.,), Ulmaceae (*Celtis* spp.,), Combretaceae (*Terminalia superba*), Meliaceae (*Entandrophragma cylindricum*, Leguminosae (*Piptadeniastrum* spp.) and Rosaceae (*Parinari excelsa*).

1.2.1 **Need for reafforestation in Cameroon**

Cameroon, like many other tropical countries exploits its forests primarily to gain foreign currency from timber but the importance of domestic consumption should not be underestimated (6th 5-year National Development Plan of Cameroon, 1986-1991). Its forests, though characterized by an enormous biodiversity of plant species, do not produce large volumes of currently utilizable timber. Of the 300 known timber species in Cameroon, only 50 are exploited on a more or less regular basis; 15 of these account for more than 70% of the total 'exploited production' (6th 5-year National Development Plan of Cameroon, 1986-1991). More of these timber species need to be planted on a large scale if export and domestic requirements are to be realised.

Attempts to encourage natural regeneration have not been documented in Cameroon. In neighbouring Nigeria, however, they have not been successful (Lowe, 1976). In W. Africa, the valuable tree species are usually very sparsely distributed, their seeds tend to be intercepted by dense vegetation and the few resulting seedlings tend to be inadequately shade-tolerant. In these circumstances good stocking and growth cannot be achieved without constant and uneconomic levels of maintenance. According to Lowe (1976) regenerating trees under these circumstances does not make intensive use of the land and silvicultural operations are generally more difficult to execute compared to the ease of plantation work. In Malaysia, in contrast, natural regeneration has been very successful resulting in adequate stands and good growth of trees (Nwoboshi, 1982).

The Cameroon Government has been more committed than ever in the reafforestation of derelict lands. It is also committed to the conservation of some existing forests; for example, the Korup National Park and several forest reserves. However, the Cameroon Government has turned to the artificial regeneration of derelict lands as opposed to natural regeneration because it avoids delays in forest establishment, allows greater opportunities for the choice of desired tree species to suit the intended market, allows genetic improvement of planting stock, facilitates full stocking and the attainment of site potential, and the management operations such as thinning and weeding are straightforward.

As pointed out by Whitmore (1981); the potential of such intensive plantation silviculture to reduce wood deficits in many Latin American nations augurs well for more of such conversions rather than less. This may well be true for Cameroon.

1.2.2 **Silviculture systems employed in reafforestation schemes in Cameroon.**

A silvicultural system, as defined by Troup (1952), is a system wherein crops constituting a forest are tended, removed and replaced by new crops, resulting in the production of wood of a distinctive form.

Natural regeneration silvicultural systems like the Malaysian Uniform System, Stand Improvement System, Post-Exploitation System, Tropical Shelterwood System, Clear(strip) Felling Selection System, Ghana Selection System and Growth Limits Systems practised in various areas of W. Africa are not practised in Cameroon (Lawson *et al.*, 1989 for full descriptions of the above-named systems). Instead, the replenishment of its forest resource is mainly achieved by artificial means, such as clear-felling and planting, enrichment planting and Taungya.

1.2.2.1 The clear-felling and planting system

Clear-felling operations are effected using a combination of chainsaws and bulldozers. All existing vegetation is cut down and removed, so exposing soil. The debris may or not be burnt. As a result of clearance the microclimate of the area becomes more extreme with wider fluctuations of temperature and moisture than would exist under intact forest canopies. Soil erosion thus becomes a likely hazard adding to that of mechanical compaction incurred during clearance. Clear-felling can also lead to an influx of weeds which compete with the desired tree seedlings. Taylor (1962) recommends that clear-felling be avoided especially in areas of high rainfall. This recommendation has been largely unheeded by many W. African countries where it is still widely used because it is simple to apply and requires no particular skills.

1.2.2.2 Enrichment planting

Where clear-felling and replanting is not desirable for economic or ecological reasons, one alternative is to underplant within the existing forest. This 'enrichment' can take place in lines or groups, classified at three levels of intensity (Baur, 1964).

a) Group planting - It is the least intensive form of enrichment planting and offers the advantage of less ground preparation, protection from grazing and improvement of tree form due to side shading. This method has been very successful in Zaire (Lawton, 1981) though it is still not widely practised, largely because of

difficulties in maintenance.

b) Line planting - Early enrichment practices in French, W. Africa (Aubreville 1938; 1947) showed that close spacing of lines (10m) necessitated the renewal of nearly all of the forest. Later, lines were spaced at wider intervals of 20m with 5m between transplants within rows. In Cameroon, the system resulted in survival ranging between 31% and 65% for planted *Khaya ivorensis* and *Entandrophragma cylindricum* and an averaged height growth of 0.5m per annum. In other African countries line planting has met with little success. For example in Gabon, the highly light demanding species *Aucoumea klaineana* suffered severe competition and intensified insect attacks (Le Ray, 1947).

c) Intensive enrichment

Dawkins (1958, 1966), based on work in Uganda, envisaged a more intensive form of enrichment, and thus defined line planting as: "the establishment of a tree crop to be closed at rotation age in lines spaced at intervals equal to or slightly greater than the estimated final crown diameter".

The Recrû ('regrowth') technique is a form of intensive enrichment and was the main treatment applied to the Mbalmayo Forest Research plots which I studied. This technique aims at providing transplants with maximum light as soon as they are planted, whilst preserving some of the undergrowth. Using this method Catinôt (1965) sets out to ensure that planted seedlings "kept their feet in a cool place and their crowns in the sun".

Between 1968 and 1985 about 12,000 hectares of forest were manually felled and planted (Manual Recrû) in the south, central, coastal and southwest provinces of the Cameroon Republic (Leakey and Grison, 1985). However, because of rising labour costs, the National Office for Forest Regeneration (ONAREF) introduced a mechanised version of Recrû (Mechanical Recrû) involving the use of bulldozers to remove about 50% of canopy trees, using a blade held above ground to minimise damage to soil.

Other similar systems to the Recrû are: the 'Martineau System' (where gradual opening of the overhead canopy takes place) and the 'Limba System' where the shrub layer is cut to a lesser height than in the Recrû (Lawson *et al.*, 1990).

1.2.2.3 Taungya

Taungya as defined by Dawkins (1958) is: "the establishment of a forest crop by planting within an agricultural crop, on land cleared for planting and weeded for the duration of the agricultural crop by the farmer".

With this system, groups of farmers are allocated blocks of land in forest reserves to clear and cultivate, given prescribed crop species and planting densities (usually 4 x 4 m) to avoid excessive competitions with young trees.

Some favoured native species for Taungya, in Nigeria include *Triplochiton scleroxylon, Nauclea diderrichii, Terminalia ivorensis, Lovoa trichilioides, Khaya ivorensis, Mansonia altissima, Entandrophragma cylindricum* and *Tarrietia utiles.* Taungya can be a satisfactory means of regenerating secondary forest provided:

- the local farmers are sufficiently short of land to undertake clearance work;
- funds are available for planting stock and supervision;
- suitable species combinations are found; and
- the soil is sufficiently stable.

Taungya however has the disadvantage of being a drastic regeneration method, with clearing almost always followed by burning (Lawson *et* al., *1990).*

1.3 EFFECTS OF SITE PREPARATION METHODS EMPLOYED IN PLANTATION ESTABLISHMENT

According to McColl and Powers (1984), the success of reafforestation projects like plantation establishment depend in part or in whole on the methods employed for site preparation especially in relation to the

soils which represent the single most critical factor affecting such schemes.

Although the tropical rainforest plant-soil system is an extremely resilient and stable ecological entity, the equilibrium between plant and soil becomes greatly disrupted when the forest is opened for cultivation to agricultural or tree crops (Adejuwon and Ekanade, 1987). Site preparation methods are often predetermined by the silvicultural systems selected and these may range from the least destructive manual methods used in enrichment silvicultural systems (e.g. The Manual Regrowth Method in Cameroon) to the extremely destructive use of bulldozers as in the clear-felling and planting silvicultural system employed by Societé de Developpement des Forêts (SODEFOR) in Ivory Coast. Drastic silvicultural methods like clearfelling have both short and long term effects on the soil's physical, chemical and microbiological properties (Smith and Sobek, 1979; McColl and Powers, 1984) which cannot be ignored as their effects on the development of any subsequent crop become better understood.

1.3.1 **Effects on soil physical properties**

Where the clear-felling system is opted for the use of heavy machinery like bulldozers is inevitable. Conventional bulldozers have been repeatedly shown to compact soils particularly sandy and loamy ultisols (McColl and Powers, 1984), cause significant changes in infiltration rates, increase bulk density and decrease porosity (Van der Weert, 1974; Suebert *et al.*, 1977; Shubart, 1977; Silva, 1979). Changes such as these, encourage surface runoff and erosion, the disaggregation of soil particles resulting from increased temperatures and the loss or rapid decomposition of organic matter, reduced root penetration and the amount of available moisture, all of which seriously affect the growth of new crops. In Nigeria, Lal *et al.*, (1975) observed that topsoil displacement (2.5m of an alfisol) and compaction resulting from the use of heavy machinery led to a 50% decrease in corn yields.

Similarly in Cameroon, Ngeh (1989) noted that more than four passes of a 24 ton bulldozer during site preparation, significantly affected the growth and establishment of a new stand of *Terminalia ivorensis*.

1.3.2 **Effects on soil chemical properties**

In the tropics, a high proportion of nutrients is tied up in the biomass, mostly in bark and foliage, with an equal amount occurring in litter, (Ruddle and Manshard, 1981). Cunningham (1963), evaluating the effects of clearing a tropical forest soil in Ghana, reported a loss of organic carbon, total nitrogen and organic phosphorus from soils of fully exposed sites. Soil temperatures in these exposed sites were raised and there was rapid decomposition of organic matter. Three years later the exposed sites still produced less mineral nitrogen, had smaller cation exchange capacities (CEC), smaller amounts of exchangeable potassium, and a lower pH than the shaded soils (Cunningham, 1963). In Ontario, Canada, trees grown on scalped sites for 10 years were as much as 20% shorter than those grown on adjacent unscalped sites (Mullin and Campbell, 1975).

Rainfall passing through the crowns of trees is known to become enriched with nutrients (Miller, 1979; Jordan *et al.*, 1980; Hilton, 1985). Mobile nutrients like nitrogen and potassium reach high concentrations in throughfall relative to incident precipitation, (Wells and Jorgensen, 1975) thus silvicultural systems like clear-felling which remove all existing vegetation, consequently eliminate the canopy enrichment effect and lose the nutrients tied up in biomass (Ruddle and Manshard, 1981).

1.3.3 **Effects on soil microbiology**

Some bacteria and fungi (e.g. *Fusarium* and *Phytopthora* spp.) cause disease and necrosis to plants while others like nitrogen-fixing bacteria, chemotrophs and chemolithotrophs, and fungi forming symbiotic associations with plant roots - mycorrhizas - have been repeatedly demonstrated as having profound positive effects on plant growth (Azcon *et al.*, 1976; Barea *et al.*, 1975; Bagyaraj and Menge, 1978; Sprent, 1979; Harley and Smith, 1983), particularly under conditions of low nutrient availability typical of tropical soils (Sanchez, 1981). Preparing sites for plantation establishment can constitute a major disturbance on the soil microbial life when heavy machinery such as bulldozers are utilized in silvicultural systems like clear-felling.

Such disturbance could lead to drastic changes including the complete elimination (Meiklejohn, 1962; Reeves *et al.*, 1979, Skujins and Allen, 1986) of beneficial symbiotic microorganisms. In fact, clearfelling has been shown to favour the rapid establishment of non-mycotrophic plant species or the facultatively mycotrophic ones which grow at the expense of obligately mycotrophic species (Janos, 1980). It is hoped that reductions in populations of mycorrhizal fungi populations could be avoided by the judicious selection of more ecologically sensitive site preparation methods for plantation establishment schemes.

1.4 IMPORTANCE OF VAM IN TROPICAL FOREST ECOSYSTEMS

Frank (1885) first defined the term 'mycorrhiza' to describe the symbiotic association of plant roots and soil inhabiting fungi. Of the two main groups of mycorrhizas, ectomycorrhizas and endomycorrhizas, 95% of the world's vascular plants bear mycorrhizas of the latter group.

Within the endomycorrhizal group, the vesicular-arbuscular mycorrhizas (VAM) are the commonest, being found in temperate, tropical and arctic regions. They also possess a broad ecological range being found in ecosystems such as dense rainforests (Janos, 1980) savanna (Khan, 1975) woodlands (Schenck and Kinloch, 1976) grasslands (Allen and Allen, 1980; Sparling and Tinker, 1975) sand dunes (Koske and Halvorson, 1981), deserts (Reeves *et al.*, 1979), heathland (Sward *et al.*, 1978) and coal spoils (Daft *et al.*, 1975).

In Java, Janse (1896) found VA-mycorrhizal infection in 69 of 75 species of woody plants and herbs belonging to 56 families. In Trinidad, 80 or 93 species of woody and herbaceous plants representing 33 families had VA mycorrhizas (Johnston, 1949). All of the forest trees examined were affected and had the highest average intensity of infection. In Nigeria, Redhead (1968) found that the majority of 66 species from 25 families had VA mycorrhizas. He thus speculated that all woody species of lowland rainforest form this type of mycorrhizas. In addition, VA mycorrhizas have been reported on a large number of strictly tropical crops including coffee (Janse, 1896) sugarcane (Cifferi, 1928), tea (Butler, 1939), citrus (Reed and Fremont, 1935),

oil palm (Rayner, 1939), date palm (Sabet, 1940), cocoa (Laycock, 1945), coconut (Johnston, 1949), avocado (Ginsburg and Avizophar-Hershenson, 1965), Brazilian rubber tree (Wastie, 1965), litchi nut (Pandy and Misra, 1971), papaya (Ramirez *et al.*, 1975) and pejibaye palm (Janos, 1977).

1.4.1 **Tropical plant dependency on VAM**

Mycorrhizal dependency is defined as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of fertility (Gerdemann, 1975).

In the lowland tropics most forest trees form mycorrhizas of the vesicular-arbuscular type (VA) (Janos, 1980). The prevalence of VA mycorrhizas in tropical forest ecosystems suggest their importance in plant growth in soils of low or imbalanced nutrient availability.

Plant species, are categorised according to their dependencies on mycorrhizas as:

a) non mycotrophic
b) facultatively mycotrophic, or
c) obligately mycotrophic

Plant dependency on mycorrhizas reflects not only differences in plant mineral nutrient requirements (Baylis, 1971; Cooper, 1975) but also in root/shoot ratios, root distribution, geometry, morphology and the possession of roothairs (Bowen, 1980; Janos, 1980; Mosse and Hayman, 1980).

Some plant families such as *Commelinaceae, Cyperaceae, Juncaceae* among the monocotolydon; *Aizoaceae, Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Famuriaceae* among the dicotolydons, have species that are independent of mycorrhizas for mineral uptake (Gerdemann, 1968). Members of these families possess very highly branched root systems with abundant root hairs to effectively take up mineral nutrients from soils.

The mycotrophic plant species on the other hand, can be facultatively

mycotrophic or obligately mycotrophic; the former group containing plant species which can attain reproductive maturity without mycorrhizas in more fertile soils (Janos, 1980) or may form fewer mycorrhizas in fertile soils than in nutrient poor soils (Daft and Nicolson, 1966; Mosse, 1973; Cooper, 1975). Pioneer species like *Trema orientallis* and grasses (Baylis, 1975) belong to this group.

As seen from surveys of some tropical areas (section 1.4) most tropical forest trees possess all the characteristics of obligately mycotrophic species with roots that have low orders (3-4) and rates of branching, coarse ultimate rootlets lacking roothairs (the magnolioid root system), high lignin and tannin, low root turnover rates (Janos, 1977, 1980) and more importantly, the soil environments in which they grow are generally very poor in available nutrients (Sanchez, 1981).

In fact, the correlation of dependence on mycorrhizas with large diameter ultimate rootlets lacking roothairs is very strong for tropical plants. The correlation of independence with the presence of roothairs, however, was poor, like-wise root/shoot ratios were not related (Baylis, 1975; St John, 1980; Howeler *et al.*, 1982).

Because both tropical tree roots and soil characteristics have led to extreme dependence on mycorrhizas, damage/diminution of mycorrhizal populations or soil fertility would have considerable consequences for continued tree growth and ecosystem recovery.

1.4.2 **The role of VAM on plant growth and survival**

1.4.2.1 Nutritional benefits

Physiological studies of VAM/plant interactions have revealed that the plant benefits in many different ways, although the most widely reported is the acquisition of mineral nutrients (Tinker, 1978) principally through the interchange of carbon and phosphates between plant and fungal symbionts (Cox *et al.*, 1975; Harley and Smith, 1983).

Growth responses observed in VAM infected plants are ascribed generally to increased nutrient absorption although the primary effects are the

improvement of phosphorus supply to plants (Abbott and Robson, 1977; Menge *et al.*, 1978; Tinker, 1978).

Being an immobile element phosphorus (P) is frequently present in small amounts in soil solutions as phosphate ions: they rapidly become bound to soil colloids or are fixed as iron or aluminium phosphates (Tinker, 1975). This is particularly true for tropical soils which are predominately ultisols and oxisols, and are as a result inherently low in available phosphorus because of the high capacity of these soils to convert soluble phosphates to insoluble forms by combining with exchangeable aluminium, iron and aluminium and hydrous oxides (NAS, 1982; Sanchez, 1976). Direct (Rhodes and Gerdemann, 1985) and indirect (Sanders and Tinker, 1971) evidence shows that VAM hyphae absorb P and translocate it to host where maximum flux values of P near entry points into roots of 4×10^{-8} cm^{-2} s^{-1} have been recorded (Pearson and Tinker, 1975).

As a result of increased P uptake, plant growth in mycorrhizal plants is greatly enhanced as opposed to their non-mycorrhizal counterparts (Mosse, 1957; Pope, 1980; Gianinazzi-Pearson *et al.*, 1981). Several recent reviews (Gianinazzi-Pearson and Gianinazzi, 1983; Harley and Smith, 1983; Abbott and Robson, 1984) have highlighted the beneficial effects of VAM on plant growth. Kleinschmidt and Gerdemann (1972) working in citrus nurseries observed that mycorrhizal citrus plants were as much as 25 to 30 times larger than their non-mycorrhizal plants.

Saif and Khan (1977) recorded 3-4 fold increases in wheat and barley yields and noted that mycorrhizal infections were as effective as adding P-fertilizer.

La Rue *et al.*, (1975) observed a 79% increase in height of peach seedlings inoculated with *Glomus fasciculatum* in a sterilized zinc deficient nursery soil, and mycorrhizal inoculation was more effective in stimulating yield than combined Zn and P fertilizers applied together at 1.3 and 50 kg ha^{-1} respectively.

Menge *et al.*, (1978) observed decreased transplant shock in mycorrhizal rather than non-mycorrhizal avocado plants.

Owusu-Bennoah and Mosse (1970) in field conditions, obtained a 14-fold increase in onion dry weight on P-deficient soils inoculated with VA mycorrhizas.

Dramatic results like these are especially evident in severely disturbed substrates like mine spoils (Daft and Hacskaylo, 1977; Lambert and Cole, 1980; Khan, 1981) and show how much inoculation with VAM improves plant growth in disturbed ecosystems and therefore how essential they are to plant growth.

The uptake of several other elements is enhanced by inoculation with VAM fungi (Mosse, 1973; Gildon and Tinker, 1983). Zinc and copper deficiencies in plants have also been alleviated through plant inoculation with VAM fungi (Gilmore, 1971; La Rue *et al.*, 1975; Cooper and Tinker, 1978).

Increased uptake of ^{45}Zn by mycorrhizal *Araucaria* roots (Bowen and Theodorou, 1979) at a translocation rate of 2.1×10^{-12} mol $m^{-2}s^{-1}$) appeared adequate to eliminate zinc deficiency. Similarly Zn deficiency in peach (Gilmore, 1971) was less in mycorrhizal plants.

That notwithstanding, reduced or negative growth responses of plants have been observed following inoculation with VAM fungi (Harley and Smith, 1983; Cooper, 1984; Koide, 1985) which might be ascribed to competition between symbionts for limited supplies of carbohydrates (Furlan and Fortin, 1973; Smith, 1980).

Similarly, it is argued that not all growth effects following infection by VAM fungi are nutritional (Kormanik *et al.*, 1977; Schultz *et al.*, 1979; Verkade and Hamilton, 1983).

1.4.2.2 VAM water relations

Studies by Menge *et al.*, (1978); Nelsen and Safir, (1982); Sieverding, (1983); have established that mycorrhizal plants can cope with water

stressed conditions better than their non-mycorrhizal counterparts.

Effects of VAM on plant water status may be especially important for canopy trees and crops because of their potentially high transpiration rates and the low moisture availability in many tropical soils. VA mycorrhizas decrease the susceptibility to wilting and transplant shock in tropical trees (Menge *et al.*, 1978; Janos, 1980; Levy and Krikun, 1980) and have improved water utilization in *Eupatorium odoratum* a tropical shrub (Moawad, 1980; Sieverding, 1981, 1983) and tropical crops (Sieverding, 1984). Although VA mycorrhizas occur in both aquatic and desert habitats (Cooper, 1984) and appear capable of tolerating very high and very low moisture availability, infection of temperate zone plants is reduced very greatly by high or low water potentials (Reid and Bowen, 1979; Nelsen and Safir, 1982). Lower infection levels of a tropical tree in the field during the dry season in an arid zone (Diem *et al.*, 1981) and maximum infection of seedlings of an African Mahogany (*Khaya grandifolia*) at intermediate moisture levels are consistent with temperate zone findings (Redhead, 1975).

The mechanisms by which VAM fungi improve host water relations are uncertain. Improved water utilization has resulted most often as a secondary effect of improved phosphorus nutrition (Nelsen and Safir, 1982; Cooper, 1984; Graham and Syvertsen, 1984; Fitter, 1985, 1988; Koide, 1985; Anderson *et al.*, 1988). Also improved water use may reflect decreased resistance to water flow in roots, stems and leaves (Levy and Krikun, 1980; Nelsen and Safir, 1982). Drought resistance however cannot always be duplicated in the absence of mycorrhizas by added phosphorus (Levy and Krikun, 1980).

On the contrary, severe water stress has also been observed in mycorrhizal plants compared with non-mycorrhizal plants (Levy *et al.*, 1983) though there is evidence that such stressed mycorrhizal plants often recover more rapidly than their non-mycorrhizal counterparts (Sweatt and Davies, 1984; Ellis *et al.*, 1985).

1.4.2.3 Resistance to pathogens

Reduced susceptibility to, or increased tolerance of host plant roots to, certain soil borne fungi is frequently associated with both ectomycorrhizal and VAM infections (Dehne, 1982). The increased synthesis of secondary metabolites like lignin, ethylene and phenols (Dehne, 1982, 1986) as well as phytoalexins (Morandi, Bailey and Gianinazzi-Pearson, 1984; Morandi and Gianinazzi-Pearson, 1986) may all contribute to these 'protective' effects.

Bartschi *et al.*, (1981) have shown mixed populations of VAM to markedly reduce root rot development by *Phytophthora cinnamomi* in *Chamaecyparis lawsoniana*, while *Glomus mosseae* alone delayed the onset of the disease. Viral infections also seemed to be reduced by VAM infections (Bagyaraj, 1984), with the disease resistance of the root linked to the improved nutrient status of the mycorrhizal plant (Daft and Okusanya, 1973; Schonbeck and Dehne, 1979).

Although it is the thought that increased monoculture through reafforestation could lead to increased disease incidences, this might be overcome by ensuring that plants become adequately VA mycorrhizal.

1.4.2.4 Other benefits

In soils of low P status (typical of tropical soils) VA mycorrhizas strongly stimulate nodulation and hence the growth of legumes (Crush, 1974; Mosse, Powell and Hayman, 1976; Azcon-Aguilar *et al.*, 1979; Redent and Reeves, 1981).

According to Mosse *et al.*, (1976) legumes, even if inoculated with appropriate strains of *Rhizobium* in very P-deficient soils, only nodulate if mycorrhizal fungi are present.

Additionally, other studies have reported enhanced Nitrogen fixation following inoculation with mycorrhizal (Azcon-Aguilar *et al.*, 1979). In *Hedysarum boreale*, a legume native to the western United States, mycorrhizal fungi stimulated both growth and N_2-fixation (Azcon-Aquilar *et al.* 1979). In an unpublished account by McGraw, Jarstfer and

Miller quoted in Miller (1984), *Cassia fasciculatum*, a legume that can grow in a broad range of environments, but more characteristic of disturbed sites was found to grow vigorously and flower when inoculated with mycorrhizal fungi whereas when inoculated with *Rhizobia* alone or left uninoculated, became stunted. Dual inoculation with VAM and *Rhizobia* therefore can greatly enhance plant productivity and the sometimes obligate nature of this association (Rhizobia and VAM), as shown above, in legumes associated with disturbance cannot be ignored. Damage on the VAM flora would inevitably lead to a reduction in nodule formation and N_2-fixation consequently causing stunting in such plants.

VAM fungi similarly stimulate the production of phytohormones (Barea and Azcon-Aguilar, 1982), though evidence to this effect is scanty. Nevertheless, the production of gibberellin-like substances by *Glomus mosseae* has been reported (Barea and Azcon-Aguilar, 1982). Allen *et al.* (1980) also observed the levels of cytokinins in *Bouteloua gracilis* to increase substantially with infection by VAM fungi, while Dodd *et al.*, (1983) reported the increased production of pepper buds in pepper plants was ascribed to a joint hormonal and nutritional effect caused by infection with VAM fungi.

Studies by Nicolson (1959) Sutton and Sheppard (1976) Koske and Halvorson (1981) showed that VAM fungal hyphae improved the soil physical structure of a sand dune by binding the soil particles together into more stable aggregates. Through similar mechanisms, Went and Stark (1968) supposed that nutrients were held together in areas of high rainfall, and this was responsible for the closed nutrient cycling observed in tropical forests.

1.5 EFFECTS OF SOIL DISTURBANCE

Disturbance events on soils arise from the use of heavy machinery like bulldozers and destructive silvicultural systems like clear-felling in site preparation during plantation establishment. Although knowledge of the impact of soil disturbance upon mycorrhizal systems is still at its infancy (Read and Birch, 1988), studies carried out in some climatically and nutritionally stressed ecosystems like tropical rainforests (Janos, 1980) temperate deserts (Reeves *et al.*, 1979) and

alpine grasslands (Allen and Allen, 1984) support the view by Skujins and Allen (1986) that soil disturbance can lead to a reduction or even the complete elimination of VAM fungi from ecosystems. This it does by changing the quality, quantity and effectiveness of the indigenous mycorrhizal inoculum (Black and Tinker, 1979; Reeves *et al.*, 1979; Allen and Macmahon, 1985; Allen and Allen, 1986; Jasper, Robson and Abbott, 1987; Thompson, 1987; Miller, 1987; Evans and Miller, 1988; O'Hallaron *et al.*, 1986; Read and Birch, 1988 and Stahl, Williams and Christensen, 1988). As a result damage to an indigenous VAM flora may have profound effects on the composition of the associated plant community (Janos, 1980; Allen and Allen, 1984).

1.5.1 **Changes in plant succession**

According to Janos (1980) and Allen and Allen (1984) plant communities depend on the availability of mycorrhizal propagules (spores, hyphae) of appropriate types. If these are eliminated as a result of extreme soil disturbance, a successional sequel in which recolonisation of the disturbed area by mainly non-mycorrhizal plant species or those little infected by mycorrhizas such as members of *Cyperaceae* (Puga, 1985) or *Brassicaceae* (Yost and Fox, 1979) is achieved, rather than recolonisation by plant species strongly responsible to mycorrhizal infection (Janos, 1980).

Following extreme disturbance (eg Mount St Helens) sites may become colonised by only non-mycorrhizal plants, which slowly give way to ecto-mycorrhizal and lastly VA mycorrhizal plants. The endomycorrhizal plants take longest to recover from such disturbance events as they do not produce aerial dispersed spores (Allen and Allen, 1984).

If damage on a moist tropical forest is very severe, trees introduced to such areas with vastly reduced VAM inoculum may find it difficult to establish, and following disturbance the ecosystem is likely to take a long time to recover.

1.5.2 **Changes in the quality, quantity and effectiveness of mycorrhizal inoculum**

Following disturbance, the inoculum level at a particular site will be determined by the survival of residual inoculum and/or the immigration of inoculum from surrounding areas (Warner *et al.*, 1987). The quantity of the residual inoculum will probably be low, with a very diffuse spatial distribution (Allen and Macmahon, 1985) which could result in an inoculum with a lower potential for establishing new infections (Black and Tinker, 1979; Allen and Macmahon, 1985).

Immigration is also slow as VAM do not produce aerial spores, and are only moved by animals especially rodents.

A progressive decline in Inoculum Potential - the potential of a specific amount of inoculum to cause root infection under a standard set of conditions (Dimond and Horsfall, 1960) - has often been observed following disturbance.

Francis *et al.*, (1986) observed that in disturbed ecosystems, the VAM network of hyphae which form the functional interconnections between plants at intra and interspecific levels failed to initiate and spread mycorrhizal infections (Birch, 1986; Evans and Miller, 1988; Fairchild and Miller, 1988; Read and Birch, 1988).

In an unpublished account of work by Birch and Read quoted in Read and Birch (1988) roots from *Plantago lanceolata* plants growing on disturbed soils were observed to have very few infection units (of lengths < 5mm) as opposed to infections units of 5 mm - 13 mm when plants were growing in undisturbed soils.

Reeves *et al.*, (1979) noted that infection levels of roots of plant colonisers in disturbed soils were just 1-2% compared to infection levels of 77-99% observed in roots of colonisers in undisturbed soils.

Stahl *et al.*, (1988) working in field and glasshouse conditions, found that soil disturbance affected VA mycorrhizal fungi in the following ways:

- Under disturbed conditions native VAM produced low mycorrhizal infection (about 20% of total root length) and for two years following disturbance VAM had no significant effects on the establishment, growth and survival of sagebrush plants. Similarly, but in a more controlled (glasshouse) environment, mycorrhizal development after 45 days remained low in disturbed soils (10%) compared with 50% in undisturbed soils.
- Significant increases in biomass production (± 70%) and tissue P content (+ 70%) were noted in the undisturbed sagebrush grassland compared with plants growing in the disturbed soil.
- Inoculum densities differed greatly in the undisturbed soils (19) compared to those in disturbed soil (0.5).
- Five VAM species were present in undisturbed soils whereas only two were found in disturbed soils.

Results by O'Halloran *et al.*, (1986) showed a reduction in P-absorption in *Zea mays* seedlings which was linked to the inhibition of VAM development after disturbance (Evans and Miller, 1988; Fairchild and Miller, 1988). Jasper *et al.*, (1989) in two glasshouse experiments designed to study the effects of disturbance on VA mycorrhizas also observed that the fungi were almost eliminated and the infectivity of the external hyphae under disturbed conditions was reduced to 15% of total root length as opposed to 55% in undisturbed soils.

Changes in spore numbers and spore viability contribute to the reduced incidence of VAM observed in areas subjected to severe and extended disturbance (Reeves *et al.*, 1979; Miller, 1977).

In Bangladesh Khan *et al.*, (1988) always found higher spore numbers associated with incidences of abundant mycelia, which became greatly reduced when soils are disturbed.

Similarly, Ahmad (1989) reported significant changes in numbers of infective VAM propagules (spores and root fragments) by 30-50% following severe disturbance of a Malaysian forest during logging activities as a result of mechanical compaction, exposure and erosion of the soil.

1.5.3 **Temperature related effects on VAM**

Normally tropical soil temperatures closely approximate to air temperatures changing by less than 5°C. When cleared of forests however, topsoil temperatures may increase by 7 to 11°C (Sanchez, 1976). Such drastic changes in soil temperatures can affect the metabolic activity and survival of indigenous VA mycorrhizal fungi and the development of infection (Moawad, 1979, 1989; Parke *et al.*, 1983; Sieverding, 1983).

Therefore such changes may have important implications for reafforestation. VA mycorrhizas of several tropical crops have been shown to have their greatest effect on host growth and phosphorus uptake at 30°C soil temperatures (Moawad, 1979, 1980). An investigation by Sieverding (1983) of the combined effects of temperature and moisture similarly indicated that optimum mycorrhizal efficiency at 30°C was attributable to enhanced fungus activity.

In contrast, at soil temperatures above 29.5°C the infection by VA mycorrhizal fungi from Southwestern Oregon was greatly reduced or prevented (Parke *et al.*, 1983). Temperatures may affect infection through its direct effect on symbiont metabolism or indirectly by influencing leakage of root metabolites necessary for fungus activity (Graham *et al.*, 1982).

It is probable therefore by laying soils bare of vegetation (as with the clear-felling system) that soil temperatures become greatly increased above the limits usually experienced by indigenous VA mycorrhizas thus leading to a change in flora and an enhancement of those fungi which prefer much higher temperatures.

1.6 **SUMMARY OF EVENTS LEADING TO RESEARCH OBJECTIVES:**

a) Tropical forests are fast declining; being exploited extensively to meet demands on them made by increasing populations, increasing demands by developed countries for tropical hardwoods and increased domestic consumption.

b) There is a growing need for tropical countries like Cameroon which rely heavily on wood export to increase the productivity of its forests. This can be achieved by conserving the existing forests or reafforestating of exploited areas or both.

c) The success of reafforestation schemes such as plantation establishment depend to a large extent on what silvicultural systems are employed and to what extent the soils physical, chemical and microbiological properties are altered.

d) Of particular interest is what happens to the microbial changes of VA mycorrhizal fungi which most moist tropical indigenous hardwoods rely on in order to acquire sufficient mineral nutrients to grow in generally poor tropical soils.

e) Severe disturbance of soils is likely to lead to the complete elimination or decrease in quality, quantity and effectiveness of VA mycorrhizal inocula resulting in drastic consequences like reduced mineral uptake and stunted growth of the tree introduced to the site.

Thus, the ecology of VA mycorrhizal fungi is clearly linked to environmental, edaphic as well as host factors. Sequential observations of the short and longterm influences of site preparation would hopefully elucidate population dynamics of these fungi essential to plants growing in nutrient poor soils. For these reasons, the thesis objectives were developed.

THESIS OBJECTIVES

1. To develop methods to extract and separate VAM fungi from soils of a moist tropical forest ecosystem in Cameroon.

2. To characterize and identify the VAM fungi of a moist tropical forest, by examining soil samples collected from the Mbalmayo Forest Reserve in Cameroon.

3. To assess the temporal and spatial occurrences of VAM fungi at Mbalmayo with special reference to naturally occurring *Terminalia superba*.

4. To assess effects on the occurrence of VAM fungi of different methods of site preparation causing different amounts of disturbance.

5. To assess impact of replanting plots prepared in different ways with *Terminalia ivorensis* on occurrence of VAM.

6. To consider the link between VAM fungi surviving site preparation and the subsequent establishment and growth of planted seedlings of *Terminalia ivorensis*.

CHAPTER 2

ASSESSMENT AND DEVELOPMENT OF METHODS TO SURVEY VAM FUNGI IN A NATURAL TROPICAL FOREST

2.1 SURVEY OF VAM FUNGI - BY MEANS OF HYPHAE AND SPORES

The distribution and abundance of vesicular-arbuscular mycorrhizal fungi (VAMF) in soil can be estimated by (a) the intensity of hyphal colonisation of roots of infected plants, (b) number of VAM spores in soil around infected roots or (c) quantities of extramatrical hyphae extending from infected root to the surrounding soil (Schenck, 1982).

In most surveys, however, spores have been used as indicators of abundance of VAM populations (Mosse and Bowen, 1968b; Khan, 1971; Johnson, 1977; Walker *et al.*, 1982) because they are relatively easy to count and more importantly they are the only stage in the life history of a VAM fungus which can be used to characterize and thus establish the population dynamics of each VAM fungal component of the ecosystem under study.

Although desirable, there are at present insufficient diagnostic characters to identify VAM fungi from their intraradical or extra matrical hyphae. As a result, in field conditions where more than one VAM fungus may colonise plant roots, identification of the different fungi becomes almost impossible.

Most of the methods developed using hyphae (Becker and Gerdemann, 1977; Hepper, 1977; Giovannetti and Mosse, 1980; Ames *et al.*, 1982; Arias *et al.*, 1987) have therefore only assessed the presence or absence of infection or the proportion of root colonised by internal hyphae without establishing which of the VAM fungi was responsible.

Morton (1988) attempted to identify these hyphae using stains but realised that some VAM fungi formed hyphae which did not stain readily, making identification difficult.

Extramatrical hyphae are also not easily quantified, because they can

form thick and thin walled elements differing in diameters (Nicolson, 1959). For example, VAM fungi such as *Acaulospora* spp. form thick-walled hyphae as opposed to the thin-walled hyphae formed by the 'fine endophyte' (Nicolson, 1959).

Even by using the membrane filter technique to directly measure the quantities of external VAM fungal hyphae in soil, Abbott *et al.* (1984) have encountered numerous difficulties when trying to distinguish VAM hyphae from those of other fungi present in soils. Kough and Linderman (1986) addressed this problem using immunofluorescence assays, but found that they were more successful in mineral soil than in peat/sand mixtures where background fluorescence of the latter, prevented accurate measurements of the extramatrical hyphae.

Spores of VAM fungi in contrast to their hyphae are more readily quantifiable. With the development of a range of spore extraction methods (Jenkins 1964; Furlan *et al.*, 1980; Porter 1982; Verkade, 1988) the clarity of spore suspensions has been improved so facilitating counting.

With spores being the only structures presently used in identification, the methods for identifying VAM fungi using them have been extensively developed and reviewed (Walker, 1983, 1986; Berch and Koske, 1986; Morton, 1988; Schenck and Perez, 1987). It is recognised however that there are several drawbacks associated with the reliance upon spores both for the identification and quantification of VAM fungi. Legitimate arguments by Read *et al.*, (1976), Sparling and Tinker, (1978) hold that not all VAM fungal species sporulate (e.g. *Glomus tenue* the fine endophyte) and thus cannot be quantified from structures they do not form. Also the spore characteristics used in identification are often very variable (Morton, 1988).

That notwithstanding, assessment of the distribution and abundance of VAM fungi via spores still provides the best available option. Available techniques have provided invaluable information on the spatial and temporal distribution and population dynamics of VAM fungi as influenced by seasons, associated vegetation, soil depth and soil physical and chemical characteristics (Walker, 1979; Koske and

Halvorson, 1981; Sylvia, 1988). Reasons such as those mentioned above, resulted in the decision to study the population dynamics of the Mbalmayo Forest via spore assessment.

However, before being able to do so, there was a need for careful consideration of the sampling techniques, extraction and assessment methods of spores especially as several surveys even of the moist tropics (Redhead, 1968) have recorded a high degree of heterogeneity.

2.2 SAMPLING VAM SPORES

Few systematic comparative studies have been made testing the validity of sampling methods (Tews and Koske, 1986), although the aggregated spatial distribution of spores and mycorrhizas of VAM fungi in soils has now been widely accepted (Porter, 1982; St John and Hunt, 1983).

To minimise the effects of heterogeneity within plots and soil samples St John and Koske (1988) suggested that large sample sizes be taken, though they realised this often met practical limits short of the ideal.

Large sample areas were also proposed by Anderson *et al.*, (1983) to provide meaningful correlations between spore counts, plant cover and environmental data for the area. By laying out up to seven plots (20m x 20m) spread over an area of 550 m^2, (Anderson *et al.*, (1983) found that the variance of spore counts was found positively correlated to the size of the sample area.

An alternative approach aimed at reducing the large numbers of samples suggested by St John and Koske (1988) is the bulking of composite samples followed by subsampling in order that the values obtained from each subsample are close to the mean of the individual core comprising the sample. Subsampling increases the precision of estimates of spore numbers (Reich and Barnard, 1984).

By bulking samples however (Southwood, 1966; Green, 1979) some statistical information used in describing spatial distribution of propagules is lost. However, St John and Koske (1988) argue that the

principle underlying bulking is related to that of the Central Limit Theorem (Snedecor and Cochran, 1969) which states that 'the distribution of a series of means, tends towards a normal distribution regardless of the underlying original data'. Combining cores therefore was thought to reduce the skewness arising from the aggregated distribution of ecological units such as spores.

Other considerations that need be taken into account when sampling VAM spores include soil depth, seasons and associated vegetation.

Most VAM spores are concentrated in the top horizons (20 cm) of the soil profile (Sutton and Barron, 1972; Redhead, 1977) where fine roots are abundant. Redhead (1977) observed an exponential decline in spore numbers and percentage infection with depth.

The relative abundance of each plant species is also reported to show a positive correlation with spore numbers (Anderson *et al.*, 1984). Similar observations in a cold desert shrubland, showed mycorrhizal infectivity was positively correlated with the numbers of spores present and hence the degree of mycorrhizal plant cover (Miller, 1987). On the contrary however, in a survey of soil from the rhizosphere of little bluestem (*Schizachyrium scoparium* (Michx.)), the abundance of VAM fungal spores was least at sites with the greatest proportion of little bluestem and greatest at sites with the lowest proportion of little bluestem (Dickman *et al.*, 1984).

Abiotic environmental conditions including season also affect VAM propagules (Hall, 1977; Gould and Liberta, 1981) and should be taken into account when monitoring VAM populations. Seasonal changes on VAM fungal populations have been widely reported (Hayman, 1970; Koske and Halvorson, 1981; Walker *et al.*, 1982; Giovannetti, 1985). In a study of VAM fungi associated with perennial grasses on sand dunes, seasonal variations in mycorrhiza formation was closely correlated with seasonal variation in spore numbers (Giovannetti, 1985). The percentage root length colonised and spore numbers of a population dominated by two *Gigaspora spp* were maximal when their host plants were flowering. Gemma and Koske (1988) observed similar variations in spore numbers due to seasonal effects on spore germination. In assessing VAM population

dynamics, therefore, it is important that the number of sampling occasions cut through several stages in the life cycle of the botanical composition of the site (e.g. period of active root and shoot growth and periods of flowering and seed production) all of which may give comparable variations in mycorrhiza formation (Daniels-Hetrick and Bloom, 1983; Jacobsen and Nelson, 1983). For spores associated with annual plants, the commonest picture is a decline in numbers during the growing season and an increase in numbers at the end of the growing season (Hayman, 1970; Smith, 1980). It was therefore of interest to see if a similar or different pattern of spore dynamics occurred in the moist semi-deciduous tropical forest at Mbalmayo dominated by perennial plants.

It is also clear that the accuracy of methods used to quantify VAM fungi, as spores, depends on the intensity of sampling within this study area.

2.3 EXTRACTION OF VAM SPORES

The earliest method developed to recover VAM spores was the Wet sieving and decanting method of Gerdemann and Nicolson (1963). With this method, a known volume of soil is mixed with a known volume of water and poured through sieves of different mesh sizes ranging from 710 µm - 45 µm. The different sized sieves are used to collect spores of corresponding sizes.

With this method, however, spore numbers could easily be underestimated as spores are often obscured by soil debris which make spores enumeration extremely tedious and time consuming. The advantage of this method, on the other hand, is that it causes no damage to the spores and their attachments and it is therefore desirable for setting up starter pot cultures. Also, large volumes of soils can be processed in contrast to the plate method of Smith and Skipper (1979) which involves mixing small quantities of soils with tapwater and viewing the mixture directly a the dissecting microscope. This method, though fast, is only accurate where spore numbers exceed 20 spores per gram soil (Daniels and Skipper, 1982). It, like the wet

sieving and decanting method, has the disadvantage of being very tedious and time consuming, resulting in possible underestimation of counts because of the amount of soil debris associated with it.

The adhesion flotation method (Sutton and Barron, 1972), as with the plate method, requires that soils are mixed with tapwater. The mixture however is poured through a separatory funnel where the spores adhere to the sides. As with the preceding technique, only small amounts of soils are sampled at a time. In addition spores that sink rapidly in water are lost, leading to a possible underestimation of spore populations.

Following wet sieving and decanting, Mosse and Jones (1968) employed the differential sedimentation method using gelatin columns in which spores settled differentially, depending on their sizes and density. This method as well as being time consuming is only suitable for processing small quantities of soil while loss of spores is an inherent disadvantage leading to the underestimation of spore populations.

In contrast to wet sieving and decanting, a much more efficient method of extracting spores was developed by Furlan *et al.* (1980). They developed the density gradient centrifugation method to further purify the spores. Using sucrose gradients suggested by Ohms (1957), Ross and Harper (1970) and Mertz *et al.*, (1979), a layer of 50% sucrose was placed in a 50 ml. centrifuge tube. Another layer of 25% sucrose was placed upon on the 50% layer. A suspension of sievings was then gently and carefully added to the centrifuge tube and spun in a centrifuge at 3100 rpm (approx 1100 g) for 5 minutes. Using a syringe the middle layer containing the spores was removed into a fine sieve and washed with tapwater. This method was more effective than the previous methods and though time consuming gave clear spore suspensions making counts relatively easy and at the same time reduce the chances of underestimation. However, it was noted that with this method the sucrose exerted osmotic shock on the spores: they were damaged especially when left in sucrose solution for long periods.

Later however Furlan *et al.*, (1980) suggested the use of radiopaque

giving fairly clear spore suspensions for easy counting. The sucrose centrifugation method however had an edge over the density gradient centrifugation in that the single gradient it employed made it less time consuming. Based on these considerations, the sucrose centrifugation method was adopted for use in this survey.

2.4 ASSESSMENT OF VAM SPORES

Various methods have been developed for counting VAM spores some of which count whole populations (Walker, 1979; Perez, 1987) while others count only a portion of the total number of spores (Daniels and Skipper, 1982; McKenny and Lindsey, 1987).

Walker (1979) used a round petri dish (5.5 cm diameter) scored into squares to fit the field of view of a dissecting microscope. The petri dish is systematically scanned and the spores are counted using incident or transmitted light for illumination (Mosse and Bowen 1968b). For spores within the water suspension less than 100 µm radius, 50 were removed and mounted in lactophenol for closer examination and the proportions of different kinds used to estimate total numbers (of the different kinds). This method is very good for quantifying spores in large samples, however it is also more time consuming than the method of Perez (1987) in which a petri dish of similar dimensions (5 cm diameter) is scored with parallel lines narrower than the field of view of a dissecting microscope. The entire spore population in water is counted by going up and down the parallel lines to the end of the dish.

With an eelworm counting slide (Daniels and Skipper, 1982) only 1 ml of the extracted spores can be assessed. To obtain an estimate of the entire spore population the number of spores in the 1 ml. spore suspension is determined and that number is multiplied by the remaining volume of spore suspension. This method has the tendency of overlooking spore types which are present in very small numbers. Although simple, some spores may also be missed as the larger spores settle faster than others during sampling. The method by McKenny and Lindsey (1987) is similar to that of the eelworm counting slide where just a proportion of the entire spore population is sampled and counted. Twenty ml of spore suspension is placed on 0.45 µm membrane

filters marked with square grids and pulled under a vacuum to give even spread. The filters are then examined under the microscope and counts made. With this method, like the preceding one, the most numerous spore types are likely to be over represented at the expense of the less numerous types, resulting in misleading counts.

Of all the methods, that by Walker (1979) and Perez (1987) provided the best options, enabling one to sample the entire population. However because the method by Perez (1987) eliminated any need for subsampling which as stated above could be selective and misrepresent the VAM spore composition, it was thought to be appropriate for the present study.

2.5 MATERIALS AND METHODS

2.5.1 Site description

2.5.1.1 Location

The site, a natural secondary moist evergreen forest is close to the road to Ebogo in the Mbalmayo Forest Reserve. Mbalmayo is one of the three sub-divisions (Nyong et Soo) which makes the Centre Administrative Province of the Republic of Cameroon. Located at 3°31'N, 11°30'E and an elevation of 640m, Mbalmayo is the economic centre of the province, playing an important role in forest exploitation and regeneration.

2.5.1.2 Rainfall

Two seasons, dry and rainy, characterize the area. The rainy season is bimodal with the first rains extending from March to June with a peak in May while the second rains span mid August to November with a peak in October (Tchienkoua, 1987) (Figure 2.1). The dry season spans from November to March with the driest month being February. Yearly rainfall ranges from 1990 mm to 1017 mm with an average of 1522mm (Tchienkoua, 1987).

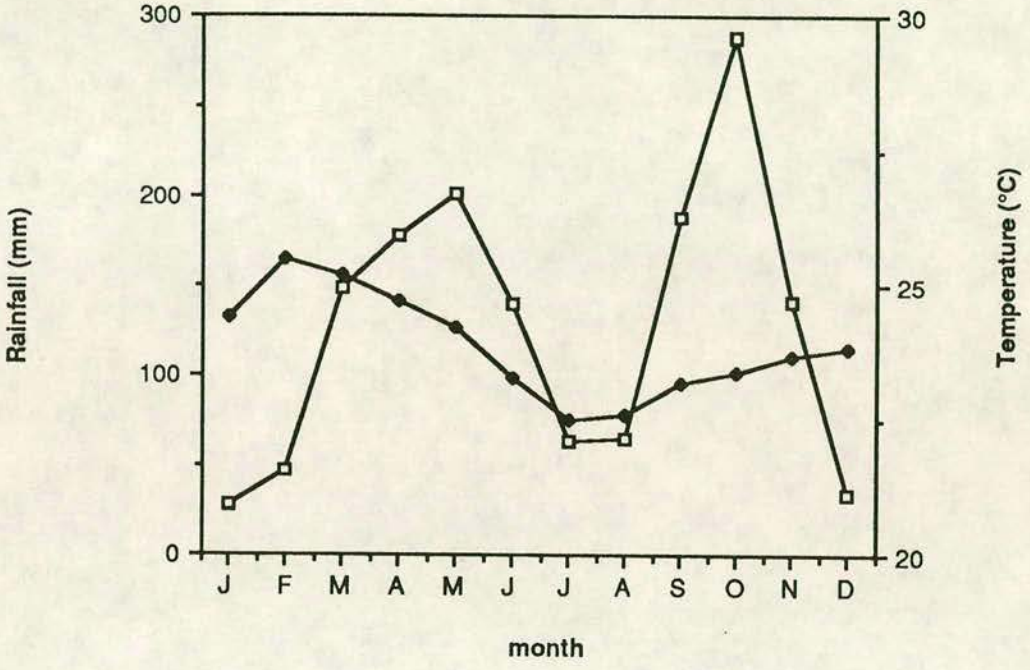


Figure 2.1
Mean monthly rainfall (mm) and temperatures (°C) distribution in the Mbalmayo area.
(Tchienkoua, 1987).

2.5.1.3 Temperatures

Because temperatures, relative humidity, insolation, have not been consistently and continuously recorded for the Mbalmayo area, Tchienkoua (1987) used temperatures recorded in Yaoundé (about 45km away from Mbalamyo). Daily mean temperatures range from 22.5°C in August to 25.5°C in February (Figure 2.1).

2.5.1.4 Relative humidity

The relative humidity values average 54% to 73%, with midday values approaching 100%, but much less at 50% in the mornings (Tchienkoua, 1987). Seasonal variations in relative humidity show negative correlation with insolation and temperatures.

2.5.1.5 Insolation

Two distinct insolation periods are recognised. The first one from mid-October to May with monthly average values greater than 145 hours (see Table 2.1). The second covers the remaining part of the year with values less than 145 hours per month.

2.5.1.6 Soils

The predominant soil types are ultisols and oxisols. They are dark brown to dark yellow with a sandy texture that becomes increasingly clayey with depth (36.9% at 0 cm to 55.5 at 150 cm). The soils are strongly weathered (clay content > 50%, low ratio of silt clay fractions, low base saturation and low cation exchange capacity). The pH range of 4.4 to 4.9 indicates they are highly acidic (Lawson et al., 1990).

2.5.1.7 Drainage

This is entirely to the River Nyong and its main tributory, the Mefou, originating from the high Inselberg region, west of Yaoundé (Tchienkoua, 1987). Other main north/south flowing tributaries are the Negese, Kongolo, Kilitsam and Messoro. The main south-north

Table 2.1 Relative humidity and sunshine hours of the Mbalmayo area

PARAMETER	MONTHS											
	J	F	M	A	M	J	J	A	S	O	N	D
Relative humidity (%)	76.2	73.7	77.9	79.9	81.4	82.6	83.5	83.8	82.5	82.0	79.2	77.6
Sunshine (hours)	195.0	179.4	174.5	160.5	164.1	124.1	89.8	72.9	103.3	146.4	161.9	196.9

* All values are means of daily readings taken over a month
(After Tchienkoua, 1987)

flowing tributory is the Mbeme. The streams are permanent due to the good rainfall distribution during the year. Heavy rains of long duration may lead to flooding in some major river valleys such as Mefou, Ezala, Negbe.

2.5.1.8 Vegetation

The vegetation is semi-deciduous, moist evergreen characterized by an abundance of plants from the Sterculiaceae and Ulmaceae families (Letouzey 1968), with economically important species such as *Triplochiton scleroxylon*, *Mansonia altissima*, *Nesorgodonia papaverifera*, *Cola cordifolia*, *Celtis zenkeri*, *Terminalia superba*, *Etrandrophragma cylindricum*, *Chlorophora excelsa* etc.

An inventory of plant species was effected during the course of the study by Dr Caroline Sargent and Mr P Mezilier (see Table 2.2).

2.5.2 **Plant material**

Terminalia ivorensis was chosen as the plant material for the plots because it is the commonest forest tree planted in Cameroon and in addition, it was supposed it would utilize the same mycorrhizas as the naturally occurring *T. superba* in the Mbalmayo forest. Seeds of *Terminalia ivorensis* collected from Kumba in the south-west province of Cameroon, where the species occurs naturally, were used to raise seedlings in the nursery at the National Office for Forest Regeneration in Mbalmayo. The seeds were soaked in water for one week to break the hard seed coat and hasten germination, then they were potted in 4½ inch transpots filled with unsterile nursery soil for six months. At the time of planting the seedlings were about 40 cm tall.

2.5.3 **Selection of research plots**

The site for the research plots in the Mbalmayo Forest Reserve was chosen in January 1987, with the assistance of the National Office for Forest Regeneration in Cameroon (ONAREF) providing the required labour to help demarcate plots and plant out. The choice of site was based on easy accessibility as well as the exclusion of all farmed areas.

Table 2.2 Numbers (ha^{-1}) and species of trees in a secondary moist tropical forest in the Mbalmayo Forest Reserve, Cameroon (Aug 1987) also records of the occurrence of mycorrhizas lodged in the international literature. Data are given for three plots enumerated **after site clearance**

Family	Genera and species	Number of trees ha^{-1} Mechanically	Manually	Control	Average	Mycorrhizal assocations	Source of record
ANACARDIACEAE	*Lanaea welwitschii*	1	1	2	1.3	endo	Hogberg, 1982
ANNONACEAE	*Cleitopholis patens*	1	-	1	0.6	arbuscular	Redhead, 1960
	Enantia chlorantha	-	2	-	0.6		
	Hexalobus crispiflorus	-	1	-	0.3		
	Polyalthia suaveotens	-	1	-	0.3		
	Xylopia sp.	4	-	6	3.3	endo	De Alwis and Abeynayke, 1980
APOCYNACEAE	*Alstonia boonei*	7	4	5	5.3	arbuscular	
	Funtumia elastica	1	-	1	0.6		
BOMBACEAE	*Bombax brevicuspe*	-	2	-	0.6		
	Ceiba pentandra	2	-	1	1.0	arbuscular	Readhead, 1960
BORAGINACEAE	*Cordia aurantiaca*	1	-	-	0.3		
	C. platythyrsa	-	1	1	0.6		
BURSERACEAE	*Canarium schweinfurthii*	1	2	-	1.0	endo	De Alwis and Abeynayake, 1980
	Santiria trimera	-	1	-	0.3		
CESALPINACEAE	*Afzelia bipindensis*	1	-	-	0.3		
	Amphimas pterocarpoides	-	1	-	0.3		
	Dialium macrocarpum	-	1	-	0.3		
	Distemonanthus benthamianus	4	-	8	4.0		
	Erythrophleum manii	-	-	3	1.0		
	Guibortia tessmanii	-	-	1	1.0		
	Hylodendron gabonense	-	-	2	0.6	arbuscular	Redhead, 1960
COMBRETACEAE	*Pteleopsis hylodendron*	-	-	1	0.3		
	Terminalia superba	9	3	4	5.3	arbuscular	Redhead, 1960
EBENACEAE	*Diospyros bipendendense*	-	-	1	0.3	arbuscular	Redhead, 1960
ERYTHROXYLACEAE	*Erythroxylum manii*	-	-	3	1.0		

Family	Genera and species	Number of trees ha^{-1}				Mycorrhizal associations	Source of record
		Mechanical	Manual	Control	Average		
EUPHORBIACEAE	*Discoglypremna caloneura*	-	-	4	1.3	arbuscular	Redhead, 1960
	Keayodendron sp.	2	-	-	0.6		
	Macaranga cecropioides	-	1	-	0.3		
	Macaranga sp	-	1	2	1.0		
	Margaritaria discoidea	-	-	2	0.6		
	Uapaca sp.	1	5	1	2.3	endo and ecto	Hogberg, 1982
	Ricinodendron heudelotii	4	3	8	5.0	arbuscular	Redhead, 1960
FLACOURTIACEAE	*Scotellia coriaceae*	-	1	-	0.3		
GUTTIFERAE	*Allanblackia glaucenscens*	4	3	2	3.0		
	A. gabonensis	-	-	-	0.3		
LAURACEAE	*Beilschmeida sp.*	1	-	-	0.3		
LECYTHIDACEAE	*Petersianthus macrocarpus*	1	3	-	1.3		
LOGANIACEAE	*Anthocliesta macrophylla*	-	-	1	0.3		
MELIACEAE	*Carapa procera*	1	2	2	1.6	arbuscular	Redhead, 1960
	Entandrophragma candollei	1	-	-	0.3		
	E. cylindricum	1	-	1	0.6		
	Guarea cedrata	2	-	2	1.3	arbuscular	Redhead, 1960
	Lovoa trichilioides	1	1	-	0.6	arbuscular	Redhead, 1960
	Trichilia tessmanii	-	-	5	1.6		
	T. zenkeri	-	1	-	0.3		
MIMOSACEAE	*Albizia adianthifolia*	1	-	6	2.3	arbuscular	Redhead, 1960
	A. zygia	1	1	1	1.0	arbuscular	Redhead, 1960
	Capocalyx denclagii	-	1	-	0.3		
	Parkia bicolor	1	-	-	0.3		
	Pentaclethra macrophylla	-	-	1	0.3	arbuscular	Redhead, 1960
	Piptadeniastrum africanum	1	2	4	2.3		
	Tetrapleura tetraptera	-	1	1	0.6		
MORACEAE	*Bosqueia angolensis*	2	1	1	1.3	arbuscular	Redhead, 1960
	Chlorophora excelsa	-	1	1	0.6		
	Ficus mucoso	1	1	1	1.0		
	Musanga cecropioides	2	1	4	2.3	arbuscular	Redhead, 1960
	Myrianthus arboreus	1	-	2	1.0	arbuscular	Redhead, 1960

Table 2.2 continued

Family	Genera and species	No. of trees ha^{-1} Mechanical	Manual	Control	Average	Mycorrhizal associations	Source of record
MYRISTICACEAE	*Coelocaryon preussii*	1	-	-	0.3		
	Pycnanthus angonensis	1	5	8	4.6		
	Staudia kamerunensis	1	3	1	1.6		
OLACACEAE	*Coula edulis*	5	1	1	2.3		
	Ongokea gore	-	1	-	0.3		
	Strombosia grandifolia	-	-	1	0.3		
OCHNACEAE	*Lophira alata*	2	3	3	2.6	arbuscular	Redhead, 1960
PANDACEAE	*Panda oleosa*	1	-	-	0.3		
PAPILIONACEAE	*Angylocalyx zenkeria*	-	-	1	0.3		
	Erythrina excelsa	1	-	-	0.3		
	Milletia excelsa	1	-	2	1.0		
	Milletia sp	-	-	1	0.3		
	Pterocarpus soyauxii	3	-	3	2.0		
RHAMNACEAE	*Maesopsis eminii*	-	1	-	0.3		
RUBIACEAE	*Canthium palma*	4	2	1	2.3	endo	De Alwis and Abeynayto, 1980
	Nauclea diderrichii	2	1	3	2.0	arbuscular	Redhead, 1960
	Pausinystalia macroceras	-	-	1	0.3		
RUTACEAE	*Fagara macrophylla*	-	-	2	0.6	arbuscular	Redhead, 1960
	F. tessmanii	2	-	2	1.0	arbuscular	Redhead, 1960
SAMYDACEAE	*Homalium species*	3	1	1	1.3		
SAPINDACEAE	*Blighia sapida*	-	-	1	0.3		
SAPOTACEAE	*Gambeya africana*	1	-	1	0.6		
STERCULIACEAE	*Cola lateritia*	-	-	3	1.0		
	Eribroma oblongum	9	4	3	5.3		
	Pterigota bequaertii	-	-	2	0.6		
	Sterculia rhinopetala	3	-	-	1.0		
	Sterculia tragacantha	2	2	1	1.6		
	Triplochiton scleroxylon	1	1	7	3.0		

Table 2.3 continued

Family	Genera and species	No of trees ha-1 Mechanical	Manual	Control	Average	Mycorrhizal associations	Source of record
ULMACEAE	*Celtis adolfi - friderici*	-	-	2	0.6		
	C. tessmanii	-	4	1	1.6		
	C. zenkeri	-	-	1	0.3		
	Holeptera grandis	-	-	1	0.3		
VERBENACEAE	*Vitex grandifolia*	2	1	-	1.0	arbuscular	Redhead, 1960

Table 2.2 continued

Advice on plot size and statistical design was obtained from Mr R.I. Smith a statistician in the Institute of Terrestrial Ecology, Bush Estate, near Penicuik in Scotland, who suggested a plot size of 100m x 100m, large enough to minimise possible spore heterogenity. This plot size also took into account the views of Anderson *et al.*, (1983), who indicated that large sample areas were necessary in order to provide meaningful correlations between spore counts, plant cover and environmental data. This area (100m x 100 m) held, on average, five naturally established *T. superba* trees from which transects were based as part of the sampling design, because they were closest to *T. ivorensis* and therefore expected to possess close VAM flora. In addition it was the most common tree species at Mbalmayo.

The plots were demarcated, with a 10 m wide strip of natural forest left around each plot in order to separate treatments and avoid edge effects.

2.5.4 **Preparation of research plots**

Four treatments were tested. For logistic reasons and following advice from Mr R.I. Smith it was decided to establish, and sample, only one plot per treatment per year, with replicate plots to be established and sampled in succeeding years. My observations are therefore restricted to the four plots established in January 1987.

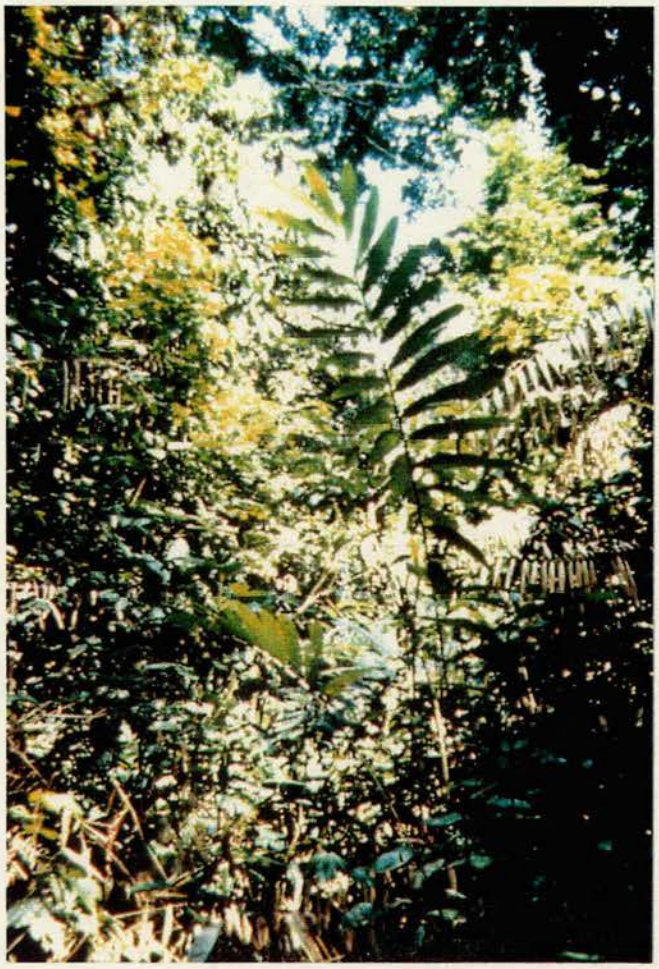
Thus, the effects on the abundance of VAM spores and infection of planted *T. ivorensis* trees into plots prepared in three different ways, ie by manual and mechanical 'recru' and complete clearance as commonly used in Cameroon, (Section 1), on the changes in VAM population dynamics were compared against events in an unchanged control area of secondary natural forest.

2.5.4.1 The Control (Natural Forest)

The control plot (100 m x 100 m) of natural forest was left unchanged for the entire duration of the study in order to provide a baseline against which to compare changes occurring in the plots prepared by the 'recru' and total clearance methods (Plate 2.1).

Plate 2.1

Above: Undisturbed
Control plot



Below: Manual
'recru' plot

2.5.4.2 Manual 'recru'

Using machetes and power saws/slashers, labourers provided by the National Office for Forest Regeneration in Cameroon, began preparing the plots in March 1987. Trees of 15-20 cm diameter at breast height (dbh) and shrubs were cut down to knee level. This diameter range of trees was chosen to open up the forest and to provide sufficient light for the young *T. ivorensis* trees to be introduced into the plot while minimising the changes to the forest microclimate. Also the tedious nature of the operation was in part responsible for the restricted size of trees removed (Catinot, 1965). Trees of greater diameters however, were subsequently debarked at breast height and poisoned with Arsenite powder. The resulting slash was left on the forest floor to slowly decompose and release nutrients important for the growth of the newly planted trees (Catinot, 1965) (Plate 2.1).

2.5.4.3 Mechanical 'recru'

This method is similar to the manual 'recru' but employed the use of a bulldozer to cut down unwanted vegetation as opposed to using hand operated tools, like machetes and power saws. The bulldozer, a DC8 straightrake dozer weighing 23.51 tons was driven in lines along the plot with the blade held at 30 cm above ground, removing trees and shrubs of 15-20 cm dbh. As in the manual 'recru' plot the slash was left to decompose and provide nutrients to the introduced tree species. With this method, more light was let into the plot (Leakey, pers. comm.) resulting from the wider tracks created by the bulldozer when cutting down trees and shrubs. The use of heavy machinery can however have adverse effects on the soil structure (Plate 2.2).

2.5.4.4 Complete clearance (clearfelling)

All vegetation was removed using a DC8 straightrake dozer weighing 23.51 tons. Every tree, irrespective of its size, was felled and unlike in the two preceding 'recru' methods, all slash was pushed to the side of the plot leaving the forest floor completely scalped. This method, because it exposes the forest floor results in extreme disturbance of the soil structure (section 1.3.1 to 1.3.3) and can lead

Plate 2.2

Above: Mechanical 'recru' plot

Below: Complete clearance plot

to the rapid invasion of the area by noxious weeds such as *Eupatorium odoratum* (Plate 2.2).

2.5.5 Planting

Three of the four plots (Manual and Mechanical 'recru' plots and the Complete Clearance plot), were planted with seedlings of *T. ivorensis* in September 1987. Prior to planting, the plots were pegged at spacings of 5 x 5m. The profuse branching habit of *T. ivorensis* led Catinot (1965) to recommend this close spacing (5 m x 5 m) in order to encourage self pruning and the formation of knot-free boles. Holes were dug at the variously pegged points to depths of 30cm. The black polythene bags in which the seedlings had been raised using unsterilized soils were removed leaving soil balls around the roots intact. Each seedling, with its soil ball, was lowered into a hole, after which soil dug from each hole was replaced. Soils were 'firmed' to ensure that there were no holes where water could accumulate and encourage root rot.

2.5.6 Maintenance

The three treated plots (excluding the control) were weeded every three months, to reduce competition particularly for nutrients between the herb layer and the planted *T. ivorensis*. No 'beating up' was done but the survival of the seedlings was recorded in each plot at regular intervals over the entire period of the study.

2.5.7 Collection of soil samples

2.5.7.1 Sampling occasions

Soils samples were collected on three occasions; the first being taken in February 1987, just before the plots were prepared by the National Office for Forest Regeneration (ONAREF) for planting later in the year when the rains had begun. Because the National Office for Forest Regeneration (ONAREF) provided the labour to assist in this study, sampling times had to be adjusted to suit their schedule of operations. This first set of soil samples, collected prior to the commencement of

treatments, was intended to provide a baseline from which inherent differences in VAM fungal populations, between the plots could be established.

The second set of soil samples was collected in August 1987 after the plots had been prepared but prior to planting *T. ivorensis* in September 1987. This occasion was chosen to test the effects of treatments on VAM fungal populations and incidentally, it was also the peak of the rains, thus introducing another important factor, seasonality.

The third set of soil samples was collected in August 1988, that is, like the second set, in the rainy season but unlike the former, one year after planting young *T. ivorensis* trees.

This was to test seasonality as well as monitor the effects of site treatments including planting.

2.5.7.2 Method of sample collection

Following advice from Mr R I Smith, (pers. comm.), six N/S transects were marked in each plot (Sylvia, 1988). Three of the six transects were based on naturally occurring *T. superba* trees so as to assess the effects (if any) of these trees on the occurrence of VAM fungi while the other three transects were aligned to avoid trees, particularly those of *T. superba*.

Along each transect, 5 sampling points were established, 2.5m to 10m from a marked *T. superba* tree (or stake where there were no trees). Previous work by Mason *et al.*, (1983) showed significant effects of distance on the distribution of different types of ectomycorrhizas. It was therefore desirable to see if similar trends could be found among types of vesicular-arbuscular mycorrizas. In the event, four of five collections or sampling points were to the north of the designated tree or stake at 2.5m, 5m, 7.5m and 10m, while one was 2.5 m to the south. The latter was included (2.5m south) to examine homogeneity/variability by comparing spore numbers from this sample with those 2.5m to the north.

A litre of soil was collected to a depth of 20 cm at each sampling point using a soil auger similar to an Edelman EL510-020 type, supplied by the Institute for Agronomic Research (IRA) Nkolbisson, Cameroon. The augerhead was constructed of two heavy duty metal steel plates which formed an open tube partly interlocked at the cutting edge and attached to a T-piece rod similar to that in EL510-020 type (Fig. 2.2) suitable for boring holes in fine cohesive soils, like those present at the research plots in Mbalmayo. The auger was used twice at each sampling point with two holes formed alongside one another, in order to obtain a litre of soil sample at the same depth of 20cm.

Thirty soil samples were collected from each plot (i.e. six transects each with five sampling points). On each occasion, they were put into separate labelled plastic bags and stored in a cool, air-conditioned room.

In August 1987, the second set of soil samples was collected from the same six N/S transects per plot. As in the first set of samples, 120 were collected into properly labelled plastic bags and stored in an air conditioned room. In the completely cleared plot where all trees had been removed sampling was effected in the same manner but with stakes placed to simulate *T. superba* trees.

In August 1988, when the third set of soil samples was collected, and following advice from Mr R.I. Smith, the sampling design was altered to cut down on the number of samples, a practical necessity. The sampling transects set up in February 1987, now cut through lines of one year old *T. ivorensis* trees planted at 5 m x 5 m spacings as well as the naturally established *T. superba* trees in the manual and mechanical recru plots, whereas in the completely cleared plot the transects cut through only *T. ivorensis* trees.

With *T. ivorensis* trees now planted 5 m x 5 m grid in the manual, mechanical and completely cleared plots it was not possible to have transects avoiding trees. The control plot was sampled in the same manner as in February and August 1987 whereas in the manually and mechanically cleared plots, samples were taken at 0m, 2.5m and 5.0m from *T. superba* trees. Those at 2.5m and 5.0m were taken from the

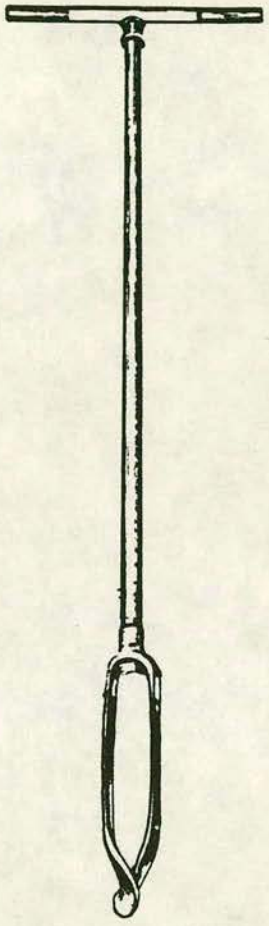


Fig. 2.2 Soil auger Edelman 520 type

north of the tree and kept separate while at 0m the four samples (N.S.E. and W) were pooled. The same sampling technique in the completely cleared plot was applied in the manually and mechanically treated plots with the only difference being that transects were based on *T. ivorensis* trees as opposed to *T. superba*. In the treated plots no samples were collected from 7.5 and 10.0m of the trees.

2.5.8 **Extraction of spores**

A centrifugation sugar flotation method, slightly modified from Jenkins (1964) by Walker (1979), was used for spore extraction. The sucrose solution was prepared by adding household sugar to 700 ml water until a litre of solution was obtained. Each litre soil sample was thoroughly mixed and sub-sampled into seven replicate 100 ml sub-samples (following advice from Mr R Smith, ITE, and supported by work done by Reich and Barnard, 1984) in order to increase the precision of estimates of spore counts. One of each set of seven soil sub-samples was weighed fresh and then put in an oven to dry at 70°C for two days. The dried sub-sample was used to determine number of spores per gram dry weight, (the generally accepted method of expressing spore numbers) while the remaining six replicate sub-samples each underwent the following spore extraction stages:

i) The 100 ml of fresh soil was put in a 2 litre capacity plastic container into which had been added a litre of cold tap water, mixed thoroughly with a wooden spoon to break all the soil peds, thus releasing spores.

ii) The resulting suspension was stirred vigorously and allowed to settle for 15 seconds after which it was gently decanted through a 710 µm mesh sieve, into another plastic container of 2 litre capacity.

iii) The 710 µm sieve was sluiced with water to ensure spores of smaller sizes than 710 µm mesh size passed into the second plastic container, while retaining bigger sized debris, or sporocarps on the sieve. The material remaining on the 710 µm sieve after this, was put into a petri dish (5.5 cm) and scanned for the presence of sporocarps.

iv) The suspension which passed into the second container was swirled vigorously, allowed to settle for 15 seconds, then it was gently poured through a 45 µm mesh sieve as all VAM spores so far recorded are larger than 45 µm.

v) Cold tap water was sluiced through the 45 µm sieve to eliminate much of the silt and smaller sized particles while the soil and spores retained by the 45 µm mesh size sieve was washed into a beaker.

vi) Using a spatula, soil from the beaker was transferred into four 50 ml plastic centrifuge tubes and topped with cold tap water.

vii) The centrifuge tubes were balanced and then spun in a horizontal rotor centrifuge for three minutes at 3000 rpm after which a soil plug formed at the bottom of each tube. The supernatant was poured into a petri dish and scanned under a dissecting microscope (X10 magnification) for the presence of spores.

viii) The centrifuge tubes with soil plugs were filled with sucrose solution bringing the soil plugs once more into suspension. The tubes were placed in the centrifuge and spun for 15 seconds at 3000 rpm.

ix) The sucrose supernatant containing the spores was poured through a 45 µm mesh sieve and quickly sluiced with cold tap water to wash away sucrose and minimise the risks of spore damage which may occur if spores are left in concentrated sucrose for too long.

x) The spores were washed from the sieves into sterilin tubes with the addition of 5% gluteraldehyde fixative to prevent bacterial growth. All the samples were stored in a refrigerator until ready for transportation to Edinburgh for assessment.

2.5.9 **Assessment**

The entire spore population in each sub-sample was assessed using the method by Perez (1987) which as indicated in section 2.4 was the best method. Small plastic petri dishes (5.5 cm diameter) were scored with parallel lines (1 mm apart) and counts were made by counting between

the lines going up and down until the end of the dish was reached. Only spores thought to be viable (i.e. cytoplasm filled) were counted. During the early phase of the study, much time was spent characterizing and learning to identify the different spore types (see Chapter 3) to ensure counts were realistic and meaningful.

Sample bottles were removed from the cold room randomly to ensure that counts were free of bias.

2.5.10 **Statistical Analysis**

Genstat was used for writing programmes for the analysis of variance. At an early stage it was realized that data needed to be transformed for statistical analysis. Spore counts were transformed to square roots, proportions converted to angles, procedures that normalised distribution, and brought about homogenity of variance and the additivity of treatment effects. Tukeys test was used to test differences between means when variance ratios indicated significance ($p = 0.05$). Simpson's diversity and equitability indices were used to test the richness, commonness and rarity of VAM fungal species (Begon *et al.*, 1990).

Graphs in this dissertation were produced using the cricket programme provided by Edinburgh Regional Computing Centre.

CHAPTER 3

IDENTIFICATION OF VAM FUNGI

3.1 INTRODUCTION

Initially VAM research was concerned with the use of unidentified mixtures of pot cultured fungi in order to establish the benefits of the mycorrhizal association to agricultural crops (e.g. Gerdemann, 1968; Mosse, 1972) instead of indulging in the extensive identification schemes of the VAM fungi producing the growth benefits (Morton, 1988).

Increasing interest in VAM ecology, especially of tropical, agricultural and forest ecosystems, of which little is yet known, coupled with mounting evidence that VAM fungi differ significantly in their effectiveness to promote growth responses in plants (Abbott and Robson, 1978; Allen and Boosalis, 1983; Allen and Cunningham, 1983; Hayman and Tavares, 1985; Aldermann and Morton, 1986) have created a fundamental need for more intensive and accurate identification of populations of vesicular-arbuscular mycorrhizal fungi, particularly when comparisons are made citing them as experimental organisms (Morton, 1988). In Walker's view (1983a, 1986a) improved identification schemes would enable researchers to be more accurate and avoid making mistakes such as have been highlighted by the almost universal misrepresentation of species *Glomus fasciculatum* (Thaxter) *sensu* Gerdemann and Trappe (1974).

3.2 PAST AND PRESENT SYSTEMS OF IDENTIFICATION

Vesicular-arbuscular fungal identification is based exclusively on spores produced by these fungi (Jeffries, 1987) although most of the spore characters utilized (such as size, shape, colour, ornamentations, walls, hyphal attachments) are variable (Morton, 1988). Hyphae (another form of propagule), produced by VAM fungi, can also be used for identification when employing immunological techniques (see Chapter 2); however, by themselves immunological techniques do not effectively distinguish between VAM fungi.

An early history of classification reveals that Link in 1809 first described the genus *Endogone* but he did not indicate whether or not the 'sporangia' or spores were the result of a sexual process. Bucholtz (1912) described the sexual origin of *Endogone* zygospores and as a result placed the *Endogonaceae* in the Order Mucorales (Zygomycetes). At the time, however, most of the known VAM fungi produced spores in clusters (a sporocarpic habit) which led to this habit being given an 'a priori' primacy in classification (Morton, 1988). Based on this habit, sporangial, zygosporic and clamydosporic VAM species were included in the genus *Endogone*.

Following Thaxter's (1922) monograph of the Endogonaceae, Kanousse (1936) erected the genus *Modicella* in an attempt to separate the sporangial from other species in the genus *Endogone*.

By observing two stages of development of the same sporocarps of *G. fasciculatum* and *G. macrocarpum* it was concluded that the clamydosporic species (for which sexual reproduction had not been observed) were in fact anamorphs of zygosporic taxa (Morton, 1988).

The genus *Endogone* thus grew into an assemblage of diverse species, even including species that only produced clamydospores singly in soil and/or root tissues (Mosse, 1956, 1959; Godfrey 1957).

Attempts were made by Gerdemann and Trappe (1974) to provide a more orderly framework from which scientists could operate. In this new framework, the sporocarpic and clamydosporic forming species included in the genus *Endogone* were separated into the genus *Glomus*, originally erected by Tulasne and Tulasne in 1845, *Acaulospora* (Gerdemann and Trappe, 1974), *Gigaspora* (Gerdemann and Trappe, 1974) and *Sclerocystis* (Berkeley and Broome, 1875). Since then two more genera have been erected; *Entrophospora* by Ames and Schneider (1979) and *Scutellospora*, (Walker and Sanders, 1986) later changed to *Scutellispora* (Almeida, 1989) and separated from *Gigaspora*. On the other hand, two of the genera formerly included in the *Endogonaceae*; *Modicella* and *Glaziella* were removed from it, with the former being transferred to the *Mortierellaceae* (Trappe and Schenck, 1982) and the latter identified as an Ascomycetous fungus (Gibson, 1985).

Until recently, the Endogonales had only one family, the *Endogonaceae* with seven genera; *Endogone* (Link, 1809), *Acaulospora* (Gerdemann and Trappe, 1974; emend Berch), *Entrophospora* (Ames and Schneider, 1979), *Gigaspora* (Gerdemann and Trappe, 1974), *Glomus* (Tulasne and Tulasne, 1845), *Sclerocystis* (Berkeley and Broome, 1875) and *Scutellispora* (Walker and Sanders, 1986).

The artificiality of this classification has long been recognised by several workers (Gerdemann and Trappe, 1974, 1975; Walker, 1987; Morton, 1988). Thus Morton and Benny (1990) attempted to address this problem of artificiality by revising the classification to reflect patterns of common descent. From recent attempts at classification (Morton and Benny, 1990), two orders have emerged; the *Endogonales* and *Glomales*, the former with zygospores and the latter without. The *Endogonales* includes only one family, the *Endogonaceae* with the type genus *Endogone*, members of which are now believed to form ectomycorrhizas rather than endomycorrhizas. Within the *Glomales*, whose members form arbuscular endomycorrhizas in contrast to ectomycorrhizas, are the suborders *Glominae* and *Gigasporineae*. In the suborder *Glominae*, are the families *Glomaceae* and *Acaulosporaceae* (represented by the genera *Glomus*, *Sclerocystis*, *Entrophosphora* and *Acaulospora*, members of which form vesicular-arbuscular mycorrhizas) whereas the suborder *Gigasporineae*, represented by one family *Gigasporaceae* consists of members of the genera *Gigaspora* and *Scutellispora* with members which form only arbuscular endomycorrhizas. The similarities and differences between the genera are given in table 3.1.

3.3 PHYSICAL CHARACTERS OF SPORES USED FOR IDENTIFICATION TO SPECIES.

3.3.1 Spore colour.

Spore colour, as a character for identifying VAM fungal species has received undue emphasis considering its variable nature, influenced by the manner in which light passes from the object to the observer (Morton, 1988; Walker, pers. comm.), by the age of the spore,

Table 3.1 Present classification of arbuscular Mycorrhizas (after Morton and Benny, 1990).

ORDERS:	*ENDOGONALES*			*GLOMALES*
	- Fungi mostly hypogeus rarely epigeus			- mostly hypogeus sometimes epigeus
	- Saprobic and free living sometimes forming associations resembling ectomycorrhizae			- Form arbuscular endomycorrhizal association
	- Have somatic hyphae with septa sometimes containing microphores			- Somatic hyphae present and are generally coenocytic
	- Reproduce sexually by forming zygospores. Both somatic and sexual hyphae are similar in sporocarps			- Frequently asexual, forming azygospores and clamydospores - rarely sexual
SUBORDERS	NONE	*GLOMINEAE*		*GIGASPORINEAE*
		- Taxa form arbuscules and vesicles in mycorrhizal roots - Clamydospores produced terminally intercalarily and laterally from fertile hyphae - Auxiliary cells not formed		- Forms only arbuscules in mycorrhizal roots - Azygospores formed on apex of sporogenous cell - Auxiliary cells formed.
FAMILY	*ENDOGONACEAE*	*GLOMACEAE*	*ACAULOSPORACEAE*	*GIGASPORACEAE*
	type genus: *ENDOGONE*	*GLOMUS*	*ACAULOSPORA*	*GIGASPORA*
	other taxon:	*Slerocystis*	*Entrophospora*	*Scutellispora*
	- Fungi form	- Form clamydospore	- Spores develop	- Spores are

Table 3.1 continued

zygospores - sporocarps bear superficial morphological similarity to those of arbuscular fungi - sporocarp development is a convergent character state in *Endogone*	like resting structures borne terminally on one or more subtending hyphae -Compact sporocarps with spores embedded in an organized (e.g.*Sclerocystis* sp) or unorganized hyphal matrix - Different types of multiple outer walls account for most diversity in spore wall structures with distinct outer walls like the expanding wall and a muculaginous evanescent outer wall which stains dextrinoid in Melzers reagent (Morton 1989) - At least one structural wall is continuous with a wall of the subtending hypha - Inner flexible spore walls rarely react with Melzers reagent, those	from (eg *Acaulospora* species) or within (eg *Entrophospora* sp) the neck of a sporiferous saccule - Sporiferous saccules and attached spores usually are borne singly but occasionally form aggregates - Spores rarely have more than one structural wall, the surface of which ranges from smooth to highly ornamental - Spores have a minimum of one flexible inner wall but usually two or more are present - Number and type of inner flexible wall accounts for most diversity in spore wall structure. Distinct wall types include the semi rigid unit wall and the	borne terminally on a sporogenous cell (Spain *et al.*, 1989) - spores generally are large usually exceeding 300 µm in diameter - spores have as a minimum a thin~ outer unit wall tightly adherent~ with a laminated structural wall; both walls appear to be continuous with the walls of the sporogenous cell (Gibson *et al.*, 1987). - Flexible inner walls form an 'endospore' with number and type of walls accounting for most structural diversity. Distinct wall types include the coriaceous wall alone or complexed with either a

Table 3.1 continued

that do are typically slow and diffuse. - Spore contents are separated from the sporophore by either an amorphous plug, a curved septum, an inner flexible wall or thickening of the structural wall - Spores of most species germinate by emergence of the germ tube through the subtending hypha - Mycorrhizal vesicles are intraradical mostly with the potential to become thick walled spores in some species	'beaded' membranous or innermost membranous wall or amorphous wall. - Spore wall material appears to seal the opening to the neck of the sporiferous saccule - Spore germination occurs with a germ tube emerging from ephemeral germination compartments (Mosse, 1970) between a rigid unit wall and a beaded membranous wall - Mycorrhizal vesicles are intraradical and do not appear to have the potential to become thick walled spores in some species	membranous or amorpheous wall - Spore contents appear isolated from the sporogenous cell by what appears to be a thin septum, not discernible in all spores - Germ tubes arise from waxy germinal wall (e.g. *Gigaspora)* (Spain *et al.* 1989) or a complex germination shield (e.g. *Scutellispora* species). - Mycorrhizae is arbuscular with thin walled auxiliary cells produced in soil, spore surfaces range from smooth to highly ornamented.

pigmentation of spore walls or cytoplasmic contents (e.g. *G. gigantea* spores with green to yellow cytoplasmic contents but with hyaline to pale colour walls) oxidation during preservation (eg in the hyaline, spores of *Scutellispora calospora* which turn yellow to yellow-green in lactophenol (Koske and Walker, 1986) or hyperparasitism (Walker *et al.*, 1984; Bhattacharjee *et al.*, 1982). Variability in spore colour exists even among spores of the same species. However although this characteristic may not be strongly diagnostic (Morton, 1988) it gives some measure of separation.

3.3.2 **Spore shape.**

This character, like colour, is highly variable even within a species, probably because it is readily affected by the soil physical environment in which spores are formed (Morton, 1988). Small to medium sized spores without rigid walls, e.g. *Glomus intraradices*, when formed in the root cortex are often ellipsoid, while larger thicker walled intraradical spores like those of *Glomus clarum* and *G. manihotis* are likely to remain globose or subglobose. The spores of *Sclerocystis* spp with their tight sporocarps, are generally oblong to clavate except in *S. coccogena, S. coremioides, S. pachycaulis* and *S. rubiformis* which have globose to ellipsoid spores. Spores of *Scutellispora* spp have a range of shapes, from globose, oblong, reniform to ellipsoid even within a species.

Generally, however, the least variable spores are those of the genus *Glomus* (< 10%, Morton, 1988) being mostly thickwalled and globose to subglobose in shape. The species that are irregular or oblong in this genus generally are those having sporocarps such as *G. boreale, G. flavisporum* and *G. radiatum* (Morton, 1988).

3.3.3 **Spore size**

Spore sizes are taken with an occular micrometer, inserted into the eyepiece of a compound microscope; with spore length taken along an axis from the point of hyphal attachment and width is measured at right angles to that axis (Trappe and Schenck, 1982). Spore dimensions within a species and between genera can vary considerably. According

to Morton (1988), the spores of 90% of *Glomus*, 67% of *Sclerocystis*, 82% of *Acaulospora* and 100% of *Entrophospora* species have mid-diameters between 50-250 µm, indicating a large overlap in spore sizes in the various genera. Even greater variability in spore size is exhibited by the larger spored species of *Gigaspora* and *Scutellispora* genera, which generally have mid-diameters exceeding 300 µm (Morton, 1988). Similarly, spores of *A. laevis* (mid-diameters of 320 µm) vary from 119 to 520 µm (Morton, 1988).

Because of the extent of interspecific and intergeneric overlap in spore size among the various taxa, undue emphasis should not be placed on spore size as a diagnostic character.

3.3.4 **Spore wall**

Spore wall, more than any other morphological character is used to characterize a particular fungus and therefore has been considered the most important character in VAM species identification (Mosse and Bowen, 1968b; Gerdemann and Trappe, 1974; Trappe 1982).

Originally four types of walls were described by Walker (1983); the laminate unit and evanescent (which are normally outermost walls) and the membranous wall (the innermost wall). Additional walls have since been added to the list as their unique characteristics become apparent. Thus, Walker (1986b) and Morton (1986) introduced the coriaceous and amorphous walls respectively, both flexible innerwalls, while Schenck *et al.* (1984) first recognised the beaded wall which Morton (1986) designated a murograph for and lastly the expanding wall (Berch and Koske, 1986) another external wall. Figure 3.1 shows the designations given each wall type.

3.3.4.1 The laminate wall

The laminate wall is recognised as a single brittle wall constructed of more or less tightly fused layers that do not differ in texture but are evident due to slight differences in refringence (Walker, 1983). This wall begins as a single layer, becoming thicker with age, by the deposition of additional laminae (Mosse, 1970; Sward, 1981). The

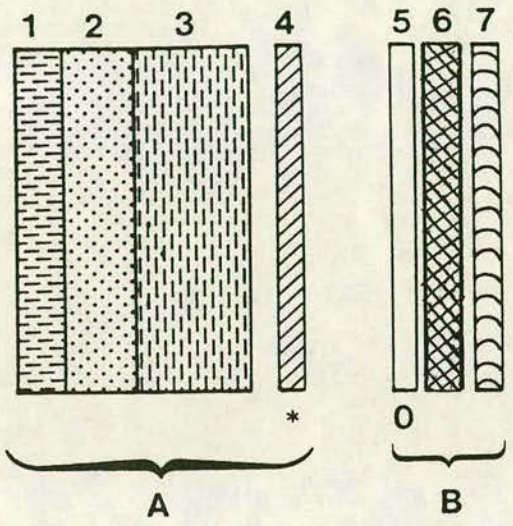


Figure 3.1 Murograph of wall type, number, group and position in a VA mycorrhizal fungus spore
(Walker 1983, 1986; Berch and Koske, 1986; Morton, 1986)
0 = ornamented, * = difficult to see
1 = expanding wall; 2 = evanescent wall; 3 = laminate wall
4 = membranous wall; 5 = unit wall; 6 = coriaceous wall
and 7 = amorphous wall. Each group is bracketed in parenthesis
with consecutive letters from outer to inner walls.

relative thickness of laminate walls varies from specimen to specimen, depending on the age and condition of the spore. In some hyaline VAM fungal species (e.g. *A. delicata*), the laminae are extremely difficult to see although the application of stains and reagents and the use of a good quality high power microscope helps surmount this problem.

Most taxa have laminate walls except those with double unit walls like *G. occultum*, *A. appendicula*, *A. longula*, *A. morrowae*, *A. myriocarpa*, *A. spinosa* and *E. schenkii*. These walls probably provide structural integrity to the spore and help it resist desiccation and even predation (Morton, 1988).

3.3.4.2 The unit wall

This is a single layered rigid wall, consistent and clearly distinguishable in spores at the same state of maturity within a species (Walker, 1983). The thickness of the unit wall may vary among spores from 0.5-3 µm thick but not relative to other walls in the same spore (Morton, 1988). Where unit walls provide structural integrity as in spores of *Glomus occultum*,, *G. aggregatum*, *G. microaggretatum*, *A. appendicula*, *A. longula*, *A. morrowae*, *A. myriocarpa*, *A. spinosa* and *E. schenckii*, two or more (unit walls) are found together - they are usually pigmented in contrast to the hyaline condition of unit walls without a structural function (e.g. as thin outermost or inner walls) (Morton, 1988).

Unit wall fractures readily when pressure is applied, sometimes separating from other walls, but in species where unit walls are tightly adherent to other walls they can be distinguished by their colour or structure (e.g. the outer walls of *Glomus caledonicum* (Nicol and Gerd) Trappe and Gerdemann. Examples of other species in which unit walls may be found include *Gigaspora gigantea*, *A. trappei* and *G. geosporum*.

3.3.4.3 The evanescent wall

Unless a relatively large series of spores of all ages is studied, evanescent walls can be incorrectly identified as unit walls,

especially in young spores (Walker, 1983). Often, as in *Glomus albidum* and *Glomus etunicatum* the outermost wall is evanescent and sloughs off as spores mature; thus these species could easily be misidentified (Becker and Gerdemann, 1977). On the other hand some species, like *Glomus gerdemannii* (Rose, Daniels and Trappe, 1979), have been shown to have two evanescent walls. Other species having evanescent walls include *Glomus ambisporum*, *G. dimorphicum*, *G. heterosporum*, *G. hoi*, *G. tenebrosum*, *G. versiforme*, *A. delicata*, *A. longula* and *A. nicolsonii* (Morton, 1988). None of the taxa of *Gigaspora* or *Scutellispora* form spores with evanescent walls, possibly indicating differences in spore ontogeny. Correct identification of this wall requires that some spores, in a population, are still young enough to retain their evanescent walls (Walker, 1985). For this, the propagation of VAM fungi in pot cultures enables populations of spores at different stages of spore maturity to be examined (Morton, 1988).

The use of preservatives such as gluteraldehyde often eliminate evanescent walls as in *G. pansihalos* (Morton, 1986).

3.3.4.4 The membranous wall

This single layered wall is very thin and flexible as a result: wrinkles or collapses in hypertonic solutions (Walker, 1983). Because of its flexibility, this wall usually does not break when spores are crushed. The membranous wall is often found as an inner wall, enclosing spore contents or sometimes other similar walls. Walker (1983) stressed that the term 'membrane' is not intended to imply a cellular or subcellular membrane, instead it is used in a broader sense to indicate a thin, more or less elastic, covering.

The membranous walls are generally 0.5-2.0 µm thick but can be 3.0 µm as in *A. foveata* and *A. tuberculata*: they are generally hyaline, although yellow membranous walls have been reported in *G. dimorphicum* (Morton, 1988).

When mounted in Melzer's reagent, the membranous walls in spores of *A. longula*, *A. mellea* and *A. rehmii* (Schenck *et al.*, 1984; Sieverding and Toro, 1987) stain light purple. Less intense coloration, possibly

attributable to differences in the polymerization of wall components, has been observed in spores of *A. foveata* and *A. tuberculata* (Trappe and Schenck, 1982).

It is not always easy to ascertain if a wall is membranous or a very thin unit wall or simply a loose inner lamina of a laminate wall. Sometimes mountants (e.g. Polyvinyl alchohol Lactophenol) or preservatives like gluteraldehyde alter the flexible nature of this wall, making it look more like a unit wall (Morton, 1986). For example, the membranous walls of *Scutellispora pellucida* become fused and inflexible, looking like laminate or unit walls when spores are preserved in 5% gluteraldehyde (Morton 1986). Hyperparasitism may also cause such inner walls to disappear (Bhattacharjee *et al.*, 1982).

3.3.4.5 The coriaceous wall.

This type of wall was termed 'coriaceous' by Walker (1986b) because of its leathery appearance, looking wrinkled in hypertonic mountants. Like the membranous wall it is an inner wall, hyaline, flexible and formed as a single layer but unlike the membranous wall, it is generally much thicker. When this type of wall was first described, thickness served as the main criterion for separating it from the membranous wall but with the considerable overlap of this aspect (e.g. in *G. globuliferum*, Koske and Walker, 1986) there is doubt as to the validity of this criterion. According to Morton (1988) the recognition of coriaceous and membranous walls as distinctly different entities can only be resolved after the limits of variation in thickness of each type of wall have been assessed.

Recent advances in mycorrhizal research (Morton, 1990) have shown that membranous walls stain pink in Melzers reagent in contrast to the coriaceous walls which do not. Like the membranous wall however, the coriaceous wall does not fracture easily when spores are lightly crushed, as seen in spores of *S. dipapillosa* and *S. weresubiae*. Several *Scutellispora* spp described by Morton and Koske (1988) have coriaceous walls adjacent to an inner amorphous wall.

3.3.4.6 The expanding wall.

This is a hyaline unit wall which expands markedlyin (2.0-10.0 µm) in polyvinyl alcohol lactophenol (PVL) (Berch and Koske, 1986), a common mounting fluid. The expanding wall forms distinctive hyaline to pale yellow, 'rough-looking' radial columns in the expanded halo surrounding the spore (Morton, 1988). So far, this type of wall has only been recognised in *G. pansihalos* by Berch and Koske (1986).

3.3.4.7 The amorphous wall.

The changing characteristics of the amorphous wall,in different chemical environments, make it difficult to define (Morton, 1986). In more acid mountants (pH < 2) eg PVL, the wall appears to be plastic (Morton, 1986). In non-acidic environments, water or glycerol, the wall seem to be a rigid unit wall, which, however, does not fracture readily when spores are 'lightly broken': the amorphous wall is difficult to see without Normaski Interference Optics (Morton, 1986). Its inner surface appears to be highly wrinkled, giving the impression of a coriaceous, or highly convoluted, membranous wall (Morton, 1988). In Melzer's reagent, the amorphous wall of spores of *Acaulospora morrowae* stains dark purple. To detect an amorphous wall the spores need to be crushed twice: on the first occasion the wall appears like a blob of cytoplasm; if this is left for 15-30 minutes before being crushed for a second time, the plasticity of an amorphous wall can be detected (Morton, 1988). Amorphous walls have been observed in 3 genera, *Acaulospora, Entrophospora* and *Scutellispora* (Morton, 1988) with records being available for *A. dilatata, A. lacunosa, A. morrowae, E. colombiana, S. pellucida* and *S. scutata* (Morton, 1988; Walker and Diederichs, 1989).

3.3.4.8 Wall ornamentations.

Surface structures (which give an ornamented appearance to some VAM fungal spores) appear on laminate, unit and membranous walls in all VAM genera but the *Gigaspora* and *Sclerocystis* VAM genera. These ornamentations are not however specific but their presence aids identification to the species-level.

In *Glomus*, (with the exceptions of *G. pustulatum* and *G. ambisporum* respectively, (Morton, 1988) ornamentation appears mainly on laminate walls. They may be rounded projections (*G. multicaule*), knobs (*G. scintillans*) crowded spines without pitted tips (*G. botyroides*), hemispherical warts (*G. pansihalos*) or tubercules (*G. tenebrosum*).

In *Acaulospora*, the laminate wall is ornamented with an alveolate reticulum overlaying echinulations (*A. elegans*), puncticulate pits (*A. dilatata*), round bottomed pits with ridges (*A. foveata, A. scrobiculata*) or round bottomed pits with raised edges (*A. lacunosa*) while the unit wall has echinulate ornamentations consisting of polygonal spines (*A. spinosa*), tubercules (*A. tuberculata*), tooth shaped projections (*A. denticulata*), colliculate projections (*A. appendicula*) or a polygonal reticulum of ridges (*A. bireticulata*).

In addition to ornamentation on laminate and unit walls of some species of *Acaulospora*, granular excrescences on the inner membranous wall give them a beaded appearance (*A. delicata, A. mellea, A. laevis, A. scrobiculata, A. rugosa*).

In *Entrophospora*, the laminate wall is ornamented with crowded spines (*E. infrequens*) and the membranous walls beaded in species of *E. colombiana*.

Ornaments are found only on the unit walls in species of *Scutellispora* and these may be in the form of patch-like warts with angular margins (e.g. *S. coralloidea*) closely packed warts with rounded tips less than 2.0 µm (*S. heterogama, S. persica, S. verrucosa*) or greater than 2.0 µm as in *S. gregaria*.

The variable degree of ornamentation on the outer walls of spores of the same species, e.g. the rounded projections of spores of *G. tenebrosum* (Berch and Fortin, 1983) and the crater-like pits on the outer walls of spores of *A. lacunosa* (Morton, 1988), exacerbates the difficulties encountered in species identification.

3.3.4.9 Wall numbers and groups

The number of spore walls is remarkably stable for most VAM fungal species (Morton, 1988). According to Morton (1988) 50% of spores of the *Glomus* genus, 100% of *Sclerocystis* and 100% *Gigaspora* usually posses just 1-2 walls while 75% of spores of *Acaulospora*, 90% of *Scutellispora* and 67% of *Entrophospora* posess 3 or more walls with the additional walls most often being flexible. A wall group (Walker, 1983) defined as an aggregation of walls which remain in close proximity to each other, after a spore has been crushed, is reliably diagnostic when comparisons are made of spores in the same medium broken with a fairly equal amount of pressure.

Before the standardization of wall terminology by Walker in 1983, early descriptions of VAM fungal walls were often confused. For example, *S. calospora*, *S. heterogama* and *S. pellucida* (Gerdemann and Trappe, 1974) were described as having 2 walls only; an outer rigid wall and an inner flexible wall. A redescription of these species of *Scutellispora* by Koske and Walker (1985, 1986) revealed that their spores actually possessed two wall groups with a total of 4, 4 and 6 wall types respectively. Great care thus needs to be exercised in differentiating correctly between wall groups and wall types when identifying species of VAM fungi.

Walker's (1983) suggestion of a standardized terminology for designating wall types and groups (muronyms) and methods of presenting them diagrammatically (murographs) have provided a focal point for wall recognition (Figure 3.1).

3.3.4.10 Wall reaction with Melzer's reagent

Melzer's reagent, an iodine based solution, appears to offer the greatest potential help at present in identifying VAM fungi. A blue reaction indicates the presence of amyloid material in the walls or contents of spores while a red colour denotes the presence of dextrinoid materials. So far, no true amyloid reaction has been observed in spore walls of VAM fungi, whereas the presence of dextrinoid material has been observed in the membranous walls of *G.*

fasciculatum, *A. scrobiculata*, *S. calospora*, *S. weresubiae* and *A. appendicula* (Morton, 1988). Some reactions however, are neither blue nor red, e.g. *G. albidum* and *S. fulgida* which have been reported to develop an orange coloration in their hyaline laminate walls. Berch (1985), Koske and Walker (1986) suggested that the reported variability in the staining reaction of laminate walls in spores of *A. sporocarpia*, *S. pellucida*, *S. verrucosa* and *S. weresubiae*, may be attributable to differences in the polymerization of wall components. The inner spore walls (membranous, coriaceous and amorphous) unlike the outer spore walls (laminate and unit) seem to vary less in their colour reactions in different environmental conditions.

3.4 SPOROCARP MORPHOLOGY

The formation of sporocarps occurs mainly in two genera, *Sclerocystis* and *Glomus*. All species of *Sclerocystis* form sporocarps, with spores arranged in highly ordered single layers around a central hyphal plexus unlike spores of species of *Glomus* which may be found freely, or of arranged randomly in loose interwoven hyphae (Gerdemann and Trappe, 1974), in soils.

Sporocarp size is very variable (Hall, 1977) although generally *Glomus* species form larger sporocarps *Sclerocystis* whose sporocarps are often less than 1mm in diameter (e.g. *S. clavispora*, 0-800 µm; Morton, 1988).

Sporocarp colour like spore colour is also variable, generally ranging from hyaline to brown and black (Schenck *et al.*, 1986). Sporocarp shape in the genus *Sclerocystis* ranges from globose ellipsoid; in *Glomus* they are mostly round. According to Morton (1988) shape appears to be a valuable character in species identification.

3.5 HYPHAL MORPHOLOGY

Hyphal characteristics are not consistent; they are therefore of little use in species identification (Morton, 1988). For instance the colour of the hyphae of most species of *Glomus*, *Sclerocystis*, *Gigaspora* and *Scutellispora* are reported to be the same as that of their spore

walls. Exceptions however, have been recorded in spores of *Glomus invermaium* which have colourless subtending hyphae (Morton, 1988). Hyphal shape also varies conspicuously, especially in species of *Glomus* where some hyphae are cylindrical, e.g. *Glomus aggregatum*, others funnel shaped, e.g. *G. mosseae* and *G. monosporum* or flared, irregular to constricted as in *G. monosporum*.

Most VAM spores have single subtending hyphae: these with multiple subtending hyphae belong mainly to the *Glomus* genus (Morton, 1988), where species like *G. glomerulatum, G. heterosporum* and *G. lacteum* have spores with 1 to 3 subtending hyphae.

3.6 MATERIALS AND METHODS

3.6.1 Extraction of spores from soils

As all spore suspensions used for counting were kept in 5% gluteraldehyde, to deter the growth of bacteria and fungi there is a possibility that the spores especially their wall components, may have changed (Morton, 1986) before examination at Bush. For this reason, additional fresh soil (4 kg) was brought back to the Institute of Terrestrial Ecology, Bush Estate near Penicuik in Scotland and kept in a coldroom (4°C) to enable fresh spores to be extracted and used for characterisation and identification purposes.

As enumeration of the spore suspensions progressed, each new spore type was characterised and identified where possible from freshly extracted spores obtained from the soil kept in the coldroom, using the centrifugation sugar flotation method already described in Chapter 2.

3.6.2 Preparation of temporary diagnostic slides.

Mountants like Polyvinyl alcohol lactophenol (PVL), alter the texture of spore walls (Morton, 1986). For this reason, and following advice from Walker (pers. comm.), temporary slides were made using water or Melzer's reagent as mountants. Observations were made of the effects of each of the mountants on the spore walls, so highlighting changes

which would not have been recognised if only acidic mountants had been used (Morton 1986).

A drop of water or Melzer's reagent was placed on each of several glass slides into which were subsequently added ten or more freshly extracted, and previously characterised spores.Round coverslips (13mm in diameter) were placed over the spores and using a dissecting microscope (Wild M5 Heerbrugg) at a magnification of x50, the spores underneath each coverslip were located and crushed lightly using a blunt edge. Changes of colour were noted after mounting in Melzer's reagent, similarly details of whether or not spore walls expanded in water were also recorded. However, because the slides dried rather quickly and yielded very few details there was a need to have more permanent diagnostic slides using viscous media like PVL or PVA which did not evaporate as readily as water or Melzer's reagent did.

3.6.3 **Preparation of permanent diagnostic slides**

In preparing permanent diagnostic slides, twin frosted glass slides were preferred as the frosted edge enabled important information to be recorded at the time of preparation. Two drops of mountant, Polyvinyl alcohol lactophenol (PVL) were placed separately on a neatly labelled slide. The number of spores put into each drop of mountant was size dependent to reduce the chances of the slides drying out; 10 or more spores if sizes were less than 100 µm, but less than 10 if spores were larger than 300 µm. These spores were picked from each characterized lot using microtweezers (to reduce the amount of water likely to be taken up from the spore suspension and mixed with the mountant) and put into each of the two drops of PVL mountant mentioned above. Round cover slips (13mm diameter) immersed in 70% alcohol to keep them clean and grease free were picked out with tweezers, dried with clean tissue and then placed over the spores in each PVL drop. By setting down one edge of the coverslip near the margin of the drop at an angle, and letting the PVL mountant spread under the coverslip surface, the chances of entrapping air bubbles within the coverslip were minimized (Schenck and Perez, 1987).

Using a dissecting microscope (Wild M5 Heerbrugg) at a magnification of

x50, slides were first checked to see if spores were beneath the coverslips, after which gentle pressure was applied on one of the coverslips using a blunt edge (preferably a pencil) to split open the spores. The spores in the other mountant drop were left intact.

The slides were left to stand for 30 minutes after which more pressure was applied to the crushed spores in an attempt to expose walls which become apparent only after crushing the spores twice such as amorphous walls (Morton, pers. comm.).

Using cotton wool dipped in ethanol, the excess PVL around the coverslips was removed. The slides were then placed horizontally in a safe place for two to three days to dry. When dry, clear nail polish was used to seal the edges of the coverslip, and so prevent the mountant from drying out.

Using a compound microscope (Wild M20 Heerbrugg) oil immersion (x150, x1000), details of the wall types, groups, numbers, sizes, colours and other diagnostic features were recorded on work sheets.

As an additional test to examine for presence of membranous walls drop of Melzer's reagent was added to each of the two drops of Polyvinyl lactophenol (PVL) mountant and mixed thoroughly prior to the additon of spores.

3.6.4 **Recording of spore details.**

Worksheets similar to those found in "The Manual for the Identification of VA Mycorrhizal Fungi" by Schenck and Perez, (1988) were used. Details on spore shape, texture, colour, size of intact spore, type of spore contents (i.e. globular or granular or oily) type of hyphal attachment, suspensor cell (if present), sporiferous saccule, germination shield, presence of a collar and any reactions with Melzer's reagent, wall types and numbers (laminate unit, membranous coriaceous, amorphous, evanescent, expanding), wall colour, and sizes (using an occular micrometer), were recorded.

Murographs were drawn, muronyms written to adequately describe the wall

configuration. In addition to provide clarity of wall details, line diagrams were also drawn. Photographs of the described species were taken to provide a record and also to aid in species identification.

3.6.4.1 Recording of photographic details.

A Zeiss photoscope with an attached camera was used for photographing spores which had earlier been mounted on slides with Polyvinyl lactophenol. Black/white panchromatic negative films with extended red sensitivity were used because of their extremely high resolving power, good latent image retention and high definition which together produced in high quality pictorial records even when low contrast developers were used.

Photographs were taken using Köehler illumination and different optical lenses such as phase contrast and Normaski Interference optics. The latter was particularly valuable in identifying types of wall and in bringing out details of wall ornamentation (e.g. amorphous walls) especially in hyaline coloured spores.

The films were developed in Kodak HC110 developer with a pH range of 8-12, following the practice recommended in the Kodak manual.

3.7 STANDARD DESCRIPTIONS

17 VAM fungal species were recovered from the research plots in the Mbalmayo Forest Reserve, most of which have been identified. For identification purposes, details of spores were recorded as seen under a dissecting microscope (Wild M5 type) using transmitted light + incident and under a compound microscope (Wild M20) with brightfield and nomarski optics. Figure 3.2 shows a collection of spores from the Mbalmayo forest as seen under a dissecting microscope.

Figure 3.2

A collection of VAM spores from Mbalmayo as viewed under a dissecting microscope.

3.7.1 C12 (*Scutellispora* sp).

A. Details of spore observations made under a dissecting microscope (at x50 magnification Wild M5 type)

a) shape: large mostly globose but sometimes ovoid, reniform or oblong. Spores form singly in soils.
b) texture: smooth appearance
c) colour: cream to red-brown
d) sporiferous saccule: absent
e) spore contents: oily
f) reaction in Melzer's reagent: positive, giving a deep red coloration.

B. Details of spore observations made under a compound micoscope at x150, x1000 magnification (Wild M20 type)

a) size: 204.0-714.0 x 204.0-459.0 µm
b) hyphal attachment: cylindrical and septate, 140.0 µm long
c) suspensor cell: present, 85.0x60.0 µm
d) germination shield: dark brown and very prominent against the cream spore colour. Oval, with invaginations at the edges. Two of them often found on a single spore with lengths of 240.0-250.0 µm. Could be peeled off and found between wall groups A and B.
e) collar:absent
f) wall characteristics: three wall groups consisting of five walls.
Wall Group A is made up of a hyaline wall (1.0 µm thick) closely adherent to a laminate wall (5.0 µm).
Wall group B consists of a membranous wall (1.0-1.5 µm thick)
Wall group C is a coriaceous wall (4.0 µm) enclosing an amorphous wall (1.0-2.0 µm) which stains deep red in Melzers reagent.

C. Distinguishing characteristics:

The bulbous suspensor cell and the flexible coriaceous and amorphous walls place this spore type in the genus *Scutellispora* (Walker and Sanders 1986). The presence of two germination shields in most of the spores however is outstanding and has not been quoted for any of the species of *Scutellispora* already described. It is thus likely that this species may not have been described previously.

D. Ecology:

Parallels in the literature can not be found primarily because of the inability to give the present fungus a species designation.
However, a wide range of species of *Scutellispora* have been recovered from several countries and habitats.

S. nigra has been seen associated with a tropical hardwood species *Nauclea diderrichii* in Nigeria Old, Nicolson and Redhead, 1973) and forms mycorrhizas in pot culture with soybeans and bahia grass.

The closest resemblance to this unidentified species is seen in *S. scutata* recovered from the Cerrrado region of Brazil (Walker and Diederichs, 1989) from the root zones of wild pineapple (*Ananus cosmosus* (L) Marr) and established in put culture with *Zea Mays* (L), *Cajanus cajan*, among other plants.

Scutellispora alborosea has been shown to form mycorrhizal associations in *Hibiscus elatus* grown in ferralitic clayey soils in a nursery in Cuba (Ferrer and Herrera, 1978).

Scutellispora calospora species are even more widely reported from Scotland (Nicolson and Gerdemann 1968) from New Zealand (Mosse and Bowen 1968a) from Oregon and Washington (Gerdemann and Trappe, 1974) and sand dunes of California (Koske 1981) associated with a broad range of plant species such as *Fragaria chiloensis, Allium cepa, Fragaria vesca, Lupinus sp* and *Zea mays* in pot cultures. Although many species of *Scutellispora* have been recovered from sand dune habitats, (e.g. *S. dipapillosa* and *S. fulgida*) this might just be a reflection of where surveys have been carried out.

E. Taxonomic source: Walker, C and Sanders F. 1986. Mycotaxon, 27: 169-182.

Plate 1

C12 (unnamed *Scutellispora* species)

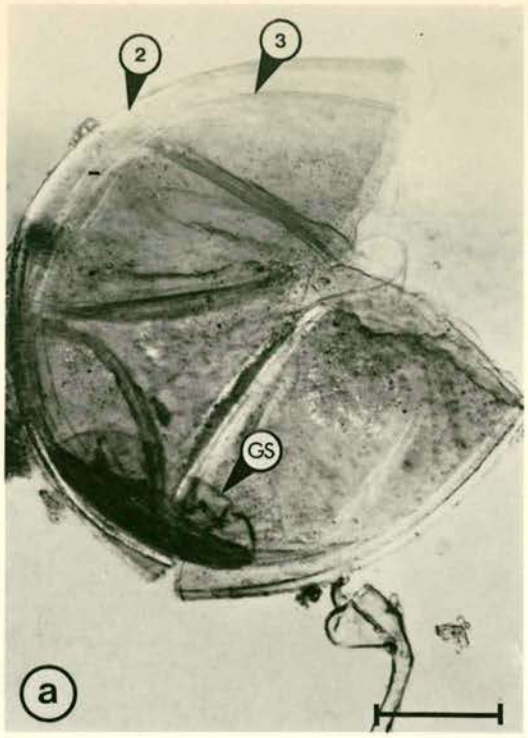
a) crushed ovoid spore mounted in PVL/Melzers showing wall 2, laminate and wall 3 membranous and a germination shield.
Scale bar 17 mm = 125 µm

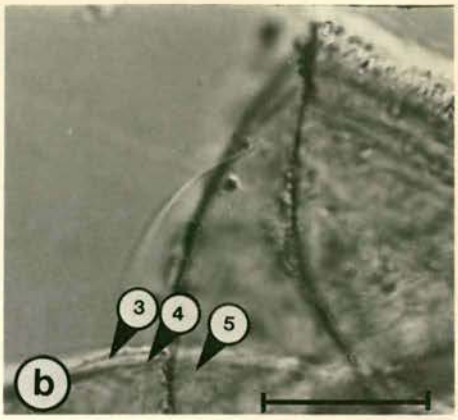
b) Enlargement of the inner walls
Wall 3 = membranous
Wall 4 = membranous, coriaceous?
Wall 5 = amorphous
Scale bar 23 mm = 125 µm

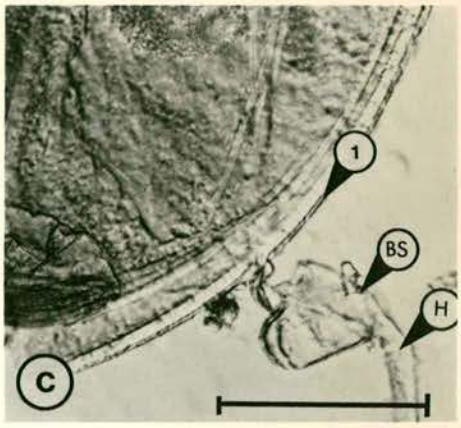
c) Showing details of bulbous suspensor cell (BS)
hyphal attachment (H)
and wall, 1 = unit wall
Scale 28.75 mm = 125 µm

d) Whole ellipsoid spore in PVL/Melzers mountant
Scale bar 33 mm = 250 µm

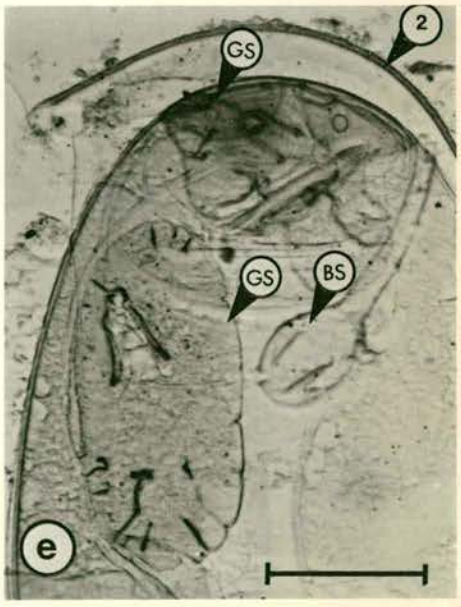
e) Section of spore clearly showing two germination shields (GS)
bulbous suspensor cell (BS)
and laminate wall 2,
Scale bar 22.5 mm = 125 µm


2
3
GS
a

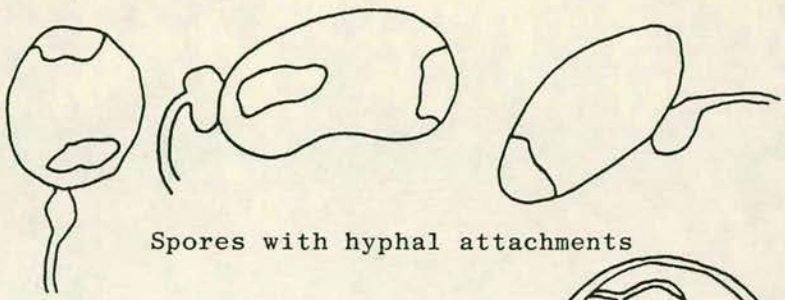

3
4
5
b


1
BS
H
c

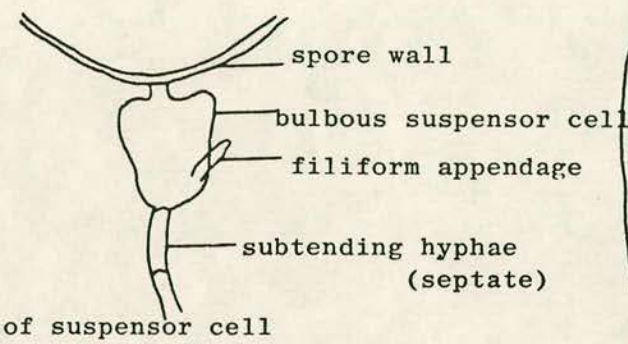

d


GS
2
GS
BS
e

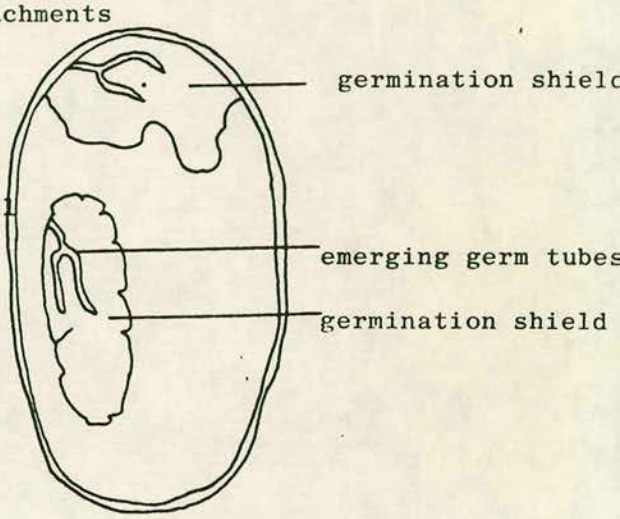
Range of spore shapes



Spores with hyphal attachments

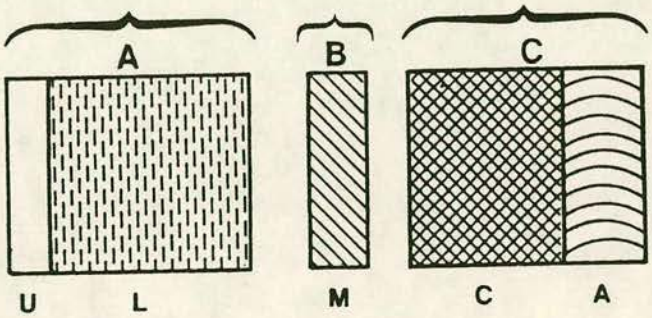


details of suspensor cell
and subtending hypha



details of germination shields

MUROGRAPH



MURONYM

A(UL) B(M) C(CA)

* Diagrams not drawn to scale

3.7.2 *Glomus etunicatum* Becker and Gerdemann

A. Details of spore observations made under a dissecting microscope (x50, magnification).

a. shape: globose to subglobose, occasionally reniform to irregular
b. texture: smooth walled
c. colour: red-brown to sienna
d. spore contents: globular and oily
e. sporiferous saccule: absent
f. reaction in Melzers: none

B. Details of spore observations made under a compound microscope (x 150, x 1000)

a. size: 120.0-146.0 (-156.0) x 96.0-116.0 (-122.4) µm
b. hyphal attachments: seldom present but on rare occasions it is seen lighter in colour than the sporse, 50.0-120.0 µm long and 10.0 µm wide at spore base, becoming thinner away from spore base to 0.5 µm. Open lumen leads through hypha into spore.
c. suspensor cell: absent
d. germination shield: absent
e. collar: absent
f. wall characteristics: two walls in one or two wall groups. Wall group A consists of an evanescent wall 0.5-1.5 µm thick and a laminate wall 5.0-10.0 µm thick while wall group B consists of a membranous wall 0.5-1.0 µm thick. These three walls do not separate readily thus the laminate and membranous two walls are often found in the same wall group as the evanescent wall.

C. Distinguishing characteristics:
Glomus etunicatum can be a very confusing species. The hyaline outer wall decomposes rapidly and presumably from the action of soil microorganisms (Becker and Gerdemann, 1977) spores containing only young or only mature spores could easily be presumed to represent two different taxa hence spores of intermediate age are necessary to establish a relationship between the extremes. Immature spores of *G. etunicatum* clearly resemble these formed by *G. caledonicum* although they are considerably larger with a persistent hyaline wall (Becker and Gerdemann 1977). The hyphae of *G. etunicatum* are thin walled and spores tend to break off just below the end of the inner wall thickening in the subtending hypha. Collections of spores wet sieved seldom occur in clusters and this serves to distinguish them from *G. fasciculatum* spores or *G. macrocarpum* which occur in clusters.

D. Ecology:
Known from a virgin sand prairie in central Illinois and agricultural fields in Illinois, Missouri and Florida, associated in the fields with *Andropogon scoparius* and *Zea mays*. It has also been reported from the rhizosphere of *Ammophila breviligulata* (Koske and Halvorson, 1981), *Mimosa biuncifera* (Bloss and Walker, 1987) from a lowland rainforest in Singapore (Louis and Lim, 1987) and from a tallgrass prairie (Gibson and Hetrick, 1988).

E. Taxonomic source: Becker and Gerdemann 1977. Mycotaxon 6: 29-32.

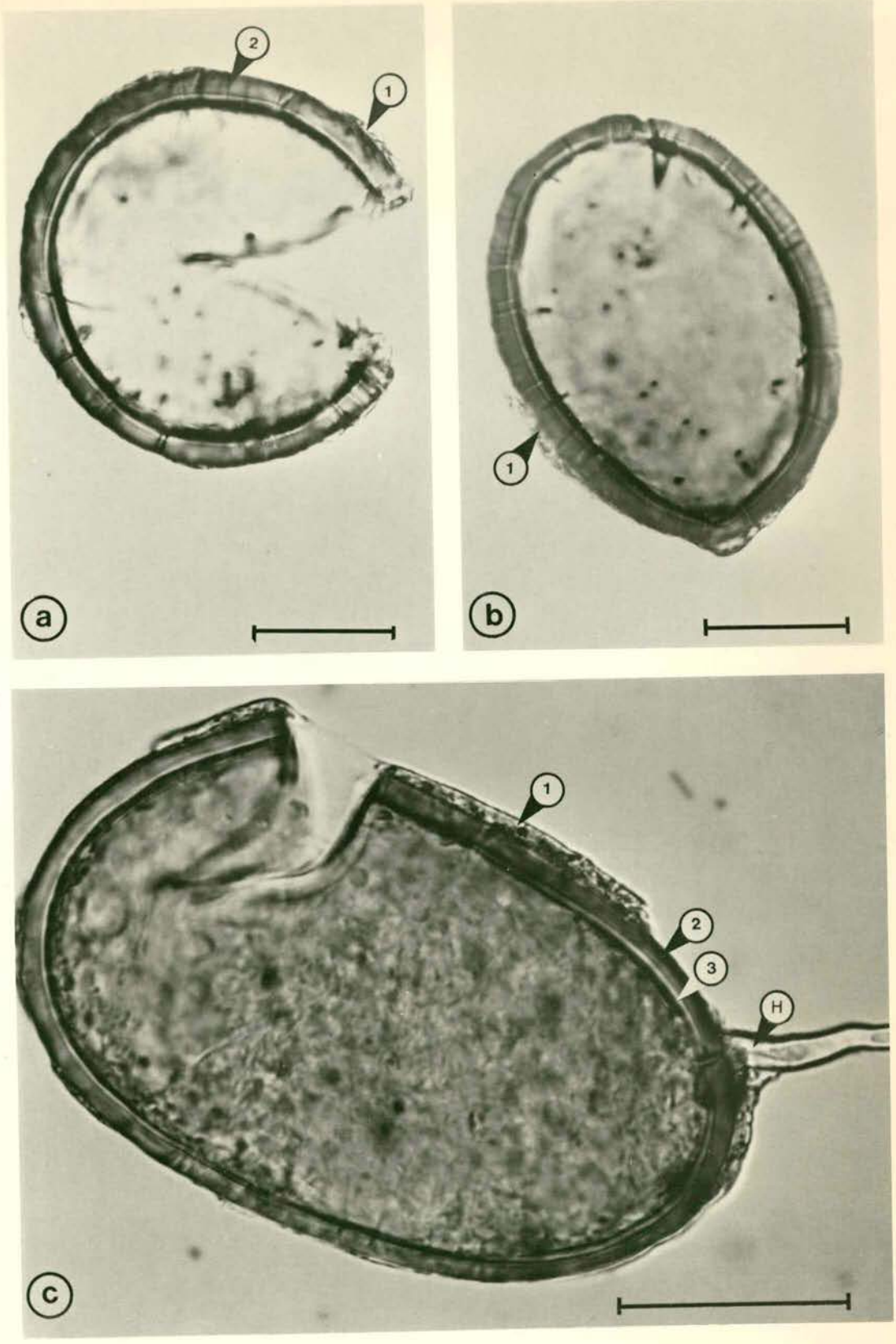
Plate 3.2

Species: *G. etunicatum*

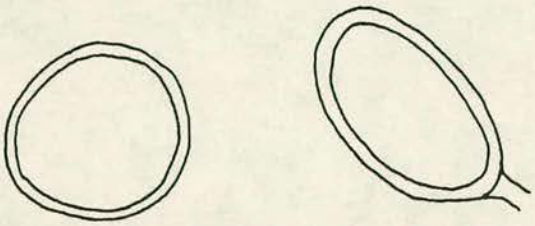
a) A globose spore of *G. etunicatum*, lightly crushed showing
Wall 1 evanescent wall
Wall 2 laminate wall
Wall 3 membranous wall
Scale bar 24 mm = 50 µm

b) A sub globose spore of *G. etunicatum*
Wall (1) evanescent wall
Scale bar 24 mm = 50 µm

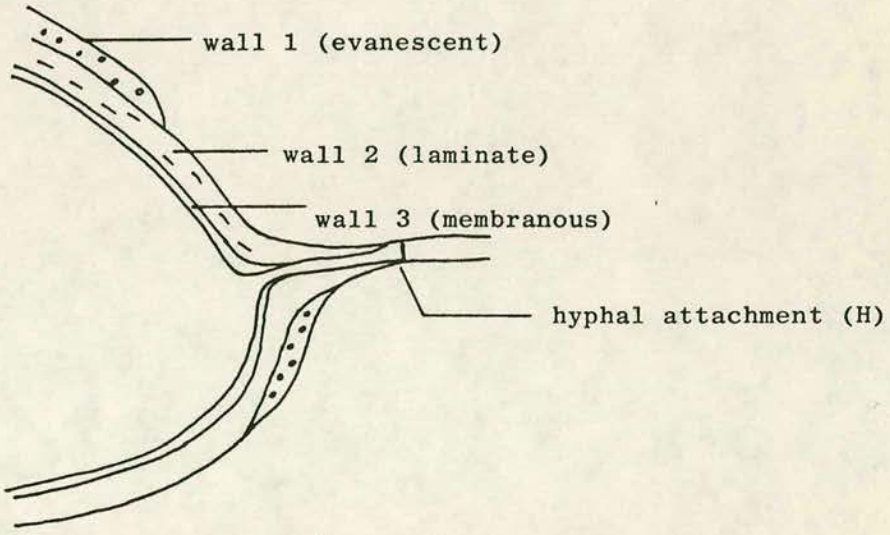
c) Crushed spore of *G. etunicatum*
showing walls 1 (evanescent wall) wall 2 (laminate) and wall 3 (membranous) and subtending hypha (H)
Scale bar 40 mm = 50 µm


2
1
a
1
b
1
2
3
H
c

Range of spore shapes



with and without hyphal attachments



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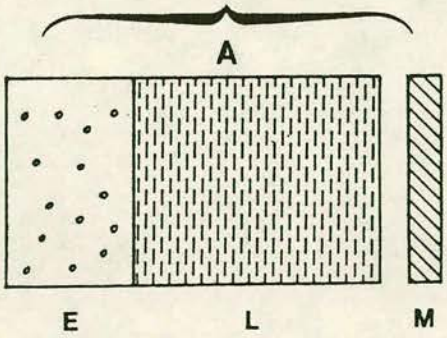


MURONYM A(ELM)

* diagrams not drawn to scale

3.7.3 *Glomus geosporum* (Nicolson and Gerdemann) Walker

A. Details of spore observations made under a dissecting microscope (x 50 magn).

a) shape: globose to subglobose
b) texture: rough, with debris adhering to the outer wall. Mucilaginous and very perforated.
c) colour: light to deep yellow
d) sporiferous saccule: absent
e) spore contents: globular in young spores but granular in older ones.
f) reaction in Melzers reagent: none observed

B. Details of spore observations made under a dissecting microscope (x 150, x 1000)

a) size: 91.8-153.0 x 91.8-153.0 µm
b) hyphal attachment: always present; short, straight or recurved with a septum which seems to separate the spore contents from the lumen. The wall of the attachment is thick nearest to the spore base and thins out away from spore.
c) suspensor cell: absent
d) germination shield: absent
e) collar: absent
f) wall characteristics: one wall group (A) with three types of walls.
Wall 1 is unit (1.0-1.5 µm) closely adhered to wall 2, a very thick laminate wall (7 µm) and a thin membranous wall 3 (1.0 µm) which sometimes is seen to collapse in PVL.

C. Distinguishing characteristics

According to Walker (1982) the only species likely to be confused with *G. geosporum* is *G. constrictum*. Although the colour ranges of these species overlap, the spores of *G. geosporum* do not become black and shiny at maturity as do the spores of *G. constrictum*. Also in *G. geosporum* spores, the inner membranous wall forms a septum closing off the spore contents; a feature not formed in *G. constrictum*.

D. Ecology: This VAM fungus has been widely reported in temperate regions unlike the tropics where they have been rarely reported; partly because species recovered from most tropical ecosystems are rarely adequately described to species level.

Reports of the presence of *G. geosporum* have been made from the rhizosphere of *Mimosa biuncifera, Muhlenbergia emersleyi* in the Santa Catalina mountains (Bloss and Walker, 1983) from a newly cleared woodland site in Florida (Schenck and Kinloch, 1980) from the rhizosphere of *Schizachyrium scoparium* (Dickman *et al.*, 1984). This species has also been reported from Great Britain by Nicolson and Gerdemann (1968), from India (Thapar and Khan, 1973 and from the southern hemisphere (Hall, 1977; Hayman 1978) *G. geosporum* can be produced in pot culture with *Lycopersicon esculentum, Zea mays* and *Fragaria* species.

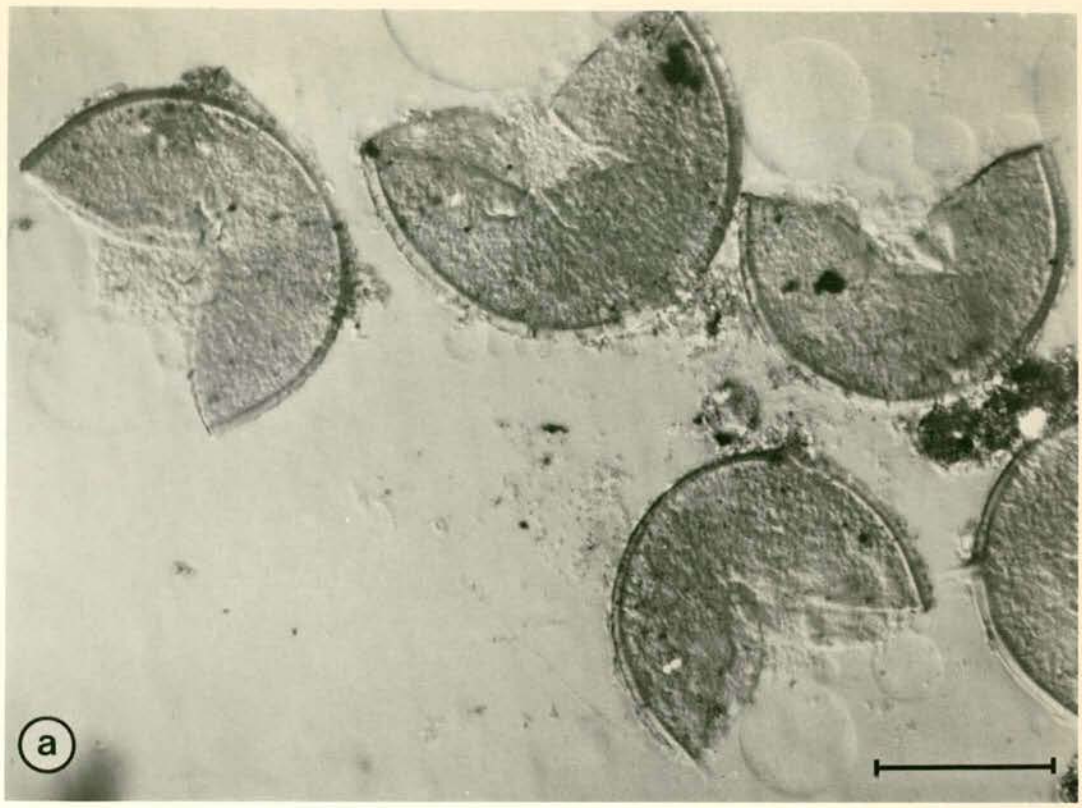
E. Taxonomic source: Walker C. 1982. Mycotaxon 15: 49-61

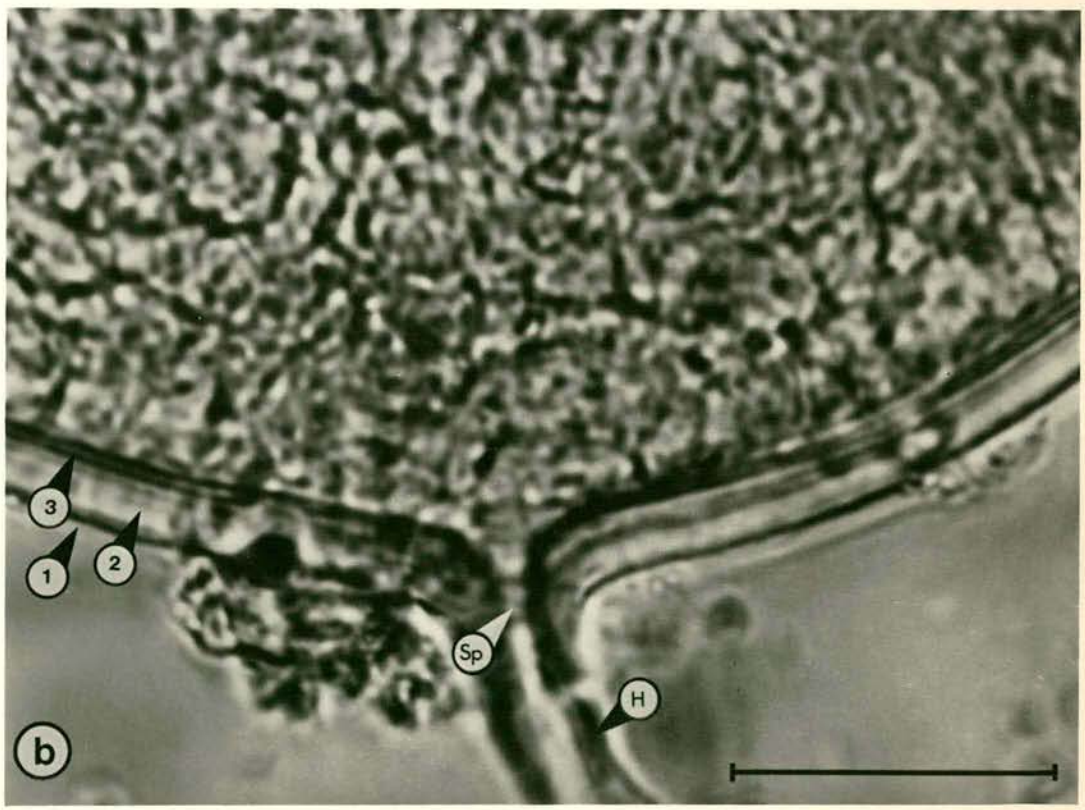
Plate 3.3

G. geosporum

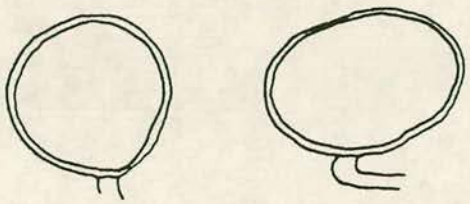
a) 5 lightly crushed spores of *G. geosporum* showing granular contents
mounted on PVL mountant only.
Scale bar 25 mm = 100 µm

b) Details of spore showing walls
Wall 1 = unit wall
Wall 2 = laminate wall
Wall 3 = membranous wall
Septum (Sp)
and hyphal attachment (H)
Scale 45.5 mm = 25 µm

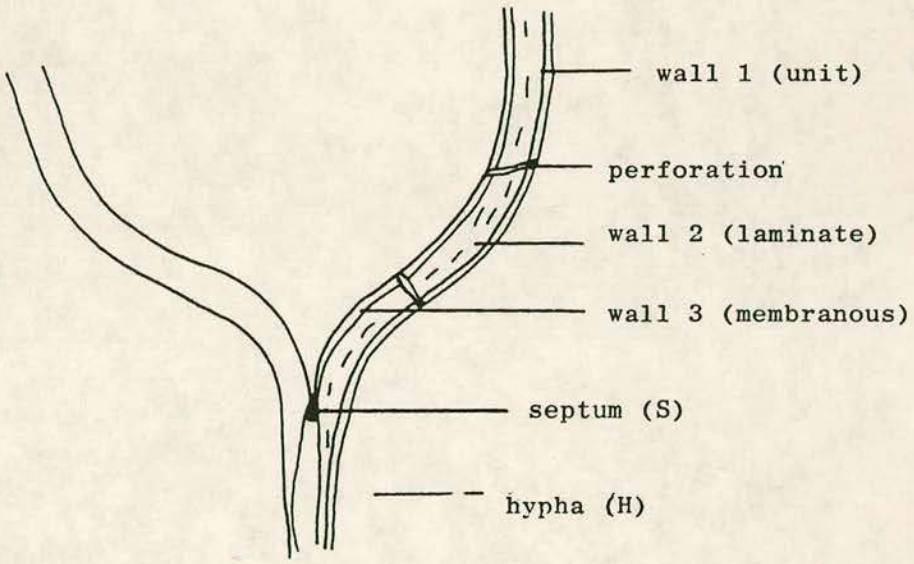

a


3
1
2
Sp
H
b

Range of spore shapes

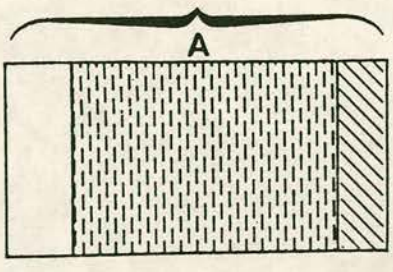


attached hyphae on spores



Details of wall structure

MUROGRAPH



MURONYM

A (ULM)

* diagrams not drawn to scale

3.7.4 *Acaulospora spinosa* Walker and Trappe

A. Details of spore observations made under a dissecting microscope (x50 magnification).

a. Shape: Globose to subglobose, occasionally ellipsoid.
b. Texture: Rough looking wall surface with crowded spines.
c. Colour: Pale yellow-brown to dark yellow-brown.
d. Spore contents: Granular.
e. Sporiferous saccule: not observed, probably detached.
f. Reaction with Melzers reagent: light pink colouration in paler coloured spores.

B. Details of spore observations made under a compound microscope (x 150, x 1000).

a. Size: 190.2-292.6 x 100.0 (-359.0) µm.
b. Hyphal attachments: Not observed.
c. Suspensor cell: absent.
d. Germination shield: absent.
e. Collar: not observed.
f. Wall characteristics: 2 wall groups consisting of three wall types.
Wall group A consists of an ornamented wall with crowded spines (3.0-8.0 µm thick) including spines 2.0-4.0 µm high, tapering at the ends.
Wall group B consisting of two membranous walls 0.5 to 1.0 µm and 0.5 µm respectively, separating easily from the ornamental wall.

C. Distinguishing characteristics.

A. spinosa close resembles *A. elegans* (Walker and Trappe, 1981) but lacks a complete reticulum superimposed on the spines at maturity and has only two thin inner walls as opposed to three in *A. elegans*. Similarly *A. bireticulata* differs from *A. spinosa* by its prominent three walls and stout polygonal projections.

D. Ecology:

A. spinosa is abundant throughout the growing season in soils around roots of annual grasses and trees on a sandy river near the Des Moines River in Central Iowa (Walker and Trappe, 1981); also from a wet subtropic of Vera Cruz state, Mexico in soils beneath roadside grasses and weeds (Walker and Trappe, 1981; Walker, Mize and McNabb, 1982) and from other tropical areas (Sieverding, 1989).

F. Taxonomic Source: Walker, C and Trappe, J.M. 1981. Mycotaxon 12: 515-521.

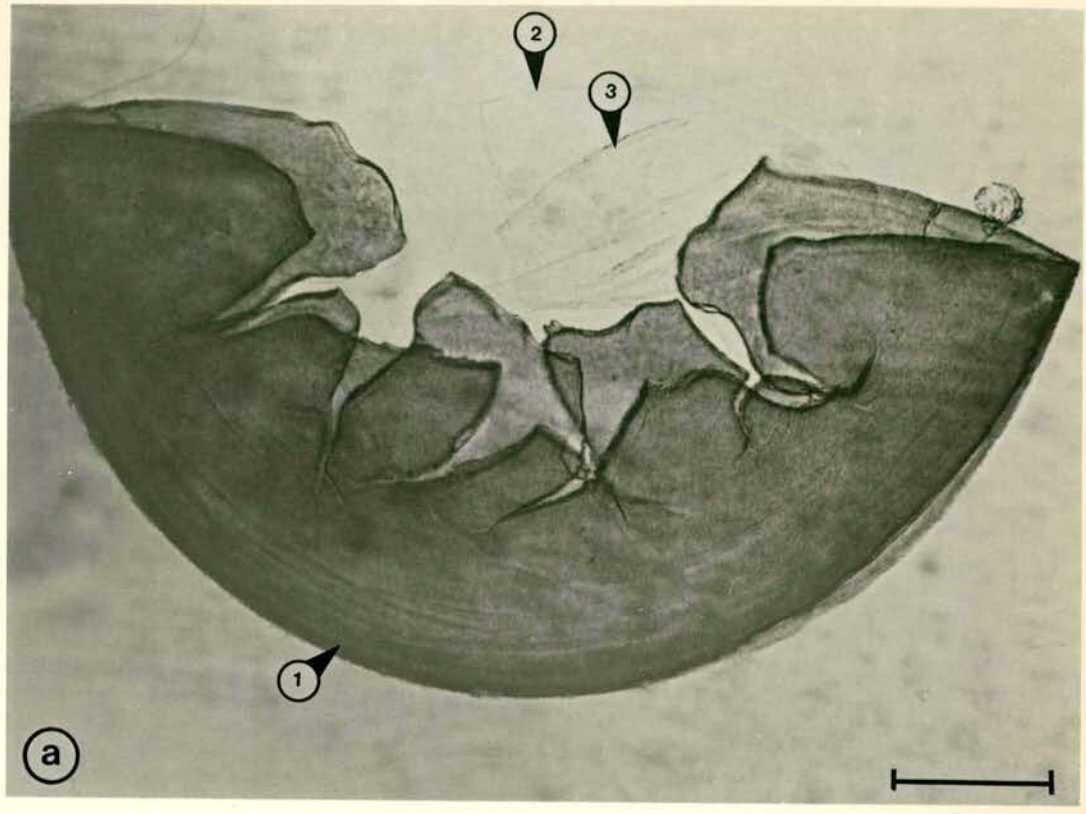
Plate 3.4

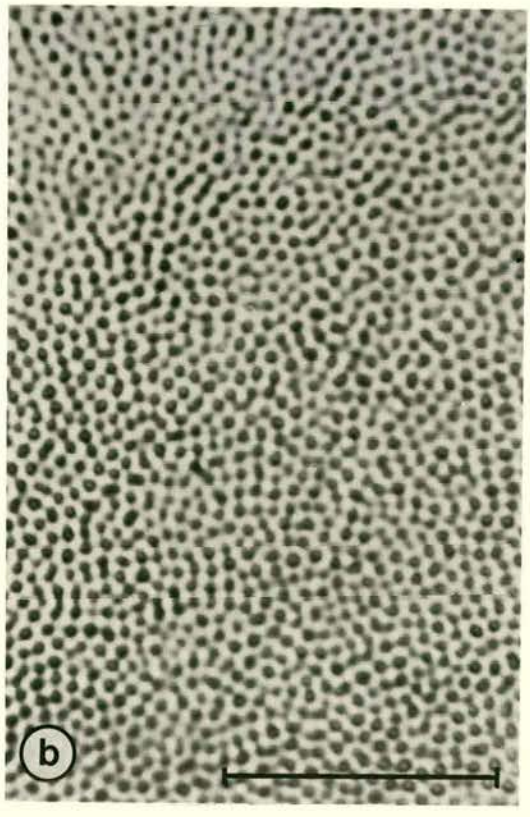
Acaulospora spinosa

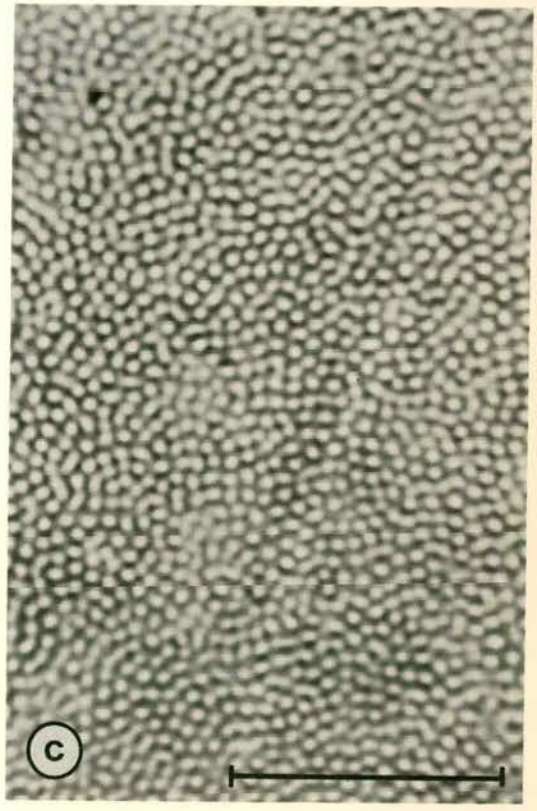
a) Crushed spore showing:
Wall (1) ornamental wall with blunt spines
Wall (2) membranous wall
Wall (3) membranous wall
Scale bar 22mm = 100 µm

b) Showing details of ornamentation; focused on the tips of the spines showing swirled arrangement.

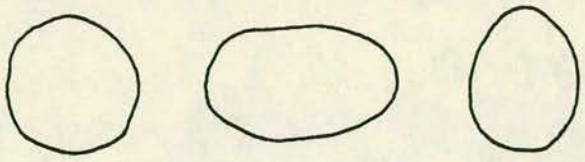
c) Focused on the base of spines


2
3
1
a


b

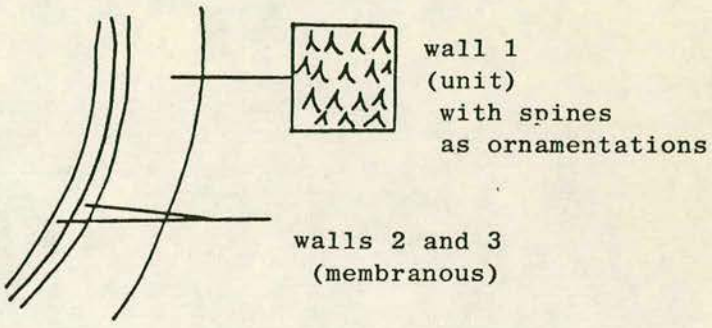

c

Range of spore shapes



no hyphal attachments

Details of wall structure



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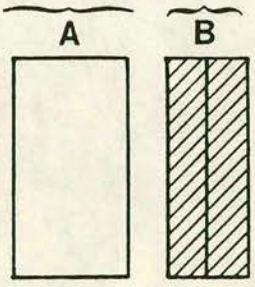


$u_{(o)}$ mm

MURONYM $A(u_o)$ $B(m,m)$

* diagrams not drawn to scale

3.7.5 *Scutellispora coralloidea* (Trappe, Gerd and Ho) Walker and Sanders

A. Details of spore observations made under a dissecting microscope (x 50 magnification).

a) shape: globose to subglobose and spores form singly in soils
b) texture: rough surface, looking like a berry
c) colour: dark red-brown
d) spore contents: globular
e) sporiferous saccule: not observed
f) reaction in Melzers: not observed probably because of dark pigmentation of spore

B.

a) Details of spore observations under a compound microscope (x 150, x 1000)

a) size: 237.5-357.0 x 175.0-357.0 µm
b) hyphal attachments: septate and paler in colour than the spore
c) suspensor cell: borne terminally on the septate hypha (20.4 -30.6 µm)
d) germination shield: very difficult to see except after bleaching as recommended by Koske and Walker (1985). 81.6-51.0 µm (LxB)
e) collar: absent
f) wall characteristics: 2 wall groups made up of 3 wall types. Group A consists of an ornamented unit wall 5.0-10.0 µm) with large widely spaced patch-like warts (10.2-40.8 (61.2) µm apart). The warts are of varying sizes ranging from 5.1-20.4 (40.8) µm and separated by ridges of 5.0-10.0 µm. The unit wall is closely adhered to a laminate wall (8.0-10.0 µm).
Group B has a readily separable membranous wall 0.5-1.0 µm thick enclosing the spore contents.

C. Distinguishing characteristics.

The patch like warts reminiscent of the scales of on the cape of *Amanita muscaria* (Fr.) S.F. Gray on the spore surface distinguish *S. coralloidea* from other dark spored species (Koske and Walker 1985). Some of the patches fuse with one another and are separated from one another by spaces of 5.0-16.0 µm. Large warts are most times dimpled at the centre giving a pitted appearance.

D. Ecology: As with most species of *Scutellispora, S. coralloidea* has been reported mainly from sand dunes (Koske and Walker, 1985). This probably reflects interests in areas and habitats under study as no reports exclude its presence from other habitats. Recovery of spores of *S. coralloidea* in the tropical rainforest at Mbalmayo reflects ubiquity. In fact Mosse *et al* (1981) noted that species of *Gigaspora* and possibly *Scutellispora* are very common in the tropics. *S. coralloidea* form mycorrhizal associations with *Fragaria chiloensis* and *Allium cepa* in pot cultures.

E. Taxonomic source: Koske, R.E. and Walker, C. 1985. *Mycologia* 77: 702-720.

Plate 3.5

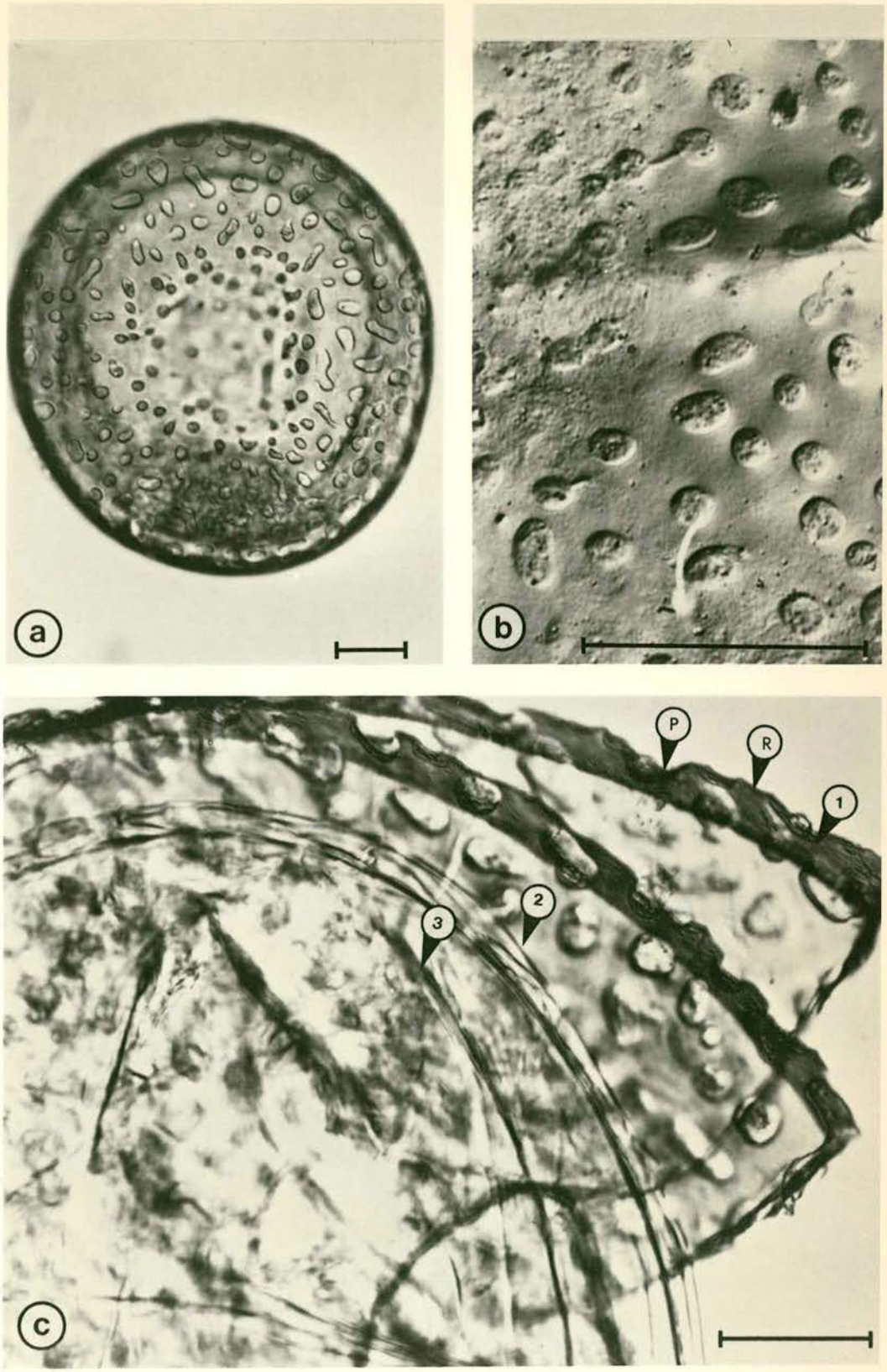
Scutellispora coralloidea

a) Intact spore of *Scutellispora coralloidea* showing patterns and distribution of flattened warts
Scale 11 mm = 50 µm

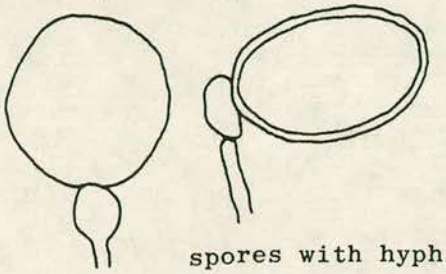
b) Focusing on the wart-like ornamentations
Scale bar 48 mm = 50 µm

c) Details of wall configuration
Wall 1 = ornamented unit wall where there are Pits (P) and ridges (R).
Wall 2 = laminate wall.
Wall 3 = membranous wall.

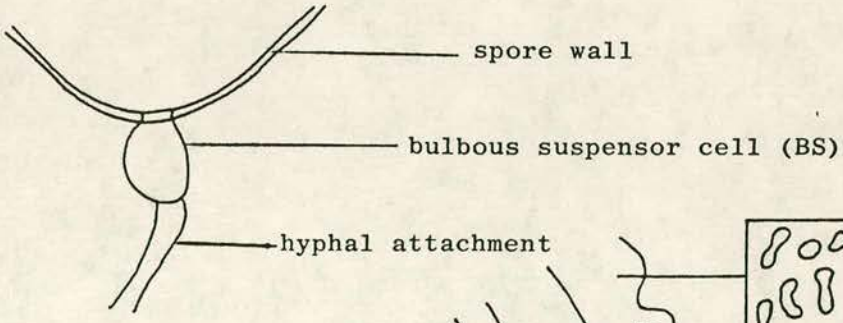
*This species needs to be propagated in pot culture to ascertain if ornamentations are truely warts or pits.


a
b
c
P
R
1
2
3

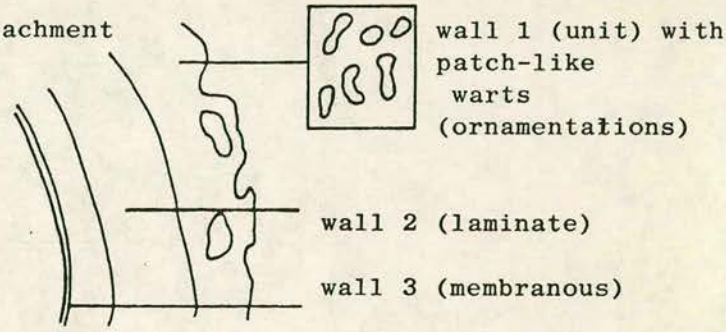
Range of spore shapes



spores with hyphal attachments



Details of wall structure



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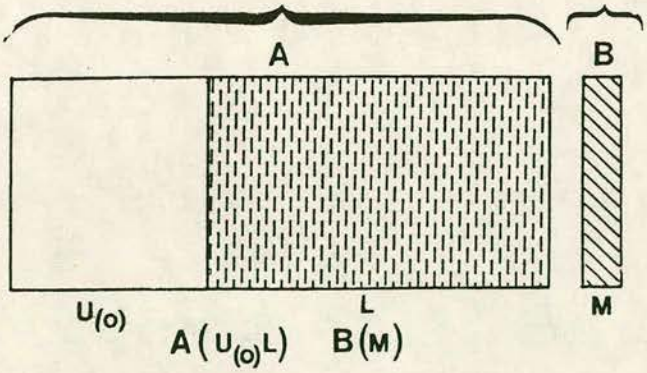


MURONYM

* diagrams not drawn to scale

3.7.6 *Acaulospora mellea/A. morrowae* (Spain and Schenck)

A. Details of spore observations made under a dissecting microscope (x 50 magnitude).

a) shape: globose to globose, ellipsoid to irregular
b) texture: smooth walled
c) colour: pale yellow to honey coloured
d) sporiferous saccule: hyaline and thin walled
e) spore contents: glistening and globular
f) reaction in Melzers: positive, staining deep maroon.

B. Details of spore observations made under a compound microscope (x 150, x 1000).

a) size: 91.8-153.0 x 81.6-132.6 µm
b) hyphal attachments: usually present on spores
c) suspensor cell: absent
d) germination shield: absent
e) collar: present
f) wall characteristics: 3 wall groups made up of five walls.
Group A consists of a unit wall (1.0 µm) closely appressed to a laminate wall (2.5 µm).
Group B consists of a membranous wall separating from Group A.
Group C consists of a beaded membranous wall (1.0 µm) and a membranous wall (1.0 µm) which stains deep purple in Melzers reagent within a very short time.

C. Distinguishing characteristics

A. morrowae is a small spored species that overlaps in spore diameters with *A. mellea* and *A. longula*. However, it is separated from these species by its glistening colour under transmitted light, and its multiple walls. Spores of *A. mellea* are deeper yellow in colour while those of *A. longula* are subhyaline to pale yellow; unlike these two spores of *A. morrowae* are bright yellow and appear to 'sparkle'. The reaction to Melzers reagent is similarly faster in spores of *A. morrowae* than in the spores of *A. mellea* and *A. longula* which also do not stain as deeply.

D. Ecology

A. morrowae has been found associated with native grasses at Carimagua in Colombia. Workers like Dr John Dodd (pers. comm.) have found it strongly associated with grasses and rather ineffective in promoting growth in kudzu, a tropical legume.

E. Taxonomic source: Schenck, N.C.; J.L. Spain, E. Sieverding and R.H. Howeler. 1984. Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia* 76: 685-699.

Plate 3.6

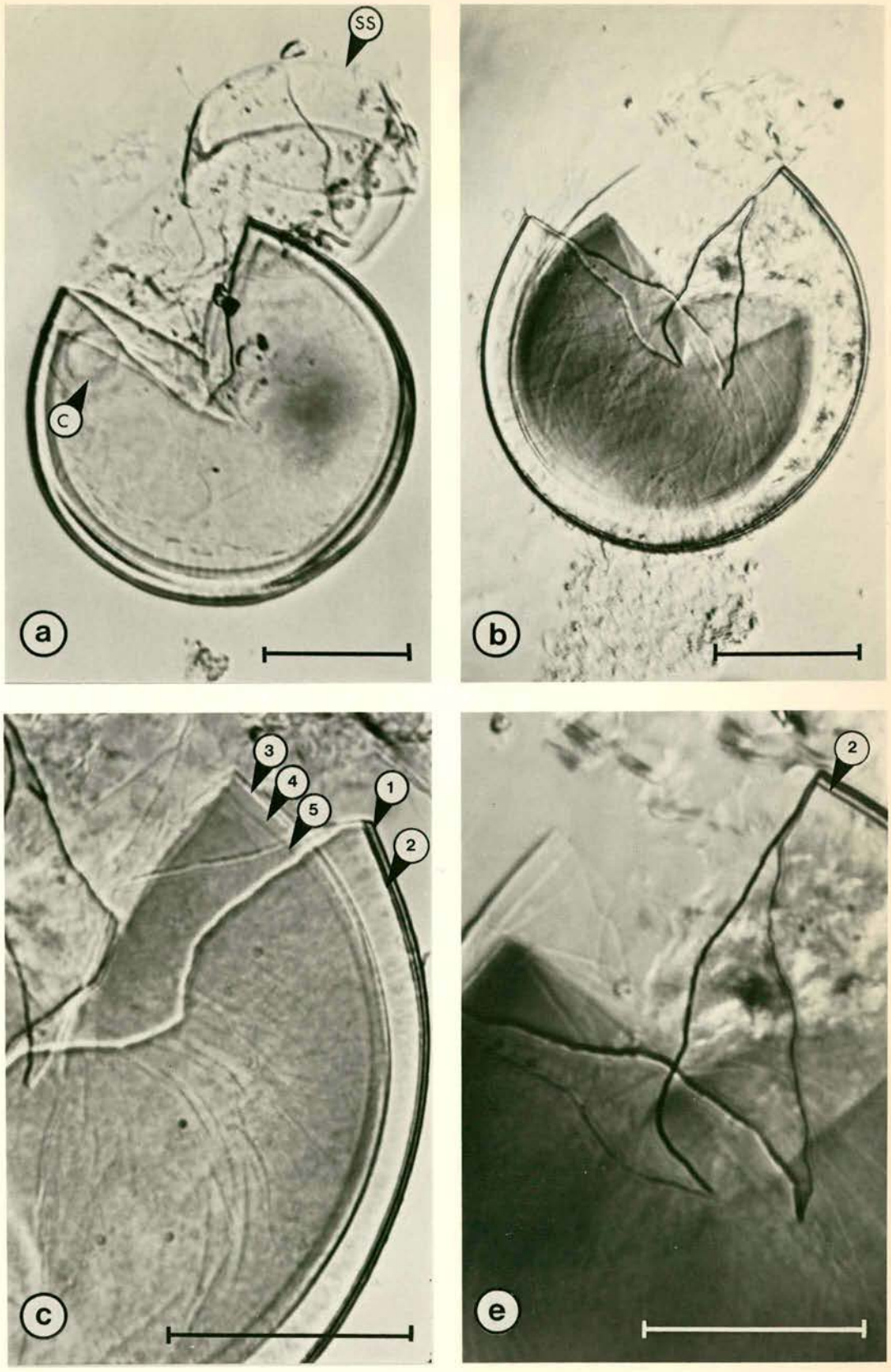
Acaulospora mellea/morrowae

a) Whole spore showing attached sporiferous saccule (SS) and collar (C)
Scale bar 25 mm = 50 µm

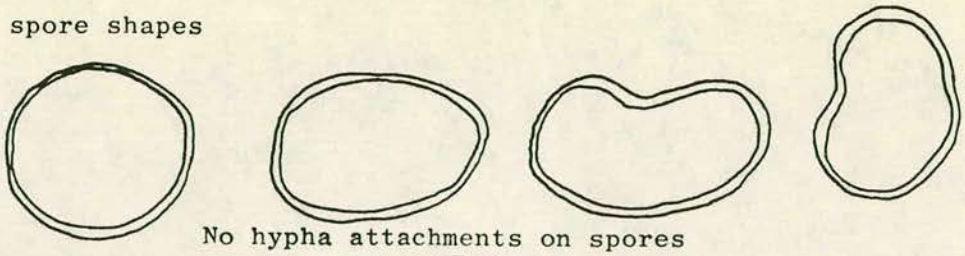
b) Single spore stained in Melzers reagent. See darkening of inner walls. Scale bar 25 mm = 50 µm.

c) Part of spore showing wall configuration
Wall 1 laminate wall
Wall 2 unit wall
Wall 3 membranous wall
Wall 4 membranous beaded wall
Wall 5 Membranous amorphous wall
Scale bar 42 mm = 50 µm

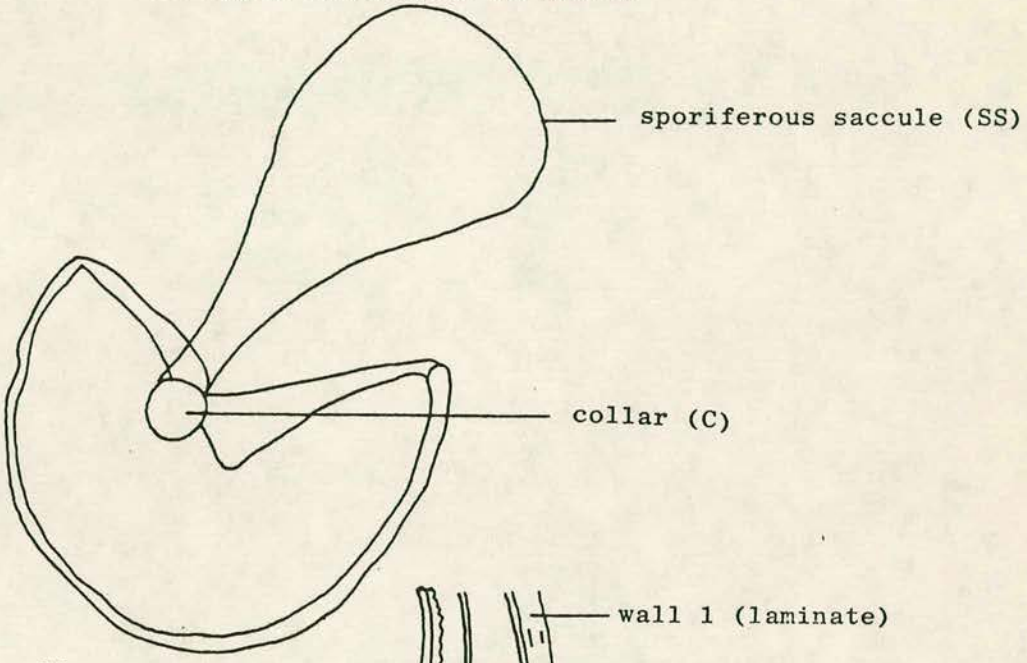
d) Spore mounted on PVL/Melzers with wall 2 shown more clearly
Scale bar 38 mm = 50 µm.


SS
C
a
b
3
4
5
1
2
c
2
e

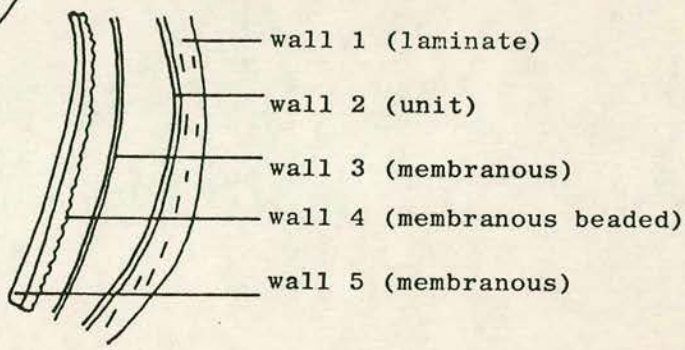
Range of spore shapes



No hypha attachments on spores



Details of wall structure



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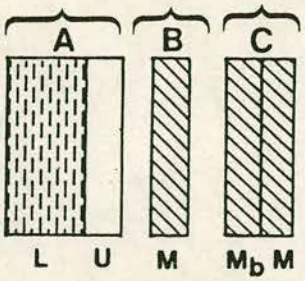


MURONYM A(LU) B(M) C(M_b M)

* diagrams not drawn to scale

3.7.7 *Acaulospora laevis* Gerdemann and Trappe

A. Details of spore observation made under a dissecting microscope

a) shape: mostly globose to subglobose, occasionally reniform
b) texture: smooth looking
c) colour: deep yellow to red-brown
d) spore contents: globular
e) sporiferous saccule: hyaline and thin walled (0.5-1.0 µm).
f) reaction in Melzer's reagent: not observed.

B. Details of spore observations made under a compound microscope (x 150, x 1000 mag)

a) size: 173.4 - 225.0 x 142.8 - 316.2 µm
b) hyphal attachments: generally absent, but points of attachments observed.
c) suspensor cell: absent
d) germination shield: absent
e) collar: present, 20-27 µm in diameter
f) wall characteristics: 3 walls observed in two wall groups. Wall group A is made up of a laminate wall (3.0-5.0 µm) readily separating from wall group B, which consists of a thin hyaline membranous wall (1.0 µm) which rarely separates from a much thicker (2.5 µm) wrinkled or beaded membranous wall.

C. Distinguishing characteristics

A. laevis spores closely resemble those of *A. mellea* or *A. morrowae* in appearance and colour, but are consistently larger in size than the above two. Also *A. laevis* has only three walls in two groups whereas *A. mellea* (Schenck *et al* 1984) has 5 walls in 3 groups and *A. morrowae*, 4-5 walls in three groups.

D. Ecology: *A. laevis* spores have been recovered from the coast of Northern California to Washington. It has been widely observed in a range of habitats including the rhizosphere of little, blue stem (Dickman *et al.*, 1984), the rhizosphere of six agricultural crops (Schenck and Kinloch, 1980), a riperian forest on the Santa Catalina mountains associated with *Agave Schotii, Dasylirion wheeleri, Mimosa biuncifera* (Bloss and Walker, 1983) from a coniferous forest in New Zealand (Johnson 1977) and from the rhizosphere of poplars (Walker *et al.* 1982). From tropical habitats, the presence of species of *Acaulospora* have been reported although the species were largely unidentified (Louis and Lim, 1987; Sharma *et al.* 1984; Redhead, 1977). However, Sieverding (1989) has reported the presence of *A. laevis* in tropical soils and noted that this species generally occurred under a narrow range of environmental conditions.

E. Taxonomic source: Gerdemann, J.W. and Trappe, J.M. 1974. Mycologia memoir No. 5 page 76

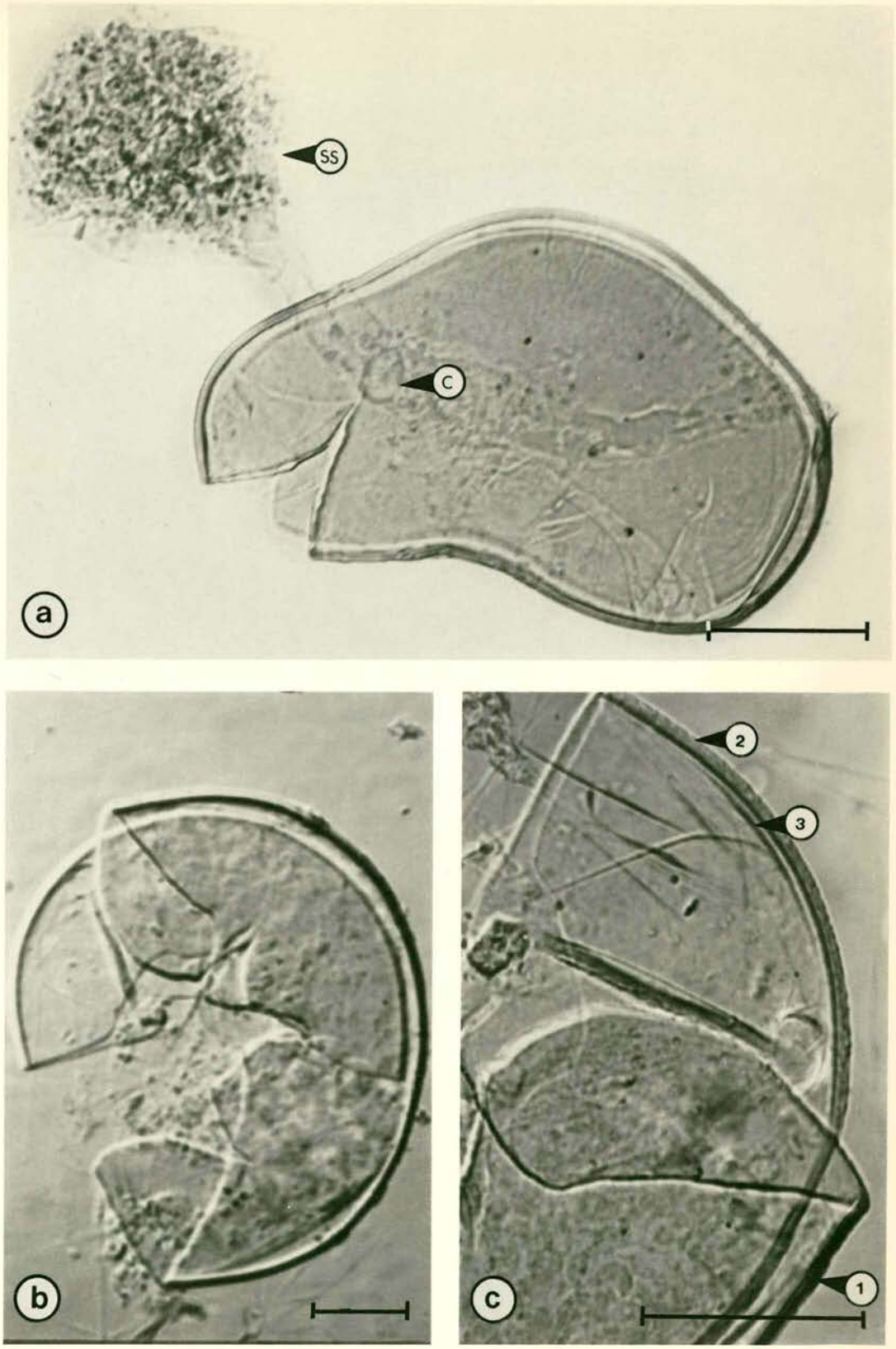
Plate 3.7

A. laevis

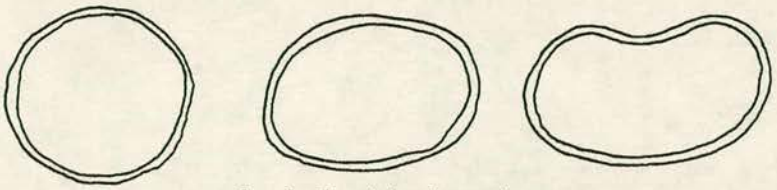
a) Whole spore with attached sporiferous saccule (SS) and showing collar (C)
Scale bar 27.3 mm = 50 µm

b) Crushed single spore showing separation of walls
Scale 16.5 mm = 50 µm

c) The different wall types: wall 1 = laminate wall
wall 2 = membranous wall
wall 3 = beaded membranous wall
Scale bar: 83 mm = 50 µm

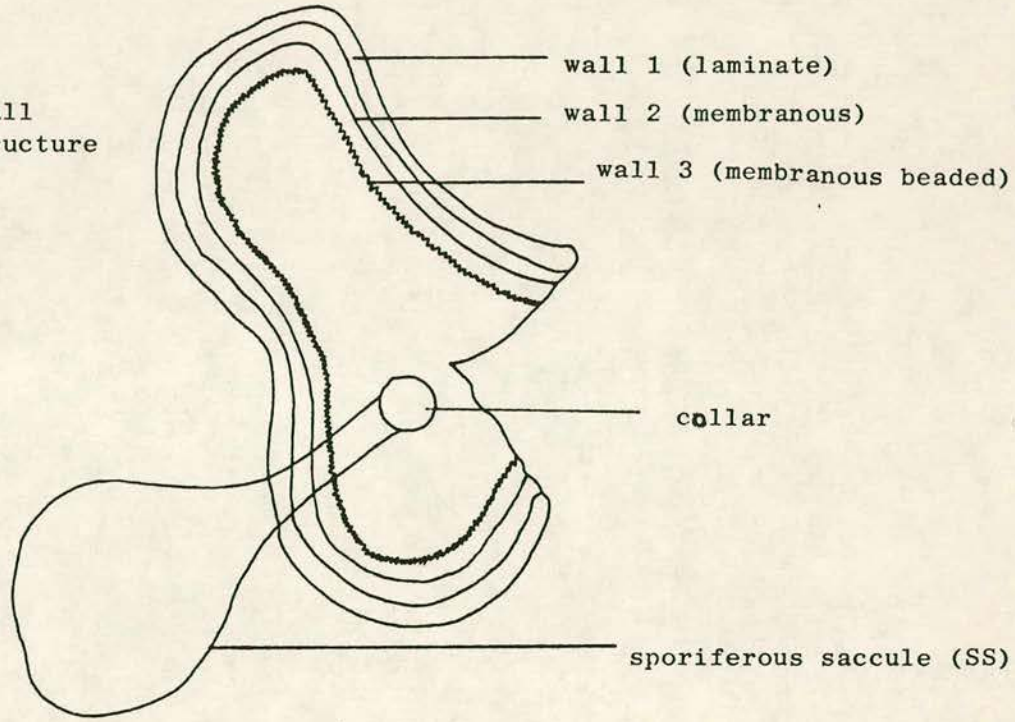

SS
C
a
b
2
3
1
c

Range of spore shapes



no hyphal attachments on spores

Details of wall structure



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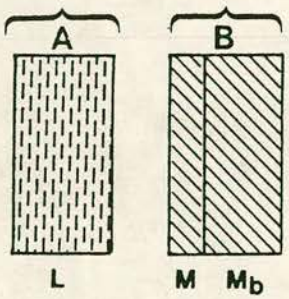


MURONYM A(L) B(MM)

* diagrams not drawn to scale

3.7.8 C22 (*Acaulospora sp?)*

A. Details of spore observations made under a dissecting microscope (x 50, magnification).

a) shape: mostly globose to subglobose, but could be irregular and formed singly in soils.
b) texture: smooth looking.
c) colour: cream to light yellow
d) spore contents: glistening and granular
e) sporiferous saccule: not observed
f) reaction in Melzer's: slight pink colouration

B. Details of spore observations made under a compound microscope (x 150, x 1000)

a) size: 102.0-122.4 (-150.0) x 75.0-100.0 (-112.2) µm
b) hyphal attachments: absent
c) suspensor cell: absent
d) germination shield: absent
e) collar: 12.0-14.0 µm diameter
f) wall characteristics: 3 wall groups with 5 walls
wall group A consists of a sloughing unit wall (1.0-1.5 µm) adhered to a laminate wall (2.5-3.8)(6.0) µm and a unit wall (1.0 µm)
wall group B consist of a thin membranous wall (2.0-2.5 µm) looking like overlaying plates. The surface view of this wall looks like polyhedral reticulation while the plate-like structures vary from 10.0-12.5 µm in diameter.

C. Distinguishing characteristics: The species C22 is identified as belonging to the genus *Acaulospora* because of the presence of a collar. As possesses plate-like ornamentations in the inner membranous wall not observed in other species of *Acaulospora* and this stains rather faintly in Melzers reagent.

D. Ecology: Recovered in this rainforest at Mbalmayo from the root zones of *T. ivorensis*, ferns and herbaceous plant species such as *Costus afer* and *Eupatorium odoratum*. This species needs to be propagated in pot culture to enable more accurate identification and perhaps later publication as a new species.

E. Taxonomic source: None at present.

Plate 3.8

Species C22 (*Acaulospora?*)

a) A group of crushed spores at low magnification (x 10)
scale bar, 10.8 mm = 125 µm
N.I. optics

b) An enlarged single crushed spore of C22
Scale bar 17 mm = 55 µm

c) An enlarged intact spore of C22
Scale bar 20 mm = 50 µm

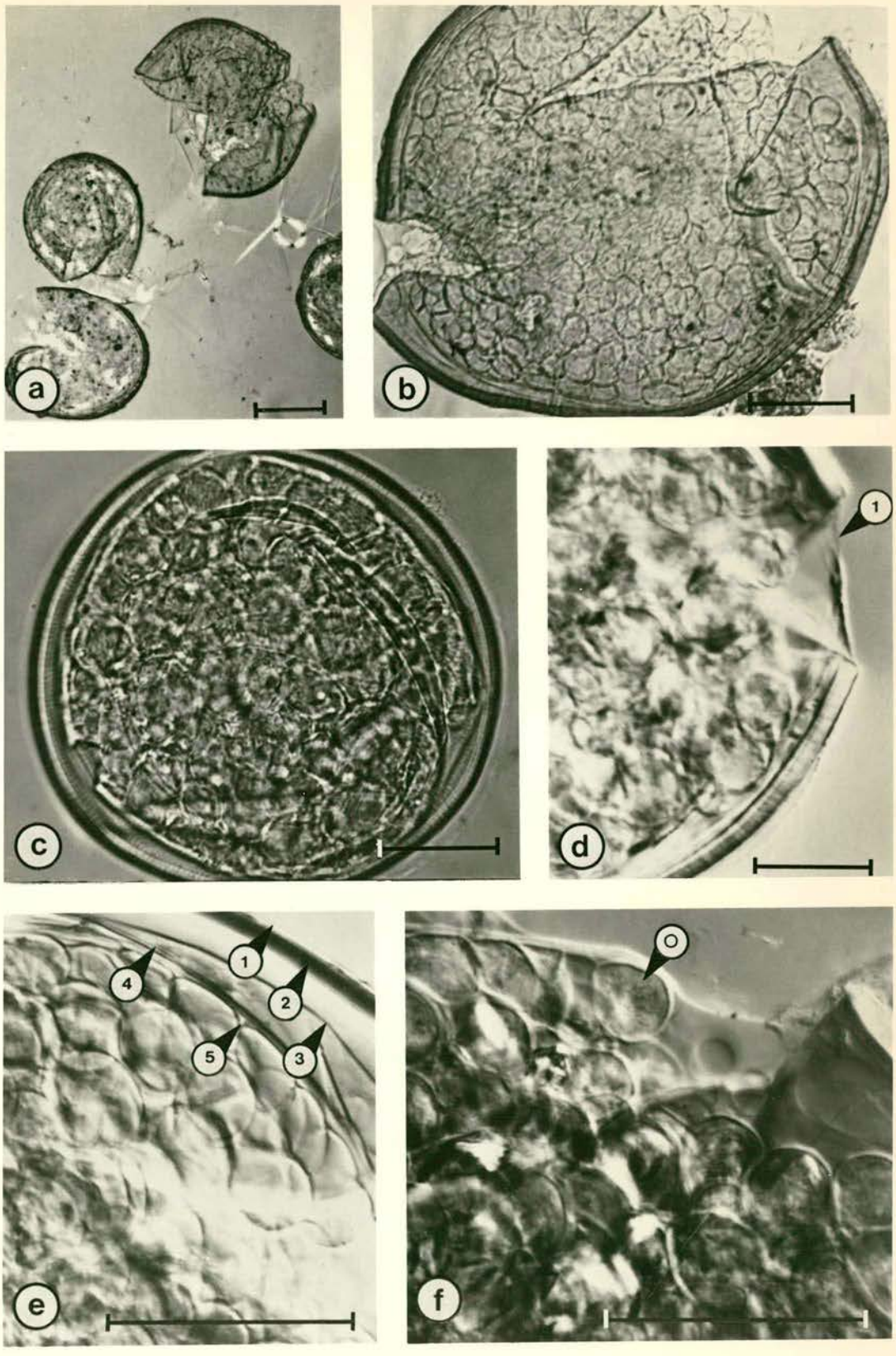
d) Part of crushed spore showing outermost wall still intact
Scale bar 19.5 = 50 µm

e) Section showing all the wall types

Wall 1, sloughing unit wall)	
Wall 2, laminate wall)	Group A
Wall 3, unit wall)	
Wall 4, membranous wall	Group B
Wall 5, ornamental membranous wall	Group C

Scale bar, 42 mm = 50 µm

f) Details of plate-like ornamentations
Arrow 0 pointing to the ornamentations
Scale bar 45 mm = 50 µm


a
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c
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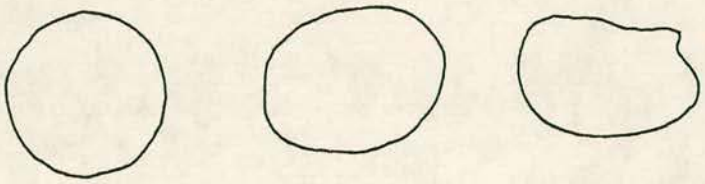
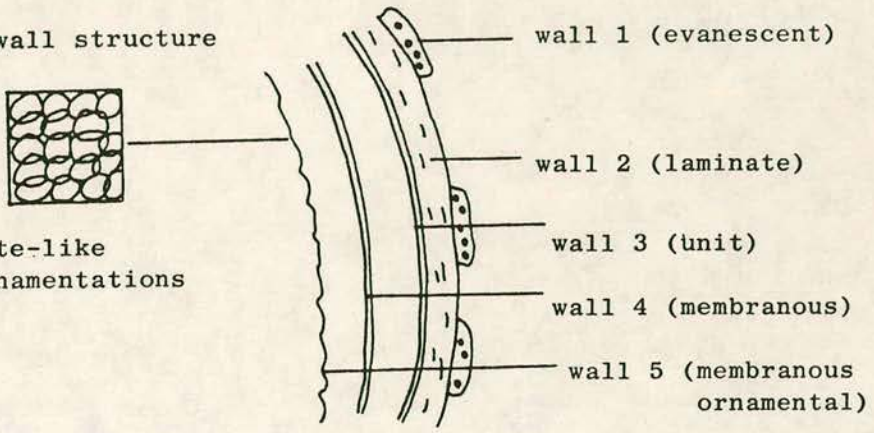
O

Range of spore shapes



Hyphal attachments rarely seen

Details of wall structure



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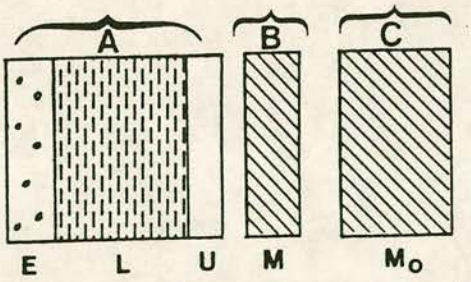


MURONYM

A(ELU) B(M) C(Mo)

* diagrams not drawn to scale

3.7.9a *Sclerocystis pachycaulis* Wu and Chen

A. Details of spore observations made under a dissecting microscope (x50 magnification).

a) shape: sporocarp shapes range from globose to subglobose while the clamydospore shapes range from globose to subglobose, ovoid to obovoid and ellipsoid to irregular.
b) texture: rough looking (berry-like) due to exposed spore tips
c) colour: yellow to brown
d) spore contents: granular
e) sporiferous saccule: absent
f) reaction in Melzer's reagent: negative

B. Details of spore observations under a compound microscope (x 150, x 1000)

a) size: sporocarp sizes, 152.8-160.1 x 152.8 - 158.6 µm while clamydospore sizes range from 60.0-80.0 x 56.0-68 µm
b) hyphal attachment: short (40.0 µm) and thickwalled at base of clamydospore (10 µm thick). Irregular thickening of hyphal attachment such that in 2 dimension, the hyphal wall on one side of the narrow lumen appears much thicker than on the opposite side.
c) suspensor cell: absent
d) germination shield: absent
e) collar: absent
f) wall characteristics: one wall group A, consisting of two walls wall 1 is a thick laminate wall 2-4 (-6) µm and a membranous wall 2 (1.0-1.5 µm) which sometimes collapses in PVL.

C. Distinguishing characteristics

Because so few *Sclerocystis* species have been described, it was relatively easy to differentiate between *S. pachycaulis* and other species of *Sclerocystis*. For example, although *S. pachycaulis* closely resembles *S. rubiformis* in spore size and gross morphology, the former has a hyaline separable outer wall not present in *S. rubiformis*, and a short thickwalled attached hypha with a very narrow lumen not present in other species of *Sclerocystis*.

D. Ecology

S. pachycaulis has been recovered from an experimental bamboo forest in Central Taiwan from the rhizosphere of *Gonestegia hirta* (Blume) miq., *Polygonum hydropiper*, *Microstegium geniculatum* and *Asplenium normale*.

E. Taxonomic source: Wu, C.G. and Chen, Z.C. 1986.

The Endogonaceae of Taiwan: 1. A preliminary investigation on *Endogonaceae* vegetation at Chi-Tou areas, Central Taiwan. Taiwania, 31: 65-68.

3.7.9b *Sclerocystis microcarpus* Iqbal and Bushra

A. Details of spore observations made under a dissecting microscope (x50 magnification).

a) shape: sporocarp globose to subglobose with spores formed radially in a single tightly packed layer. The clamydospores are clavate or cylindric to clavate.
b) texture: rough, verrucose from exposed clamydospore tips.
c) colour: dark brown
d) spore contents: granular
e) sporiferous saccule: absent
f) reaction in Melzer's reagent: negative.

B. Detail of spore observations made under a compound microscope (x 150, x 1000).

a) size: sporocarp sizes range from 306.0 - 408.0 µm while clamydospore sizes range from 61.2 - 91.8 x 30.6 - 61.2 µm.
b) hyphal attachments: thick walled at clamydospore base with an opening of the lumen to the spore.
c) suspensor cell: absent
d) germination shield: absent
e) sporiferous saccule: absent
f) collar: absent
g) wall characteristics: one laminate wall, which is very thick at the apex of clamydospores 10.0-15.3 µm and very thin at the sides 1.0-2.5 µm

C. Distinguishing characteristics: Only nine species of *Sclerocystis* have so far been described, thus it was fairly easy to find out if the *Sclerocystis* species from the Mbalmayo forest had been described earlier. *S. microcarpus* was very similar to *Sclerocystis clavispora*, however the former had smaller sporocarp and clamydospore sizes than the latter (Iqbal and Perveen, 1980).

D. Ecology: Most of the described species of *Sclerocystis* have been recovered from the rootzones of tropical or subtropical plants mainly grasses and ferns; for example *S. clavispora* recovered from Tropical Mexico and seen associated with roots of grasses and *Saccharum officinarum* (L).

A species similar to *S. clavispora* was also reported by Thapar and Khan (1973) from India while *S. coremioides* has been reported in the greenhouse of the department of Botany, Oregon State University, where it fruits abundantly on the soil of organic matter of pots of tropical and sub tropical plants, e.g. of *Musa* spp. throughout the year, and from Florida (Schenck and Hinson, 1971). It is thought to have been introduced having otherwise been reported only from the South Pacific tropics (Thaxter, 1922).

According to Sieverding (1989) *Sclerocystis* sp. may occur frequently in tropical savanna areas under scrubs or permanent crops like sugar-cane or in permanent mixed cropping systems. In addition, Sieverding (1989) noted that when native ecosystems were taken into agronomic use with one or few crops, the *Sclerocystis* sp. were the first to disappear from limed and fertilized treatments.

S. microcarpus was found in Pakistan from the rhizosphere of ferns under coniferous forests in Khanspur. In Cameroon where this species was also found, the understorey of the Forest at Mbalmayo consisted of some ferns (e.g. *Arthropteris cameroonensis*). This species thought to have a small host range (Sieverding, 1989) may have been restricted only to these ferns as their sporocarp numbers were quite few.

E. Taxonomic source: Iqbal S.H. and Perveen, B. 1980. Some species of *Sclerocystis* (*Endogonaceae*) from Pakistan. Trans. Mycol. Soc. Japan 21:57-63.

Plate 3.9

Sclerocystis species

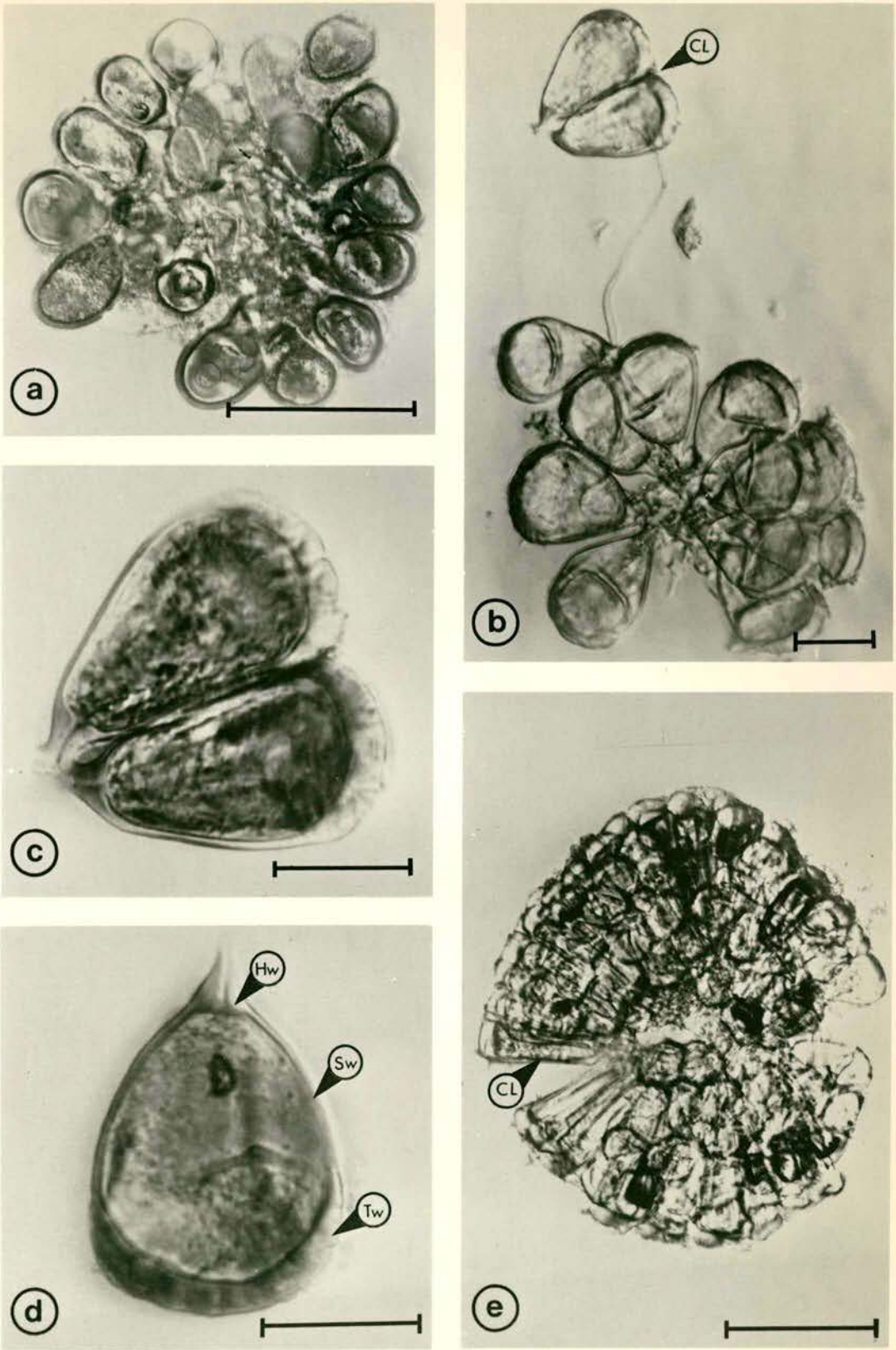
a) Sporocarp of *Sclerocystis pachycaulis* sliced into 2 open to reveal spore arrangement
Scale bar 32 mm = 100 µm

b) Teased sporocarp separating two clamydospores (CL)
Scale bar 12.75 m = 25 µm

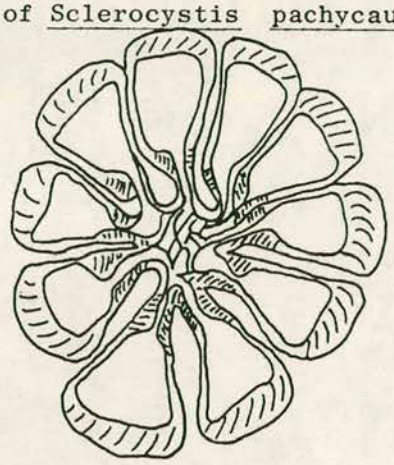
c) Two clamydospores showing interconnecting lumen, thick wall at the tops of clamydospores and thin walls at the sides. Hyphal attachments short but thickened towards the base of clamydospores.
Scale 23 mm = 25 µm

d) Single clamydospores of *Sclerocystis pachycaulis* showing thickened top wall (TW), thin side walls (SW) and thick hyphal wall (HW) at spore base.
Scale 26.5 mm = 25 µm

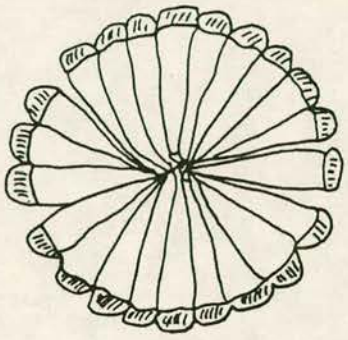
e) Sporocarp of *Sclerocystis microcarpus*
See the much slender clamydospore compared with (b)
Scale 25 mm = 100 µm

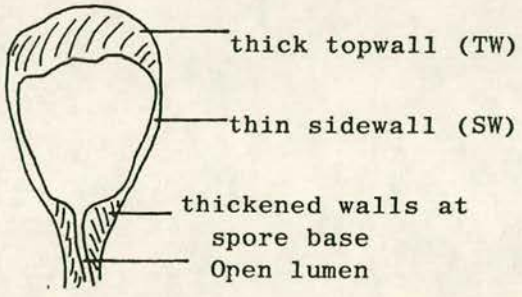

a
CL
b
c
Hw
Sw
Tw
d
CL
e

Transverse section of sporocarp of Sclerocystis pachycaulis

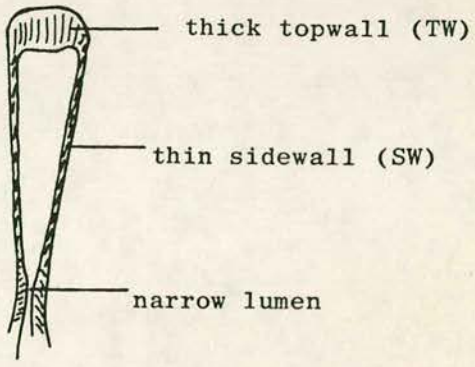


Transverse section of sporocarp of Sclerocystis microcarpus

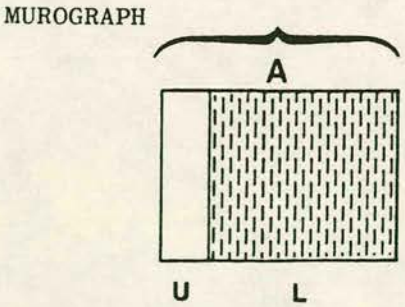




Obovoid clamydospore



clavate clamydospore



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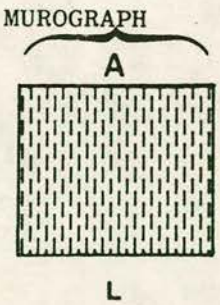


MURONYM A(L)

* diagrams not drawn to scale

3.7.10a *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend Walker and Koske.

A. Details of spore observations made under a dissecting microscope (x50 magnification).

a. Shape: Globose to subglobose. Spores formed singly or in loose clusters of 1-3 spores in soils, and always with an attached hypha.
b. Texture: smooth-looking but may have adhering debris
c. Colour: pale yellow with a greenish tinge when viewed under transmitted light.
d. Spore contents: Granular.
e. Sporiferous saccule: absent.
f. Reaction with Melzers reagent: staining red-brown.

B. Details of spore observations made under a compound microscope (x150, x1000).

a. Size: 51.0-81.0 x 55.0-77.0 µm.
b. Hyphal attachments: Always present and up to 7.5 µm wide; paler in colour than the spore.
c. Suspensor cell: Absent
d. Germination shield: Absent
e. Collar: Absent
f. Wall characteristics: 3 wall types in one wall groups A. Wall group A consisting of a unit wall 0.5-1.0 µm which is appressed to a thick laminate wlal 4.0-6.0 µm (-10 µm) and a membranous wall (1.0-2.0 µm) which sometimes recedes in PVC giving the impression of a separate wall group, and stains red-brown in Melzers reagent.

C. Distinguishing characteristics.

G. fasciculatum sensu stricta is distinguished from other species by its spores which are pale yellow to pale yellow-brown, with a broad thick walled subtending hypha (Walker and Koske, 1987).

D. Ecology

G. fasciculatum has been reported as widely distributed. It has been recovered from California to British Colombia from the coast to near timberline across the Cascade Mountains into Idaho dunes, cultivated fields, meadows, orchards and forests (Gerdemann and Trappe, 1974). The presence of this species has been reported from a rainforest in Nigeria (Redhead, 1977), pioneer grasses of a maritime sand dune (Nicolson and Johnston 1979), cleared woodland (Schenck and Kinloch, 1980), from two sites in Central Iowa (Walker et al., 1984) and from semi and zones of India (Mukerji and Kapoor, 1986). *G. fasciculatum* remains one of the species which has been widely erroneously identified (Walker and Koske, 1987).

E. Taxonomic source: Walker C and Koske R E, 1987. Mycotaxon 30: 253-262.

3.7.10b *Glomus macrocarpum* Tul and Tul emend. Berch and Fortin

A. Details of spore observations made under a dissecting microscope (x50 magnification)

a. shape: globose, subglobose to ellipsoid and formed singly in soils
b. texture: smooth walled
c. colour: yellow to brown
d. spore contents: globular oily contents
e. sporiferous saccule: absent
f. reaction with Melzers reagent: none

B. Details of spore observations made under a compound microscope (x 150, x 1000)

a. size: 125.0-150.0 x 112.5-122.5 µm
b. hyphal attachments: straight to slightly flared, 50.0-120.0 µm long and 1.5-10.0 µm wide at spore base decreasing to 2.0 µm away from spore base. Pore opening into hypha.
c. suspensor cell: absent
d. germination shield: absent
e. collar: absent
f. wall characteristics: two wall types in a single wall group. Wall 1 is a unit wall 0.5-1.0 µm thick appressed to a thick laminate wall 4.0-6.0 µm.

C. Distinguishing characteristics

G. macrocarpum spores are distinguished from those of *G. fasciculatum* by generally being bigger in size and possessing two walls as opposed to 3 of the latter, and *G. microcarpum* with one wall.

D. Ecology

Thaxter (1922) first encountered *G. macrocarpum* in earth in greenhouse pots at the Botanic Garden in Cambridge, New England associated with *Hymcnogaster Klotschii* and *Hydnangium carneum*, a habitat and association also noticed in Europe. More recently reports of the presence of this fungal species have been made by Louis and Lim (1987) from a lowland rainforest in Singapore. Like *G. fasciculatum*, *G. macrocarpum* has been widely misidentified. Certain Australian collections examined by Tandy (1975) were in fact *G. caledonium*. Nicolson and Schenck (1979) found two main forms of *G. macrocarpum* in Florida which on re-examination revealed other taxa misidentified as *G. macrocarpum*.

E. Taxonomic source: Berch, S.M. and Fortin, J.A. 1983. Leptotypification of *Glomus macrocarpum* and a proposal of new combinations: *Glomus australe, Glomus vesiforme* and *Glomus tenebrosum* (Endogonaceae). *Can. J. Bot.* 61: 2608-2617.

Plate 3.10

Glomus fasciculatum/G. macrocarpum

a) A loose network of crushed spores of a fasciculatum showing wall 1 = laminate wall.
thickening of hyphal wall (HW) at spore base and thinning away from spore base.
Scale 16 mm = 50 µm.

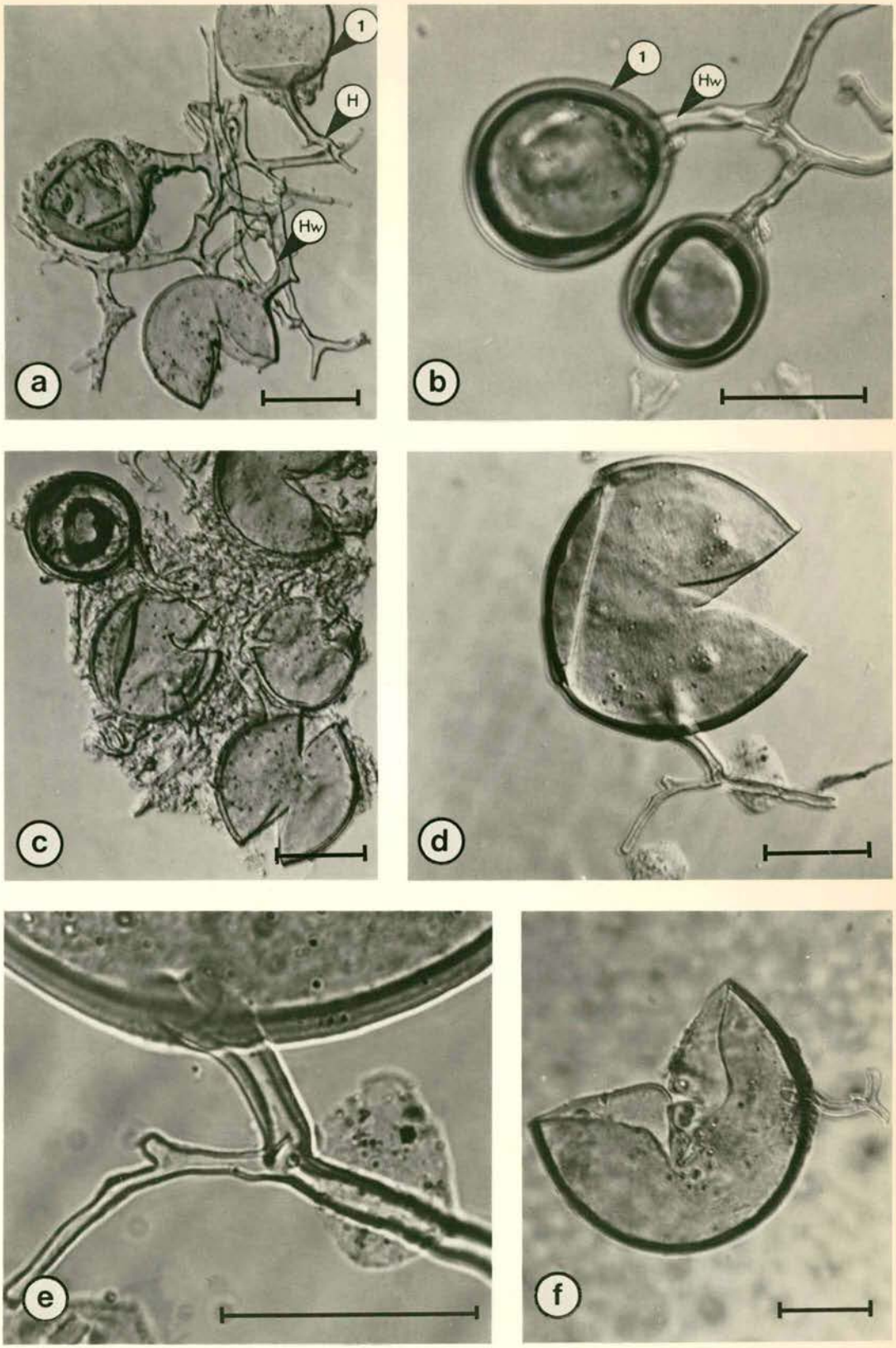
b) Two intact spores of *G. fasciculatum* showing a laminate wall (1), hyphal wall (HW).
Scale bar 24.5 mm = 50 µm.

c) Network of loose spores in PVC/Melzers mountant
Scale bar 15 mm = 50 µm.

d)&
f) single spores of *G. macrocarpum* with attached hypha.
Scale bar 17.5 mm = 50 µm and 15 mm = 50 µm.

e) Details of the walls and hyphal attachment illustrated better here.

Note - wall thicker towards spore base
and thinner towards hyphal attachment.
Scale bar 44 mm = 50 µm.


1
H
Hw
a
1
Hw
b
c
d
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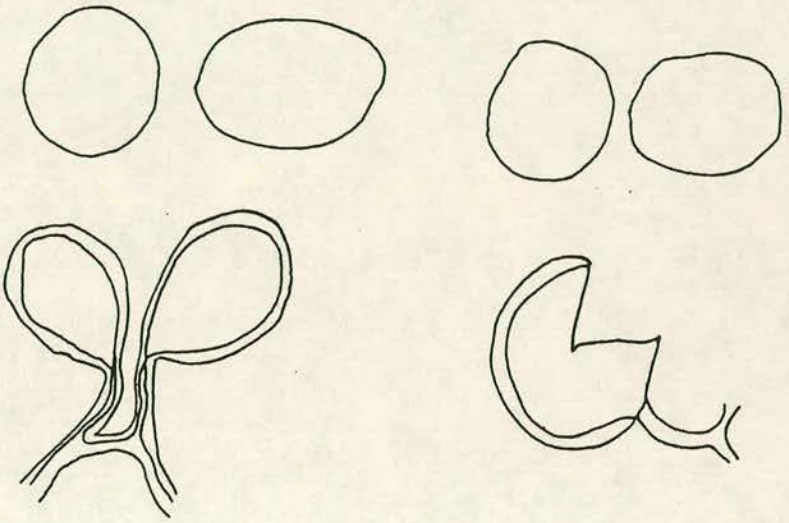
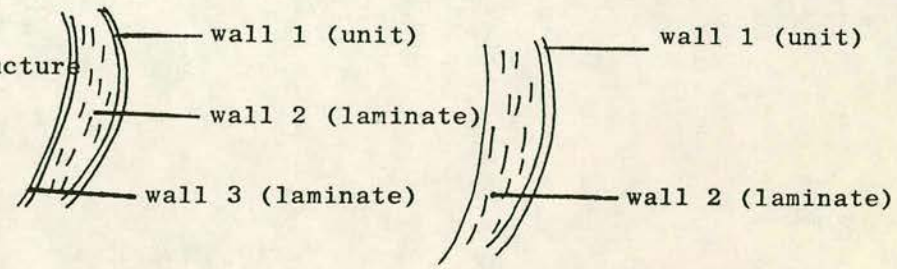
Range of spore shapes

G. fasciculatum

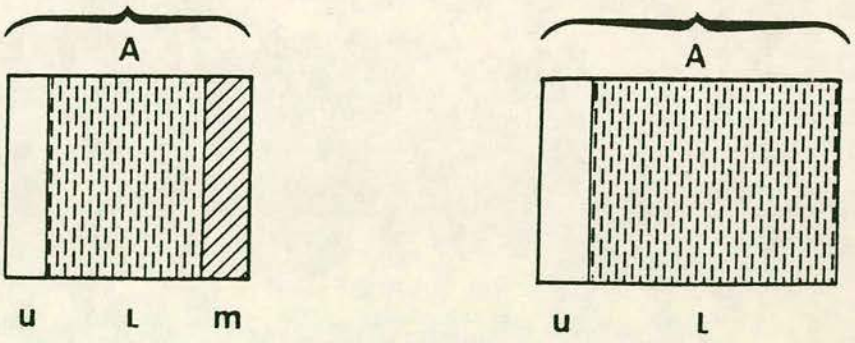
G. macrocarpum



Details of wall structure



MUROGRAPHS



MURONYMS

A(ul m)

A(u,L)

* diagrams not drawn to scale

3.7.11a *Glomus occultum* Walker

A. Details of spore observations made under a dissecting microscope (x 50 magnification).

a) shape: globose to subglobose; sometimes ellipsoid to irregular spores borne singly in soil.
b) texture: very smooth after the mucilaginous well is completely sloughed off.
c) colour: hyaline to white
d) sporiferous saccule: absent
e) spore contents: oily to granular in older spores
f) reaction in Melzer's reagent: negative

B . Details of spore observations made under a compound microscope (x 150, x 1000 magnification).

a) size: 71.4-91.8 x 7.14-81.6 µm
b) hyphal attachment: straight to recurved, non septate with septum formed along the hypha. Width of hyphae range form 50.8 µm to 52.8 µm and the lengths from 50.0-61.2 µm.
c) suspensor cell: absent
d) germination shield: absent
e) collar: absent
f) wall characteristics: one wall group made up of 3 walls. wall group A consisting of a unit wall (1.0 µm) and two hyaline indistinctly laminate walls 2.0 and 2.5 µm respectively.

C. Distinguishing characteristics: *Glomus occultum* is distinguished from other described species of the genus by its colourless ectocarpic clamydospores with the subtending hypha recurved and eccentrically attached (Walker, 1982). Compared with spores of *G. pallidum* Hall and *G. clarum* Nicolson and Schenck which also are hyaline and ectocarpic, the spores of *G. occultum* are smaller, have a narrower size range and a bilaminate wall structure (Walker, 1982).

D. Ecology

G. occultum forms VA mycorrhizas with *Sorghum vulgare, Zea mays* and has been recovered from an old meadow in Central Iowa, a polder in the Netherlands and reclaimed coal mining spoils in Yorkshire, England. *G. occultum* is associated in the fields with roots of several other plant species.
According to Sieverding (1989) this VA mycorrhizal fungus can be found under a broad range of environmental conditions (soil conditions principally) and from the root zones of a broad range of potential agronomic hosts.
In the Mbalmayo forest *G. occultum* seemed to be more associated with the herbaceous species found mostly in large numbers at considerable distances from the tree species.

E. Taxonomic source: Walker, C. 1982. Species of *Endogonaceae*. A new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon* 15: 49-61.

3.7.11b *Acaulospora scrobiculata* Trappe

A. Details of spore observations made under a dissecting microscope (x50 magnification)

a) shape: globose to subglobose or ellipsoid
b) texture: rough looking, evenly pitted wall with circular to elliptical pits (1.5 to 3.5 µm in diameter)
c) colour: white to creamy with a distinct brownish outer wall under transmitted light
d) sporiferous saccule: not observed
e) spore contents: globular
f) reaction in Melzers reagent: negative

B. Details of spore observations made under a compound microscope (x150, x1000)

a) size: 122.4-142.8 (187.5) x 122.4-142.2 µm
b) hyphal attachments: absent
c) suspensor cell: absent
d) germination shield: absent
e) collar: present (10.0 to 11.0 µm diameter)
f) wall characteristics: 3 wall groups consisting of four walls.
- wall group A is a thick sub-hyaline rigid wall with pits (1.5-3.5 µm diam)
- wall group B is a membranous wall which readily separates from group A (1.0-1.5 µm thick)
- wall group C consists of a beaded membranous wall (1.0 µm) and a membranous wall (1 µm)

C. Distinguishing characteristics

Spores of *A. scrobiculata* generally stain pink in Melzers reagent; these species from the Mbalmayo Forest although closely related in description to spores of *A. scrobiculata.* Nevertheless did not stain in Melzers reagent. Similar observations hazve been made by Christopher Walker (pers comm) on spores of *A. scrobiculata* which are not related to age or the method of preservation of the spores.

D. Ecology

Species of *A. scrobiculata* have similarly been reported from other rainforests in the tropics such as the Mexican Tropics and Central Highlands. The species occurs over a wide range of soils with pH 3.8-8.0 and diverse chemical fertility levels (Sieverding 1989) which supports my observations in Mbalmayo where the pH range was 4.4-4.9. Although reports have not been made of this species with tropical forest trees, it has nevertheless been seen associated with tropical plants such as sugarcane. In temperate regions, *A. scrobiculata* has been reported from subalpine meadows of Central Western USA and the lowlands of Japan found associated with *Festuca viridula* and wild grasses.

F. Taxonomic source: Trappe, J.M. 1977. Three new Endogonaceae: *Glomus constrictus, Sclerocystis clavispora, and Acaulospora scrobiculata. Mycotaxon* 6: 359-366.

Plate 3.11

Species: *G. occultum and A. scrobiculata*

a) *G. occultum* (whole spore) with recurved hypha (Hr) and an evanescent
wall (1)
Scale bar 34.5 mm = 50 µm.

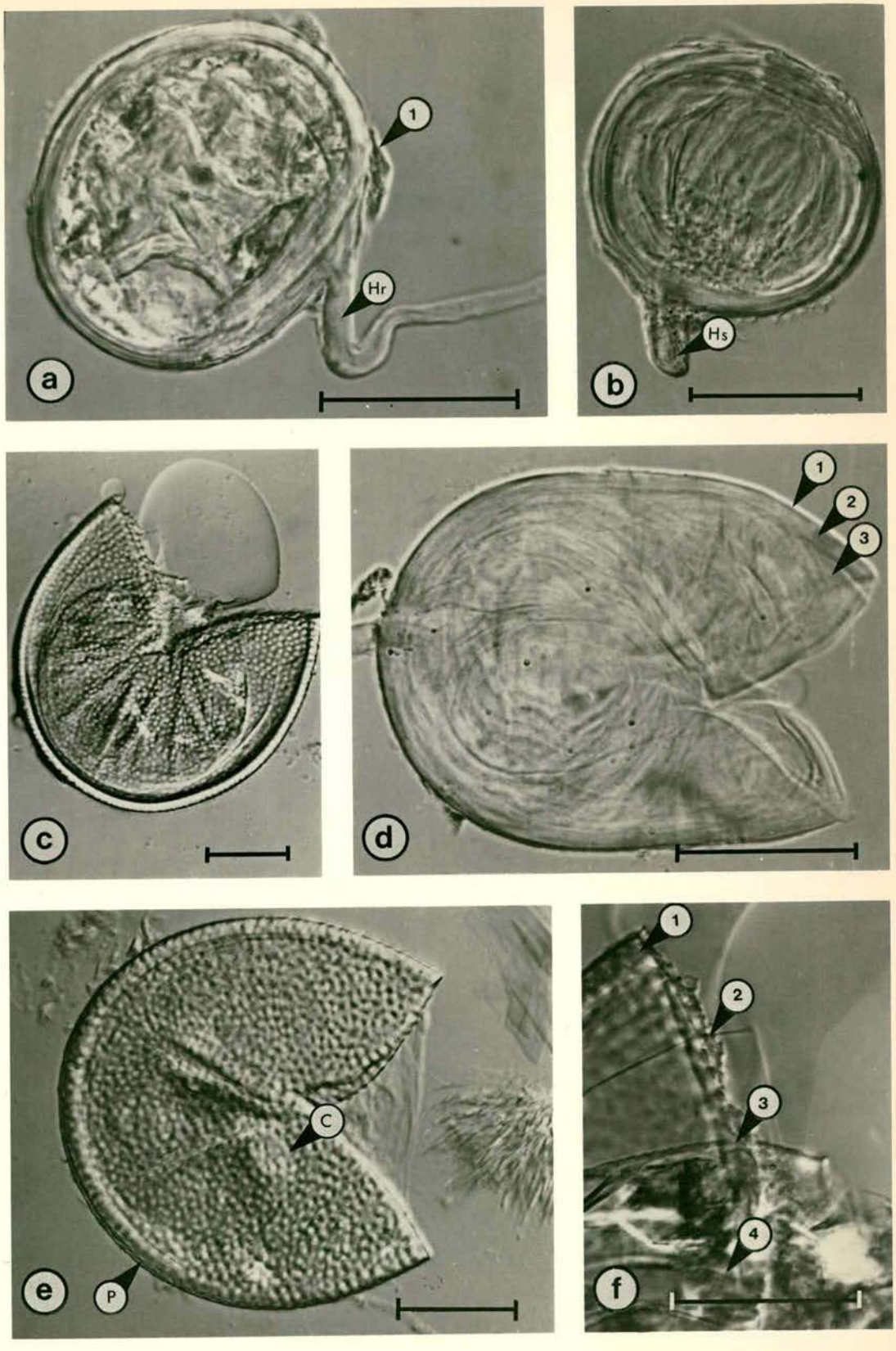
b) Whole single spore of *G. occultum* with straight hypha (HS)
Scale bar 29 mm = 50 µm.

and d) crushed spore of *G. occultum* showing walls
wall 1, unit wall
wall 2, hyaline laminate wall
wall 3, hyaline laminate wall
Scale bar 31 mm = 50 µm.

c) A lightly crushed spore of *A. scrobiculata*
Scale bar 13.5 mm = 50 µm.

e) Crushed spore of *A. scrobiculata* showing evenly distributed pits (P) and a collar (C)
Scale bar 20 mm = 50 µm.

f) Part of spore showing the separation of the different walls:
Wall 1 rigid wall with pits
Wall 2 membranous wall
Wall 3 beaded membranous wall
Wall 4 membranous wall
Scale bar 32.2 mm = 50 µm.


1
Hr
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Hs
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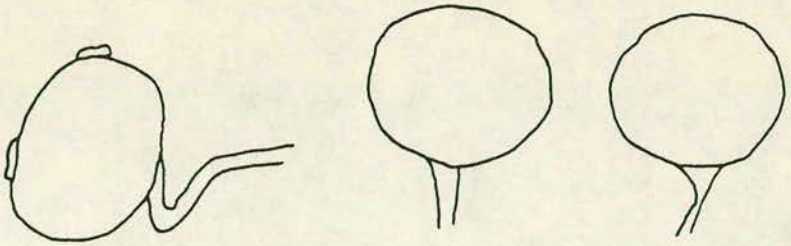
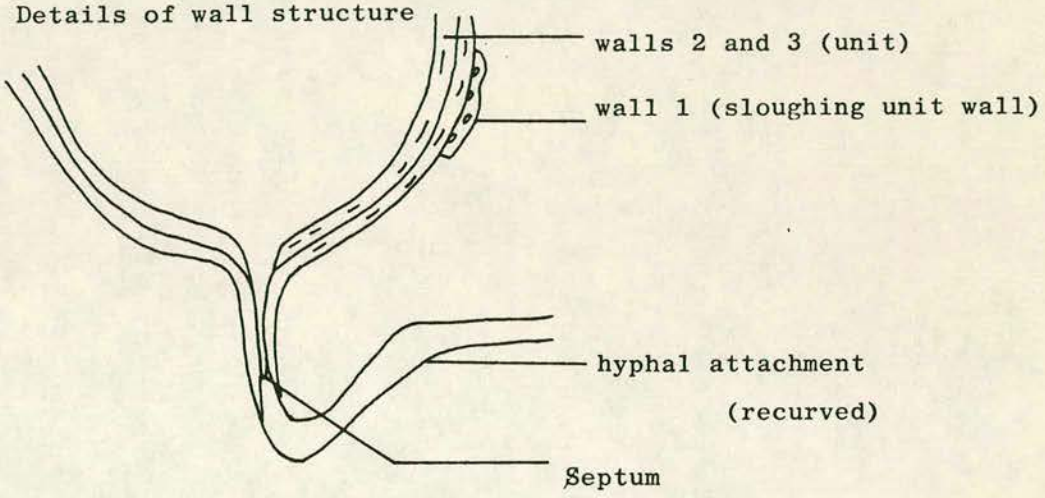
Range of spore shapes



recurved, straight, funnel shaped hyphae

Details of wall structure



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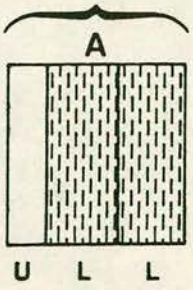
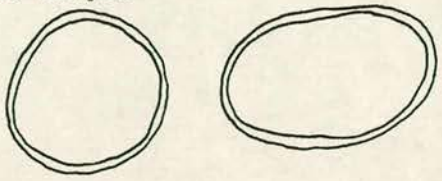


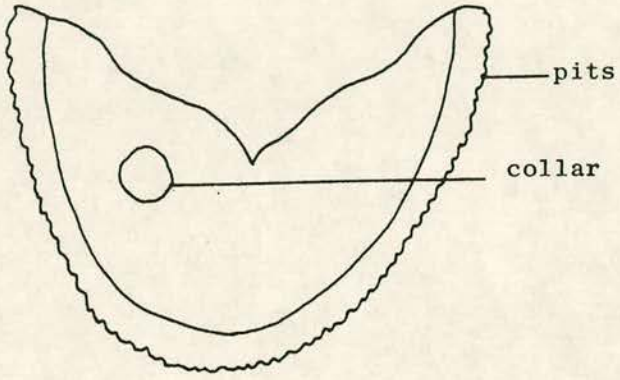
MURONYM A(ULL)

* diagrams not drawn to scale

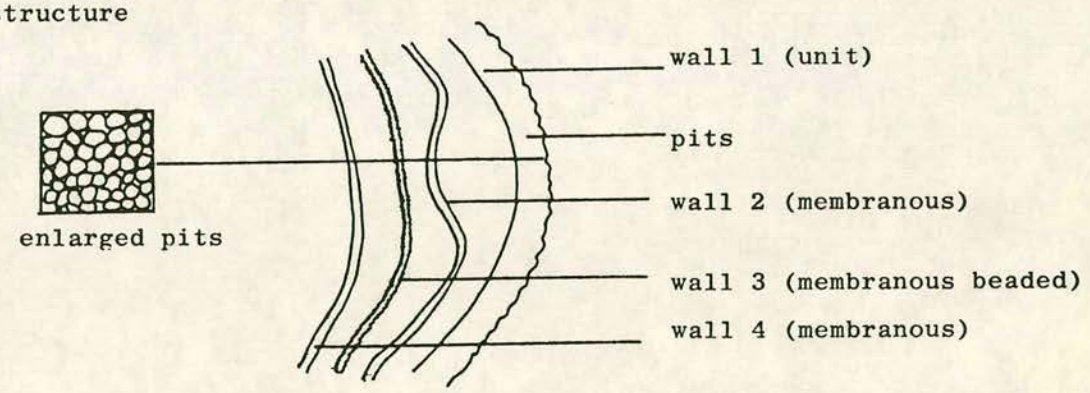
Range of spore shapes



spores with no hyphal attachments



etails of wall structure



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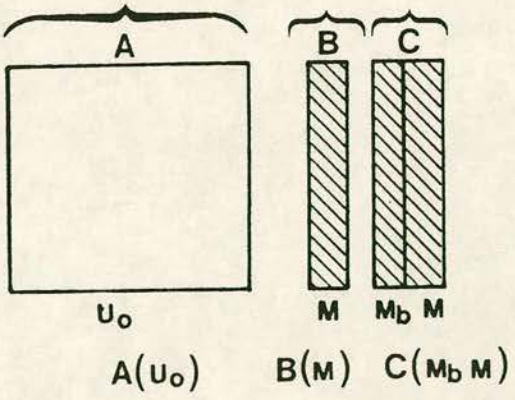


MURONYM

* Diagrams not drawn to scale

3.7.12 *Scutellispora pellucida* (Nicol and Schenck) Walker and Sanders

A. Details of spore observations made under a dissecting microscope (x50 magnification)

a) shape: globose to subglobose or ellipsoid
b) texture: very smooth
c) colour: hyaline to whitish grey
d) spore contents: glistening and granular
e) sporiferous saccule: absent
f) reaction with Melzers reagent: deep purple staining of the innermost walls.

B. Details of spore observations made under a compound microscope (x150, x1000)

a) size: 163.2-204.0 x 153.0-173.0 (-204.0) µm
b) hyphal attachments: hyaline and septate
c) suspensor cell: bulbous 51.0-74.0 µm long and 20.4-30.6 µm wide, borne terminally. The wall 10 µm thickening slightly towards spore base to 2.0 µm.
d) germination shield: very difficult to see but present with edges slightly invaginated.
e) collar: absent
f) wall characteristics: 3 wall groups made up of 5 walls.
Wall group A is a hyaline and brittle unit wall (1.0 µm) closely appressed to two hyaline unit walls (1.0 µm thick respectively).
Wall group C is an amorphous wall which stained deep purple in Melzer's reagent and varies in width from 3.0-5.0 µm.

C. Distinguishing characteristics:

Scutellispora pellucida is differentiated from other light coloured species of Scutellispora by its hyaline spore walls, and contents (Nicolson and Schenck, 1979); the outer wall b eing brittle and easily separable for the more pliable inner wall.

D. Ecology:

In the tropics Sieverding (1989) observed species of *S. pellucida* to occur over a wide range of environmental conditions with a broad range of potential hosts. Nevertheless species fo *Scutispora pellucida* have not been widely reported from tropical rainforests. Their wide range of soil pH over which this species can thrive (Sieverding, 1989) probably explains its presence in the acidic soils of Mbalmayo. In temperate regions *S. pellucida* has been recovered from a newly cleared woodland site in Florida particularly from the rhizosphere of soybeans (Schenck and Kinloch, 1980).

E. Taxonomic source: Nicolson, T.H. and Schenck, N.C. (1979). Mycologia, 71: 178-198. Redescribed by Koske, R.E. and Walker, C. (1986). Mycotaxon, 27: 219-235.

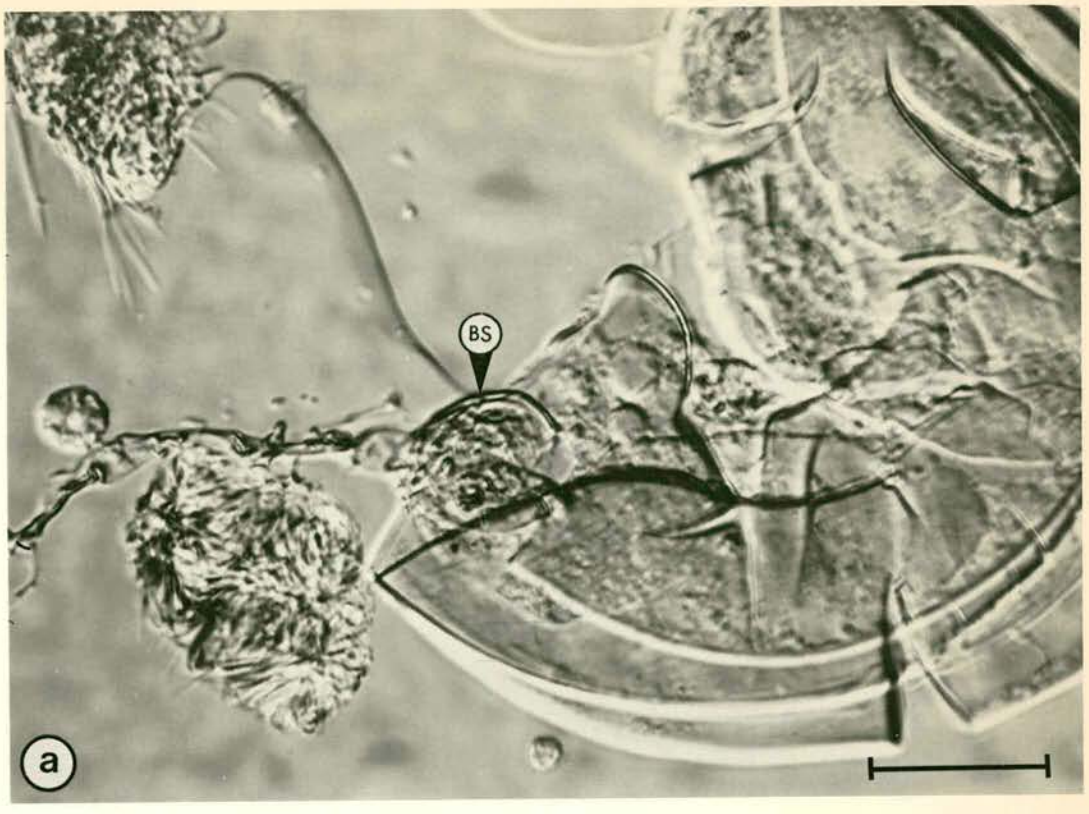
Plate 3.12

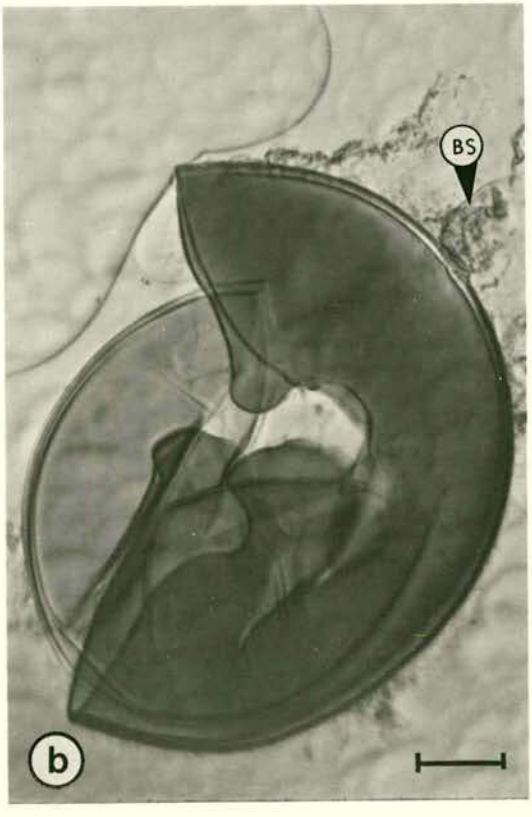
Scutellispora pellucida

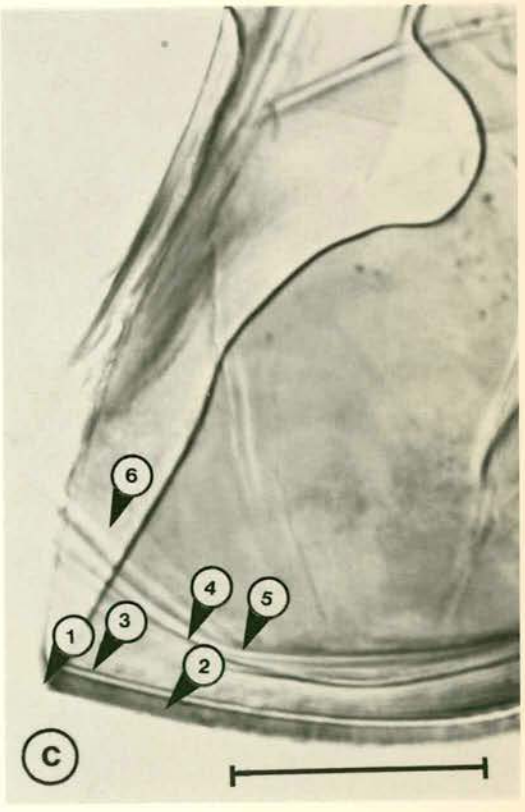
a) Crushed spore showing bulbous suspensor cell (BS) in PVC only
Scale bar 25 mm = 50 µm.

b) Single spore PVC/Melzers. Note staining of innermost walls also showing the bulbous suspensor cells (BS)
Scale bar 11.5 mm = 50 µm.

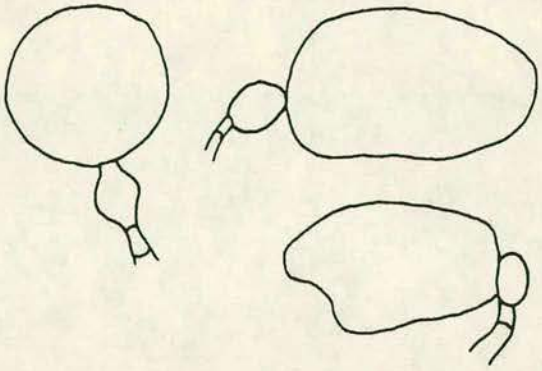
c) Section of spore showing the various wall types
Wall 1 hyaline brittle unit wall
Wall 2 hyaline laminate wall
Wall 3 membranous wall
Wall 4 unit wall
Wall 5 unit wall
Wall 6 amorphous wall
Scale bar 36 mm = 50 µm.


BS
a

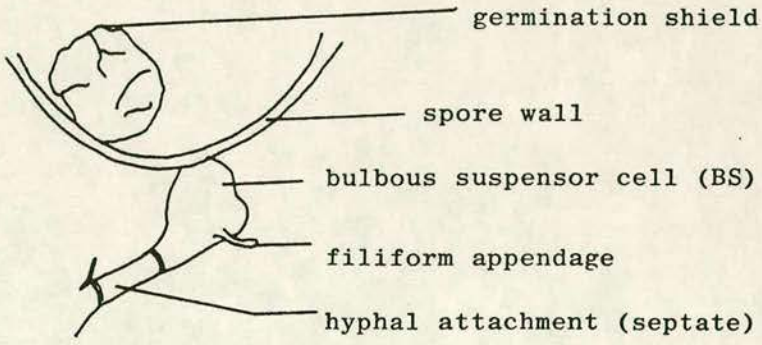

BS
b


6
4
5
1
3
2
c

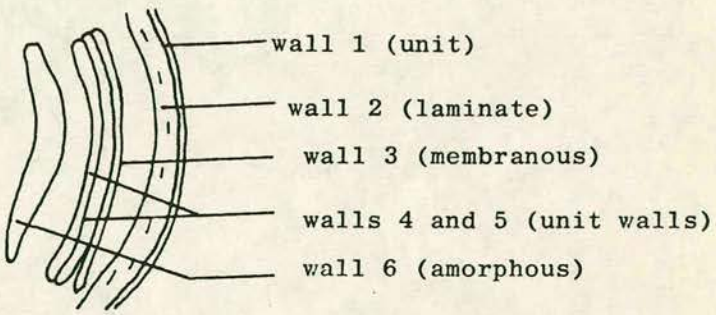
Range of spore shapes



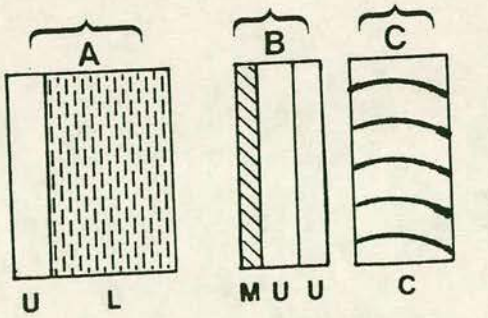
spores with hyphal attachments



Details of wall structure



MUROGRAPH



MURONYM

A(UL) B(M,UU) C(A)

* diagrams not drawn to scale

3.7.13 C4 (*Acaulospora* sp?)

A. Details of spore observations made under a dissecting microscrope (x50, magnification).

a. shape: globose to subglobose
b. texture: rough looking with adhering debris. Spore surface looking minutely alveolate.
c. colour: pale yellow.
d. spore contents: globular to granular.
e. sporiferous saccule: absent
f. reaction with Melzers reagent: stains light purple.

B. Details of spore observations made under a compound microscope (x150, x1000)

a. size: 80.0-100.0 (-112.5) x 57.5-100.0 (-112.5) µm
b. hyphal attachments: absent
c. suspensor cell: absent
d. germination shield: absent
e. collar: 5.0-10.0 µm diameter
f. wall characteristics: 2 wall groups made up of 4 walls. Wall group A is a sloughing unit wall 0.5 µm adhered to an almost hyaline laminate wall (3.0-4.0 µm). Wall group B consists of two membranous walls; the first one being 1.0 µm thick while the second is beaded (1.5-2.0 µm).

C. Distinguishing characteristics: An alveolate looking surface similar to that of *A. delicata* but not enough characteristics to place it in this species.

D. Ecology: From the Mbalmayo semi-deciduous rain forest, associated with a wide variety of trees and herbaceous vegetation.

E. Taxonomic source: None at present.

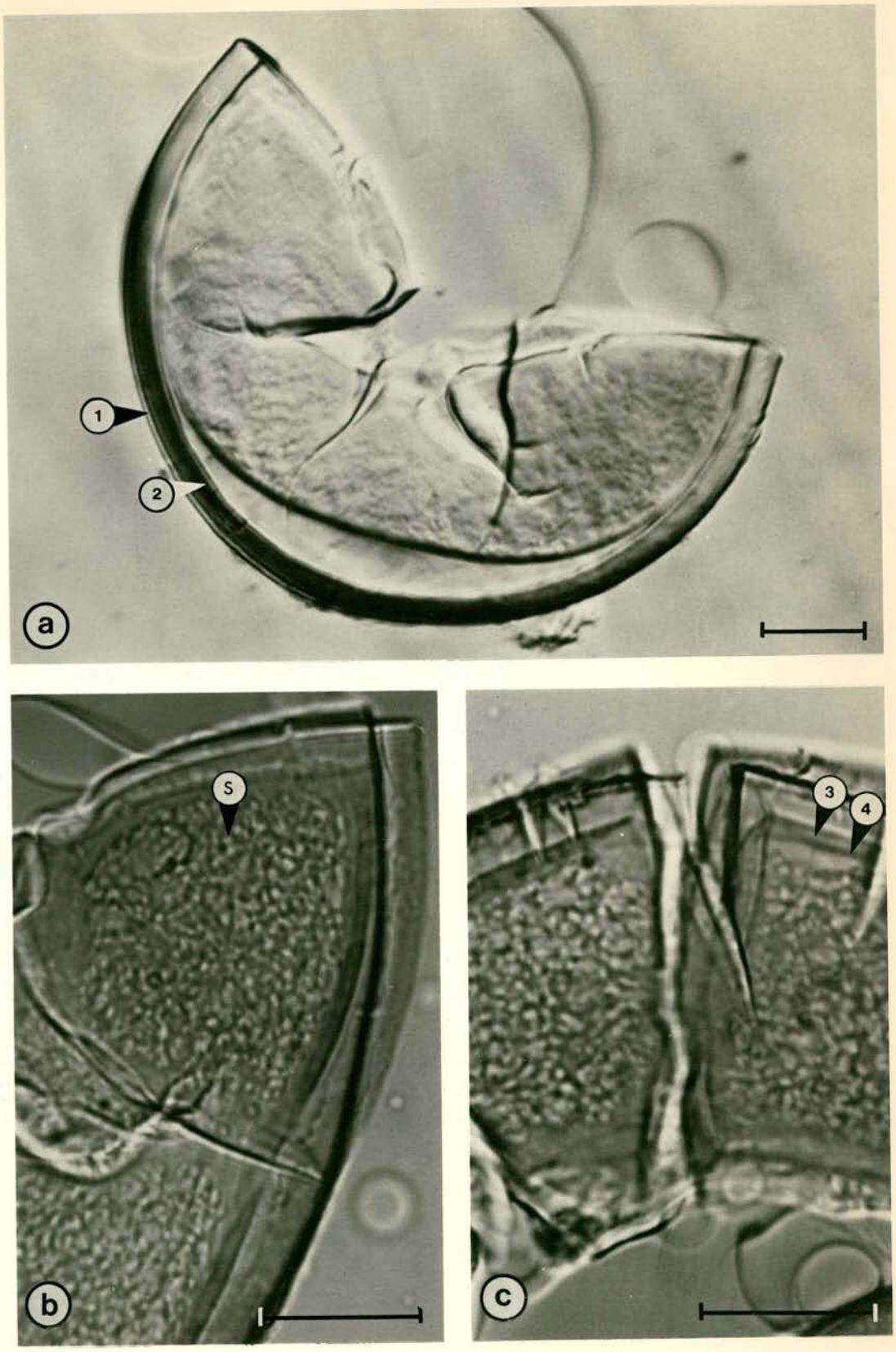
Plate 3.13

C4 (*Acaulospora* species?)

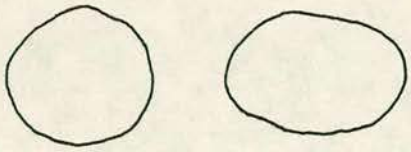
a) Crushed spore mounted in PVC only
showing wall 1 = Evanescent unit wall
wall 2 = Laminate wall
Scale bar = 17.5 mm = 25 µm

b) Surface showing irregular due to granular spore contents
Scale bar 27.5 mm = 25 µm

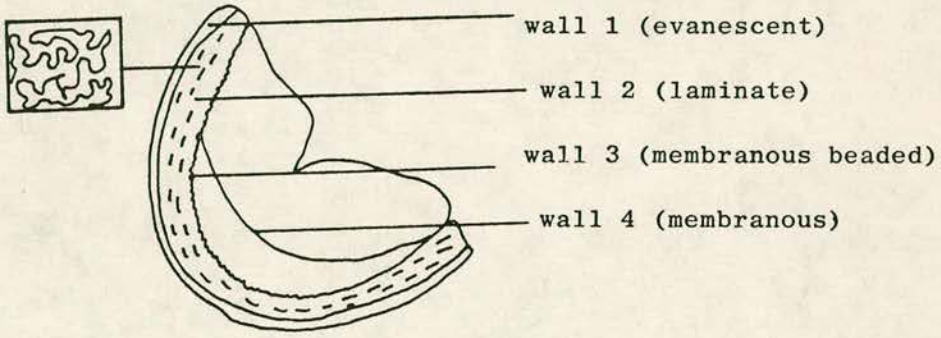
c) Wall types 3, membranous
and wall 4, beaded membranous
wall 5, amorphous wall
Scale bar 29.5 mm = 25 µm


1
2
a
S
b
3
4
c

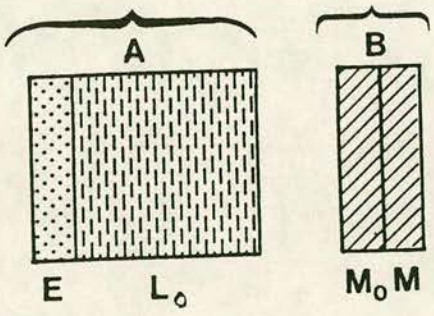
Range of spore shapes



no hyphal attachnents



MUROGRAPH



MURONYM A(E L) B(M_0M)

* diagrams not drawn to scale

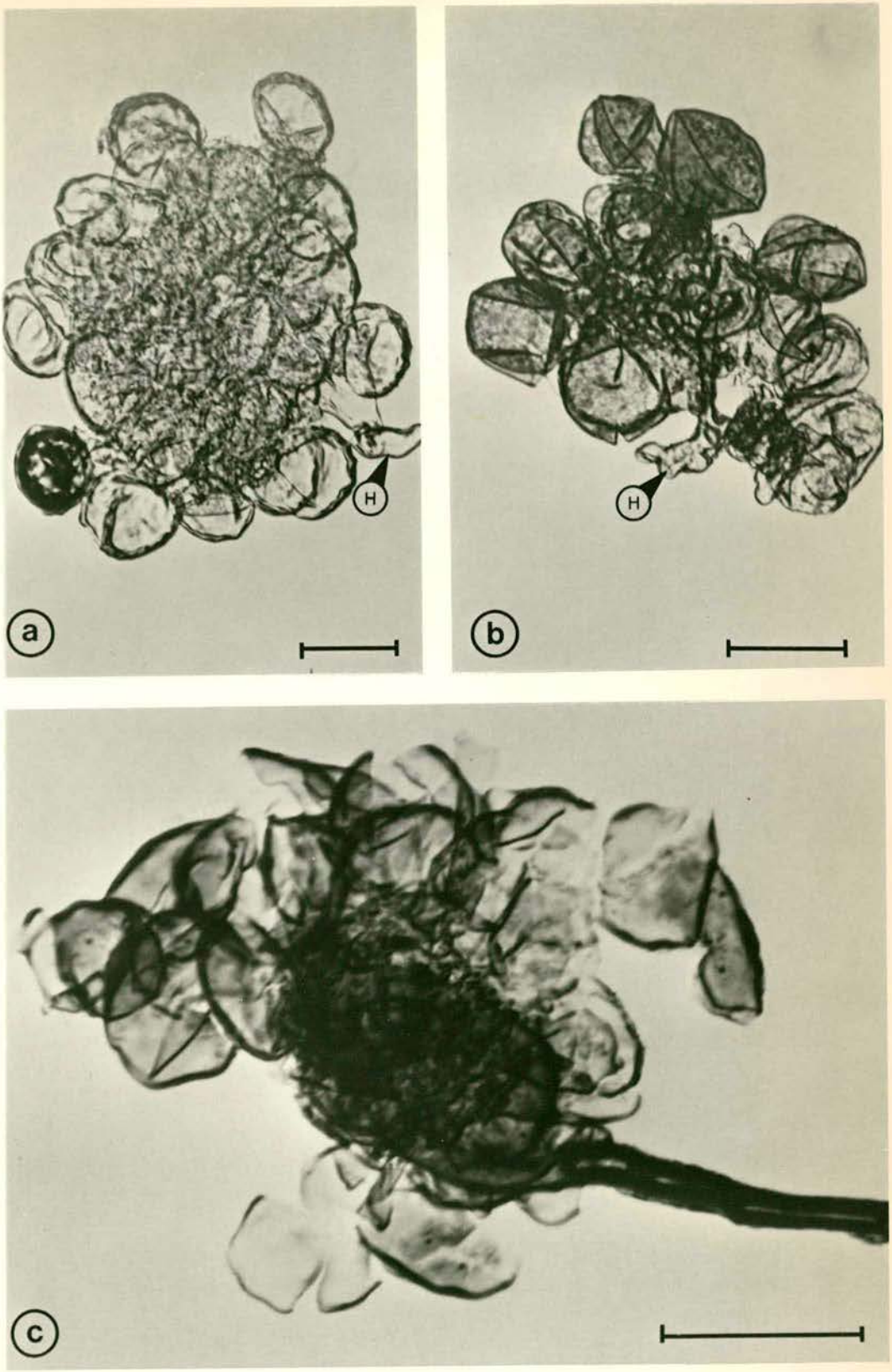
Plate 3.14

Types of auxiliary cells extracted from soils

a) Knobby auxiliary cells attached to a hypha (H)
Scale bar 15 mm = 50 µm

b) Smooth walled auxiliary cells on a hypha (H)
Scale bar 19.5 = 50 µm

c) Crushed smooth walled auxiliary cells
34 mm = 50 µm


a
H
b
H
c

3.8 DISCUSSION

Spore characters such as shape, colour, size, texture, and hyphal attachements provided useful information towards identifying the VAM fungi recovered from the moist semi deciduous forest at Mbalmayo. These characters were fairly easily ascertained under incident illumination but their overlapping nature between and within species of VAM fungi, also observed by Morton (1988) showed they could not solely be relied on for VAM fungal identification.

The wall types (laminate, unit, evanescent, expanding, membranous coriaceous, amorphous and beaded) and their arrangement between and within species have been observed by many workers to be important diagnostic features (Morton, 1988; Walker, 1983, 1986; Berch and Koske, 1986, Hall, 1977). The process of differentiating between wall types, however, is fraught with difficulties; hence most researchers except the experts tend to lump new species into existing ones from the literature (e.g. *G. fasciculatum* which has been widely misrepresented).

Of the wall types observed in spores isolated from the Mbalmayo Forest Reserve, the laminate, unit and beaded walls were easiest to decipher. In a majority of the seventeen VAM species recovered from the forest with laminate walls (except *G. occultum* and *S. pellucida*) the walls were always pigmented and had distinct laminae. However, unit walls were not as easy to distinguish from laminate walls in hyaline coloured spores and sometimes but rarely, a dislodged lamina from a laminate wall appeared similar to a unit wall.

In contrast, evanescent walls were not easily deciphered especially in those spores covered with mucilaginous substances to which soil debris had adhered. In spores of *G. etunicatum* where the evanescent wall often became completely lost as spores aged, correct identification of this species became increasingly difficult.

In the species of *Acaulospora* (*A. mellea, A. scrobiculata, A. laevis, A. morrowae*) the beaded wall with its wrinkled appearenace was easily recognised, whereas the membranous, coriaceous and amorphous walls were more difficult to decipher. The criterion for separating the

membranous from the coriaceous wall until recently, had been based on width (Walker, 1986) with the latter being much wider (2-5 µm) than the former (1-2 µm). It was observed in species such as C22, that the widths of membranous walls sometimes overlapped with those of coriaceous walls. Problems like these had also been noted by Morton (1988). Differences between the two wall types have now been resolved with the membranous wall seen to stain pink while the coriaceous wall does not stain at all (Morton, 1990).

The amorphous wall seen in an unidentified species (C12) and *A. morrowae* became apparent only after crushing the spores twice as suggested by Morton (pers. comm.). This wall type also was seen to stain deep purple in Melzer's reagent.

Wall structure being the most definitive aid to the identification of a VAM fungus has prompted VAM taxonomists to advocate for less use of fixatives and mountants such as gluteraldehyde and Polyvinyl alcohol lactophenol (PVL) which alter them. Alternatives such as 0.02% sodium azide and Polyvinyl lactic glycerine (PVLG) have been proposed instead (Morton, 1990).

The difficulties encountered in differentiating between wall types in VAM species identification call for improved and less arduous methods. Identification of a VAM fungus through the use of biochemical, serological techniques (Hepper *et al.*, 1986; Sen and Hepper, 1986) need to be improved upon. Also methods such as the gas chromatography of cell contents (Weijman and Meuzelaar, 1979), lipid and fatty acid profiles (Jabaji-Hare, 1988) should be encouraged. Hopefully methods like these would simplify the process of identifying VAM fungi, encourage more researchers into this field of study, avoid the misrepresentation of species (such as *G. fasciculatum*) as well as avoid the common mistake of lumping new finds into already existing species.

The recovery in the present study of seventeen VAM fungi from the tropical rainforest in Mbalmayo, of which fourteen have been identified and named using existing literature (Schenck and Perez, 1988) as *Acaulospora mellea, A. morrowae, A. laevis, A. scrobiculata, A. spinosa, Glomus etunicatum, G. fasciculatum, G. geosporum, G.*

macrocarpum, *G. occultum*, *Sclerocystis microcarpus*, *S. pachycaulis*, *Scutellispora coralloidea*, *S. pellucida*, C4, C12 and C22 reflect the universal distribution of these fungi shown by previous studies. For example, spore surveys previously effected in countries such as Nigeria (Redhead, 1977), Singapore (Louis and Lim 1987), Senegal (Diem *et al.*, 1981), Pakistan (Khan, 1971), Italy (Giovannetti, 1985), United States (Nicolson and Schenck, 1979; Walker *et al.*, 1982; Koske and Halvorson, 1981; Bloss and Walker, 1987) and/or in diverse habitats such as rainforests (Redhead 1977; Louis and Lim, 1987) sand dunes (Koske, 1975, 1981; Koske *et al.*, 1975), tallgrass prairie (Hetrick and Bloom, 1983; Anderson *et al.*, 1984; Liberta and Anderson, 1986) and with particular plants such as *Auracaria* (Bevege, 1971) *Festuca* spp (Molina *et al.*, 1978) and soybeans (Schenck and Hinson, 1971) have accorded the ubiquity of vesicular-arbuscular mycorrhizal fungi.

Relatively few studies, however, have been made in tropical rainforest ecosystems, probably due to the paucity of mycorrhizal researchers in the tropics, and because the process of VAM fungal identification is still fraught with difficulties. VAM species are often so inadequately described and identified that it becomes almost impossible to make comparisons between species recovered from different habitats in tropical regions.

In the Mbalmayo Forest however, the diverse VAM flora of seventeen fungi, was well above those of a tropical rainforest in Nigeria where only eight VAM fungi were recovered (Redhead, 1977) and a lowland rainforest in Singapore where Louis and Lim, (1987) reported only three identified VAM fungi with some species of *Glomus* and *Acaulospora* yet to be identified. In a Meghalayan subtropical evergreen forest, Sharma *et al.*, (1986) lumped their finds into the *Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora* genera without attempting to differentiate between the species within each of the genera. Surveys of mycorrhizal associations of tropical trees which do not mention the VAM fungi present (Janse, 1896; Thomazini, 1974; Shamsuddin, 1979; de Alwis and Abeynayake, 1980; Herrera and Ferrer 1980; Hogberg, 1982) exacerbate the problem of making valid comparisons. Notwithstanding, reports of studies effected in some natural ecosystems in Brazil (Toro and Sieverding, 1976) and Kivu, Zaire (Sieverding, 1989) have shown that

natural tropical ecosystems have a more diverse fungal flora than tropical ecosystems, in contrast to temperate natural agroecosystems which possess similar numbers of species as temperate agrosystems (Mosse & Bowen, 1968b).

In contrast to the tropics, several spore surveys have been effected in temperate regions. Nevertheless samples removed from a coniferous forest in New Zealand (Johnson, 1977), from maritime and barrier sand dunes (Giovannetti, 1985; Koske and Halvorson, 1981), grassland, shrubland and woodlands of east-Central England (Read, Koucheki and Hodgson, 1976) and raspberry, strawberry and barley fields (Mason, 1964) showed that these habitats all possessed less species than seen at Mbalmayo.

A greater diversity of VAM fungi comparable with that of the Mbalmayo Forest, was however observed from a woodland characterized by a riperian forest in the Santa Catalina montains of Arizona (Bloss and Walker, 1987) where 12-18 different VAM fungi were reported.

The high diversity of VAM fungi at Mbalmayo is due principally to the plant species rich composition of the forest (Sieverding 1989). An inventory (Mason *et al.*, 1988) established that the forest possessed more than 200 plant species, most of which are known to belong to mycorrhizal families. Observations such as these add to Janos' (1980) view that most tropical rainforest trees are mycorrhizal dependent.

The most common VAM fungi from the Mbalmayo rainforest belonged to the genus *Glomus* supporting reports by Gerdemann and Trappe (1974) that species in this genus are widespread in native grasslands as well as forests. Contrary to experimental evidence which shows species of *Glomus* to be better adapted to alkaline soils (Young *et al.*, 1985; Porter *et al.*, 1987; Abbott and Robson, 1989) the abundance of species of *Glomus* in Mbalmayo, the soils of which are acidic indicate that species of this genus are adapted to wide ranges of pH.

Other rainforest reports such as from Nigeria and Singapore (Redhead, 1977; Louis and Lim, 1987), of semi-arid regions in Senegal and India (Diem *et al.*, 1981; Mukerji and Kapoor, 1986) and subtropical montane

forest (Sharma *et al.*, 1986) accord the presence of species of *Glomus*. Of the five species of *Glomus* present at the Mbalmayo Forest (*G. etunicatum, G. fasciculatum, G. macrocarpum, G. occultum,* and *G. geosporum*) *G. fasciculatum* has been most widely reported from both tropical and temperate ecosystems. *Glomus occultum* has been reported by Sieverding (1989) as occurring under a broad range of environmental conditions and host plants. The species *G. etunicatum*, however, has not been as widely reported from tropical ecosystems as from temperate regions, which might be a reflection of an absence of skilled VAM taxonomists in the areas surveyed and not necessarily an absence/scarcity from tropical ecosystems as shown by the presence of this species in the Mbalmayo rainforest.

Interestingly, however, the recording of five species of *Acaulospora (A. mellea; A. morrowae; A. laevis; A. scrobiculata* and *A. spinosa*) in this study reflects the pattern set by earlier reports, from tropical or subtropical regions (Gerdemann and Trappe, 1974; Walker and Trappe, 1981; Schenck *et al.*, 1984; Spain *et al.*, 1984) and from the rhizospheres of some exclusively tropical plants like coffee (Janse 1896) and sugarcane (Cifferi, 1928).

According to Sieverding (1989) *A. scrobiculata, A. morrowae and A. spinosa* are able to tolerate a broad range of environmental conditions including a pH range of 3.8-8.0 and diverse chemical fertility levels, whereas *A. laevis* has a smaller environmental host range. As a result, it was not surprising to find these species of *Acaulospora* in the Mbalmayo soils with its pH of approximately 4.5.

Of the two *Sclerocystis* species recovered in this study *S. microcarpus* was previously reported (Iqbal and Perveen, 1980) to be associated with ferns. This could very well have accounted for their presence in the Mbalmayo forest where ferns (eg *Arthropteris cameroonensis*) were a regular component of the understorey.

S. pachycaulis on the other hand has only previously been reported in association with bamboo vegetation in Taiwan (Wu and Chen, 1986). Although there was no bamboo vegetation in the Mbalmayo forest, the observations made in this study did not ascertain what plant species

this fungus was associated with.

Sieverding (1989) and Mosse *et al.*, (1981) noted that *Sclerocystis* species generally occurred frequently in tropical savanna areas under scrubs or permanent crops such as sugarcane. However, *S. rubiformis* a closely related species to *S. pachycaulis* has been reported from Nigeria (Redhead, 1977), while in temperate regions it has been reported by Miller *et al.*, (1985) from apple rootstocks, Johnson (1977) from a New Zealand coniferous forest, and Gibson and Hetrick (1988) from a tallgrass prairie.

Scutellispora pellucida and *S. coralloidea* both isolated from the Mbalmayo forest are species that had been recovered in tropical as well as temperate ecosystems. Sieverding (1989) observed that *S. pellucida* thrives over a wide range of tropical soil (pH 3.8-8.0) and under diverse chemical fertility levels. This could explain their presence in the acidic soils of Mbalmayo (pH 4.5) as well as their presence in some alkaline soils of temperate regions (Gibson and Hetrick, 1988).

Species of the genera *Gigaspora* and *Entrophosphora* were conspicuously absent from the Mbalmayo forest soils. The presence of all VAM genera in spore surveys has rarely been reported, the exception being a tallgrass prairie (Gibson and Hetrick, 1988). Redhead (1977) surveyed both rainforest and savanna soils in Nigeria but never recovered species of *Scutellispora* and *Entrophosphora*. In a rainforest in Singapore, Louis and Lim (1987) did not recover species of *Entrophospora*, *Scutellispora*, *Gigaspora* nor *Sclerocystis*. In several other surveys such as from semi arid regions in Senegal and India (Diem *et al.*, 1981; Mukerji and Kapoor, 1986), a coniferous forest in New Zealand (Johnson 1977), maritime and barrier sand dunes (Koske and Halvorson, 1981; Diem *et al.*, 1981) and a subtropical evergreen montane region (Sharma *et al.*, 1986) 2 to 4 VAM genera were usually absent. The absence of certain genera from areas surveyed cannot be satisfactorily explained at present but edaphic in addition to environmental factors could dictate genera distribution. In addition the re-description of certain genera, e.g. the *Gigaspora* and *Scutellispora* genera could have accounted for the absence of species of *Scutellispora* from surveys conducted prior to Walker and Sanders (1986)

redescription of the genus *Gigaspora* (e.g. the switch in genera from *Gigaspora* to *Scutellispora* of *G. nigra* to *S. nigra*; Old, Nicolson and Redhead, 1973).

It would appear that host, environmental and edaphic conditions together determine the distribution of species of VAM fungi observed at Mbalmayo, where species belonging to the genera *Acaulospora, Glomus, Sclerocystis* and *Scutellispora* were recorded. The wide diversity of VAM fungi may be a consequence of the wide diversity of plant species recorded in this rainforest, although further studies are required to establish this relationship. Most of the VAM fungal species however had been reported in previous studies in both tropical and temperate ecosystems; thus their recovery at Mbalmayo lends credence to the non specific, ubiquitous nature of these fungi. The identification of VAM fungi however, still remains an arduous task probably responsible for the paucity of extensive ecological VAM studies in the tropics. With improved methods for identifying VAM fungi it is hoped more research on these fungi will be effected in tropical and temperate environments.

CHAPTER 4

DYNAMICS OF SPORE POPULATIONS IN AN UNDISTURBED FOREST AT MBALMAYO

4.1 Occurrence/distribution of VAM fungi in a natural tropical forest

Seventeen VA mycorrhizal species were recovered following extraction from soil samples (100 g oven dry weight) collected from the undisturbed Forest Reserve at Mbalmayo.

Of the seventeen, fourteen species have been described and named using existing literature (Schenck and Perez, 1987) while three are yet unidentified and have been designated as C4, C12 and C22 (Table 4.1). Four genera have been represented:

Acaulospora was represented by five species namely *A. laevis*, *A. mellea*, *A. morrowae*, *A. spinosa* and *A. scrobiculata*.

Glomus was represented by five species which are *G. etunicatum*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum* and *G. occultum*.

Sclerocystis by two species, namely *S. pachycaulis* and *S. microcarpus*.

Scutellispora by two species; *S. coralloidea* and *S. pellucida*.

Inexperience, coupled with difficulties encountered in differentiating between morphologically similar and closely related VAM fungal species at the initial stages of this survey, led to the inadvertent grouping of some of the fungi now realised to be distinctly different. As a result a total of 12 VAM fungal types have emerged including the following groups; *A. mellea/A. morrowae*, *G. fasciculatum/G. macrocarpum*, C12/*S. pellucida* and *G. occultum/A. scrobiculata*. With acquired experience the component species within these species aggregates would be quantified separately in future surveys.

In all plots sampled the most widespread VAM fungi were *G. etunicatum*, *G. occultum/A. scrobiculata;* they occurred in all plots and samples

Table 4.1 VAM species composition of the natural forest in Mbalmayo.

Acaulospora	*laevis*	Gerdemann and Trappe
A.	*mellea*	Spain and Schenck
A.	*morrowae*	Spain and Schenck
A.	*scrobiculata*	Trappe
A.	*spinosa*	Walker and Trappe
Glomus	*etunicatum*	Becker and Gerdemann
G.	*fasciculatum*	(Thaxter) Gerdemann and Trappe, emend Walker and Koske
G.	*geosporum*	(Nicolson and Gerdemann) Walker
G.	*macrocarpum*	(Tul and Tul) emend Berch and Fortin
G.	*occultum*	Walker
Sclerocystis	*microcarpus*	Iqbal and Bushra
S.	*pachycaulis*	Wu and Chen
Scutellispora	*coralloidea*	(Trappe, Gerd and Ho) Walker and Sanders)
S.	*pellucida*	(Nicol and Schenck) Walker and Sanders
C4	unpublished	
C12	unpublished	
C22	unpublished	

(100% occurrence), and were followed by C12/*S. pellucida*.

In contrast other exhibited a much more patchy distribution (e.g. *S. coralloidea* and C22) (Table 4.2).

Apart from *G. etunicatum*, no other VAM fungus gave rise to more than 20% of the total spore population in any one sample, with most (9 fungi) making up less than 3% of the population (Table 4.3).

Inspection of the data showed that plot 2 had the highest number of spores followed by plot 3, plot 4 and lastly plot 1: analysis of mean total spore numbers using square root transforms as recommended by Snedecor and Cochran (1967), indicated a homogenous spore distribution without statistically significant differences ($p = 0.05$) (Figure 4.1).

In contrast, however, significant differences were found in the spatial distribution of individual fungi (Figure 4.2). The species *G. etunicatum*, C4 and *A. spinosa* were significantly less numerous in spore numbers in plot 1 than in plots 2, 3 and 4, in which these fungal types were relatively homogenously distributed.

More spores of *G. fasciculatum/G. macrocarpum* were recovered from plots 2 and 3 than in plots 1 and 4 (Figure 4.2). In plots 3 and 4 there were significantly more spores of C12/*S. pellucida* than in plots 1 and 2. In contrast, spore numbers of *A. mellea/A. morrowae* and *S. coralloidea* were significantly greater in plot 1 compared to plots 2, 3 and 4.

As would be expected when total numbers of spores, in the four plots were not unduly different, the spore numbers of *G. occultum/A. scrobiculata*, *A. laevis*, C22 and *G. geosporum* were found to be more or less uniformly distributed across the four plots.

4.2 **Effects of cover on spore distribution (February 1987)**

The overall mean number of VA mycorrhizal spores across the four plots was larger, although not significantly different in those soil samples collected from transects through *Terminalia superba* trees ('with

Table 4.2 Frequency of occurrence of VAM fungal species in soil samples collected from an undisturbed rainforest at Mbalmayo, Cameroon, sampled February 1987 (Means of 30 samples; frequency expressed in percentages).

VAM fungal species	Frequency of occurrence in soil samples
G. etunicatum	100.0%
C4	61.3%
G. fasciculatum/G. macrocarpum	93.3%
A. spinosa	88.4%
G. occultum/A. scrobiculata	100.0%
C12/*S. pellucida*	95.5%
A. laevis	47.7%
S. coralloidea	18.2%
C22	6.5%
G. geosporum	54.4%
A. mellea/A. morrowae	73.0%
S. pachycaulis/S. microcarpus	nil

Table 4.3. Proportions of the VAM types in 100 g dry weight soil samples collected from the Mbalamyo Forest before treatments in February 1987.

Proportion (%) = $\frac{\text{No. of spores of the VAM type}}{\text{Total spore numbers extracted from 100 g dry soil}} \times 100$

VAM FUNGAL TYPES

PLOTS	*G etunicatum*	*C4*	*G fasciculatum/G. macrocarpum*	*A. spinosa*	*G. occultum/A. scrobiculata*	*C12/S. pellucida*	*A. laevis*	*S. coralloidea*	*C12*	*G. geosporum*	*A. mellea/A. morrowae*	*Sclerocystis spp*
1	55.3	0.68	1.58	0.62	19.39	4.76	1.12	0.12	0.01	2.34	14.07	nil
2	65.1	0.99	3.10	1.09	18.65	3.00	1.05	0.09	0.06	1.55	5.37	nil
3	68.3	1.38	2.71	0.82	17.17	3.61	1.79	0.06	0.03	2.83	0.36	nil
4	62.4	1.88	1.72	1.00	17.27	7.02	2.4	0.06	nil	1.26	5.04	nil

*For conformity all figures are represented as 2 decimal points, e.g. 0.99 is in effect 1.0.

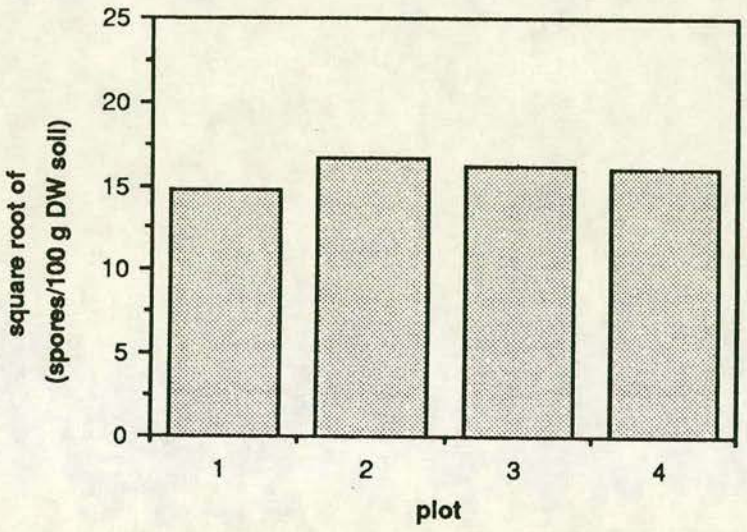


Figure 4.1
Variations in the mean total numbers of VAM fungal spores extracted from 100 g dry soils taken from four (1 ha) plots of natural forest at Mbalmayo in February 1987 (dry season). Means of 30 samples per plot
LSD indicated by error bars only when differences in means are significant at P = 0.05.

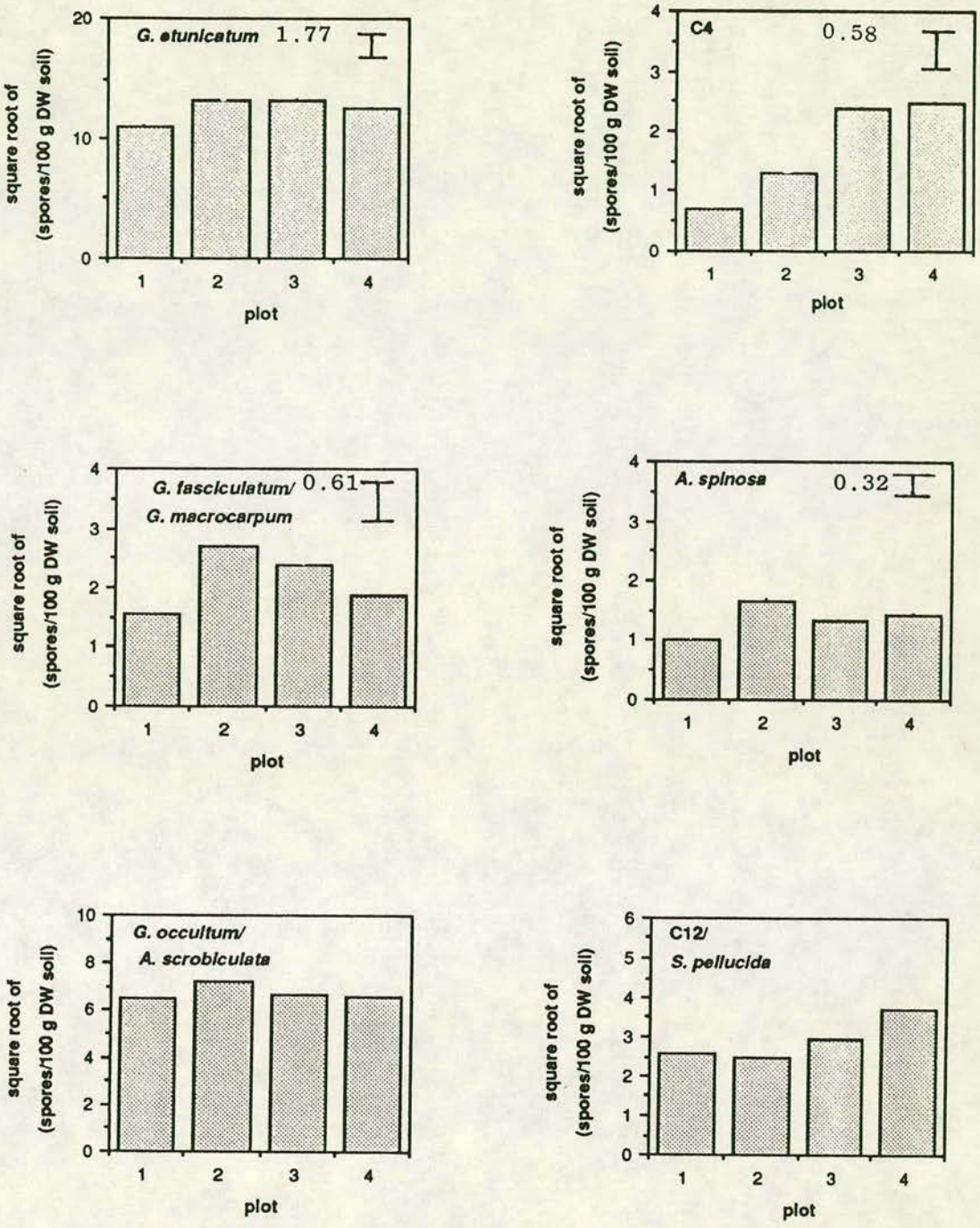
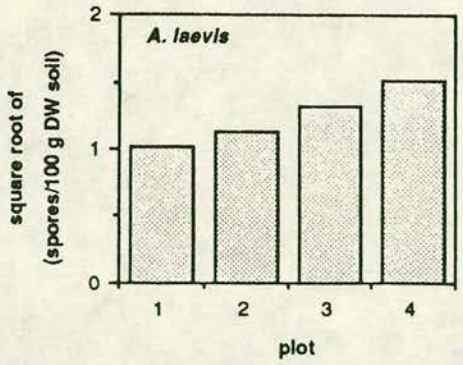
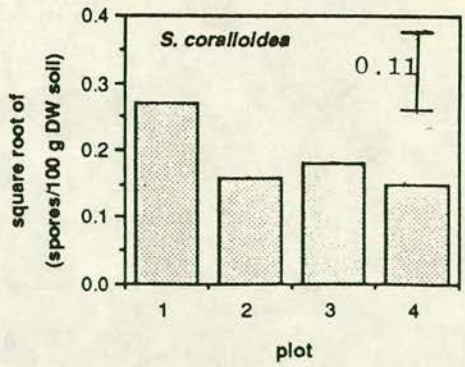
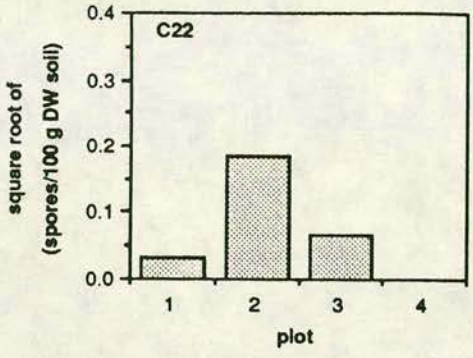
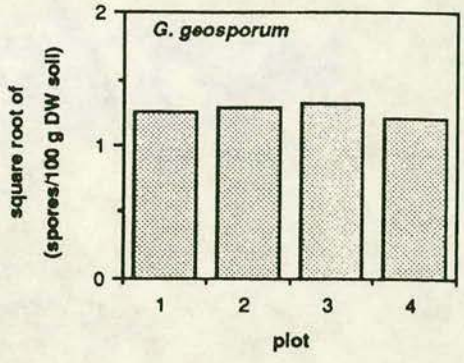


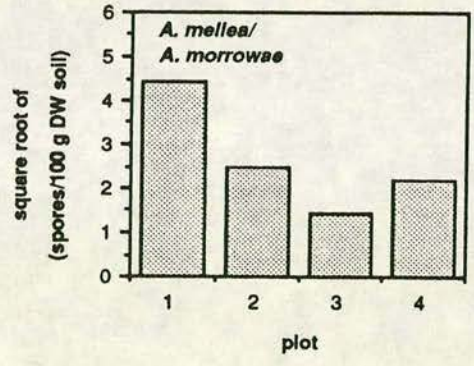
Fig. 4.2
Effects of plot variations on the mean spore numbers of 12 VAM fungi, extracted from 100g dry soil samples collected from four (1 ha) plots of natural forest at Mbalmayo in February 1987 (dry season). Means of 30 samples per plot.
LSD indicated by error bars only where differences in means are significant at p = 0.05.











4.2 continued.

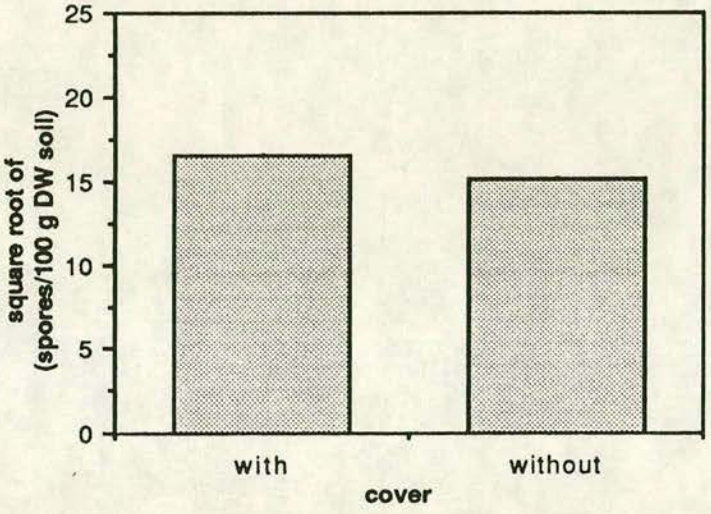


Fig. 4.3
Variations resulting from amounts of cover on the mean total numbers of spores of VAM fungi extracted from 100 g dry soils collected from four (1 ha) plots of natural forest in Mbalmayo in February 1987 (dry season).
Means of 120 samples.
LSD indicated by error bars only when differences in means are significant at p=0.05.

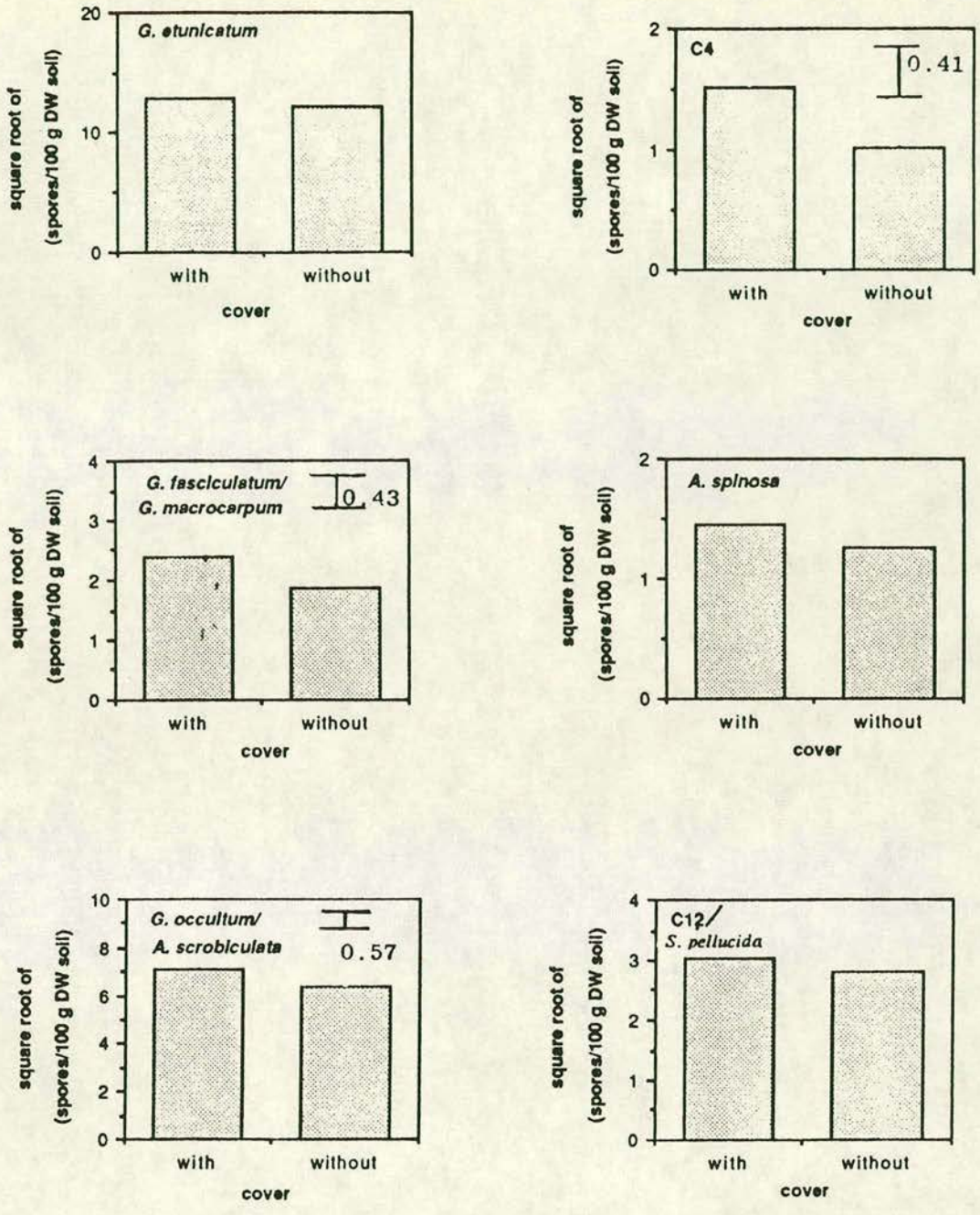
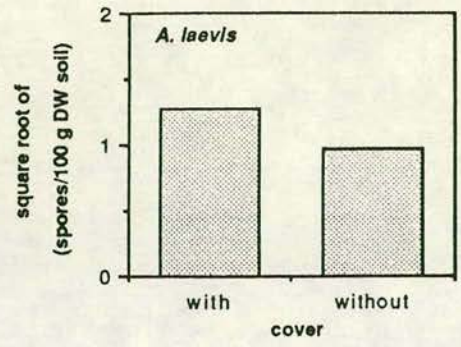
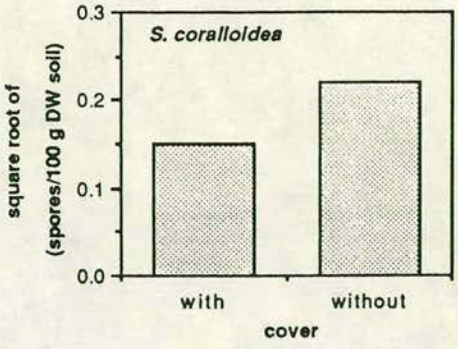
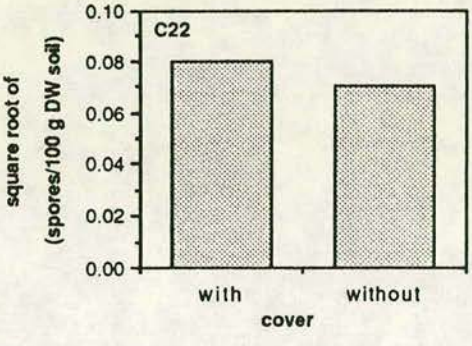
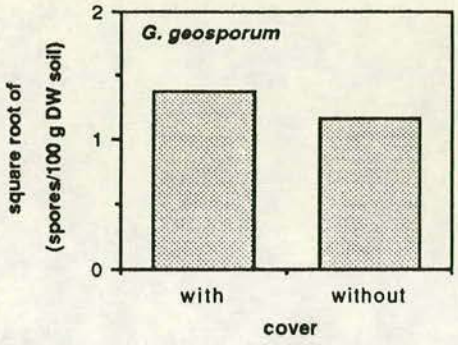


Fig. 4.4
Changes associated with or without cover on the mean spore numbers of 12 VAM fungi; extracted from 100 g dry soils collected from the four (1 ha) plots of natural forest in Mbalmayo in February 1987 (dry season).
LSD indicated by error bars are shown only when differences in means are significant at p=0.05.









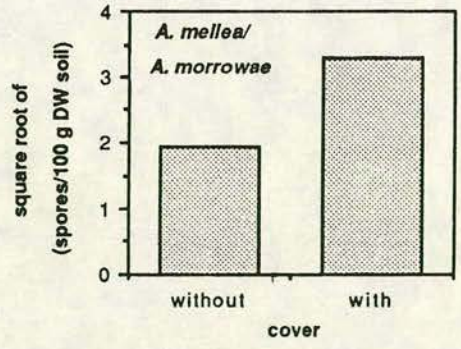


Fig. 4.4 continued

cover') than in transects 'without cover' set out in non-tree herbaceous areas (Figure 4.3).

This distribution was evident for the majority of individual VAM fungal types, although only numbers of spores of C4, *G. fasciculatum/G. macrocarpum* and *G. occultum/A. scrobiculata* were significantly larger along 'with cover' transects compared with 'without cover' transects (Figure 4.4).

4.3 **Effects of distance on spore distribution (February 1987).**

Ecological studies of ectomycorrhizal fungi (Mason *et al.* 1983) suggested that VAM fungi might be located at different distances from trees. Although the largest overall mean spore numbers were realised 2.5 m from specimen trees of *T. superba*, this was not significantly different from numbers obtained from 5.0 m, 7.5 m and 10.0 m from the trees (Figure 4.5). Despite the absence of a statistically significant overall effect, the occurrence of five VAM fungal types was not random. Spore numbers of C4, *A. spinosa*, *G. occultum/A. scrobiculata* and *A. laevis* peaked significantly at 2.5 m after which numbers of spores at the other distances 5.0 m-10.0 m were uniformly smaller. Only in C22 did the spore numbers peak at 5.0 m from specimen *T. superba* trees (Figure 4.6).

Samples were taken at 2.5 m south and 2.5 m north of *Terminalia superba* trees in order to assess the degree of variation in spore numbers likely to be encountered along sample points placed at different positions of the specimen *T. superba* trees. Observations indicated that, in 4 of the 5 VAM fungal types that exhibited significant distance effects, the two compass positions consistently gave significantly different numbers of spores confirming earlier significant variations in spore numbers across plots of individual trees. The number of spores at 2.5 m from *T. superba* trees were mostly larger in a northward, than in a southward, direction.

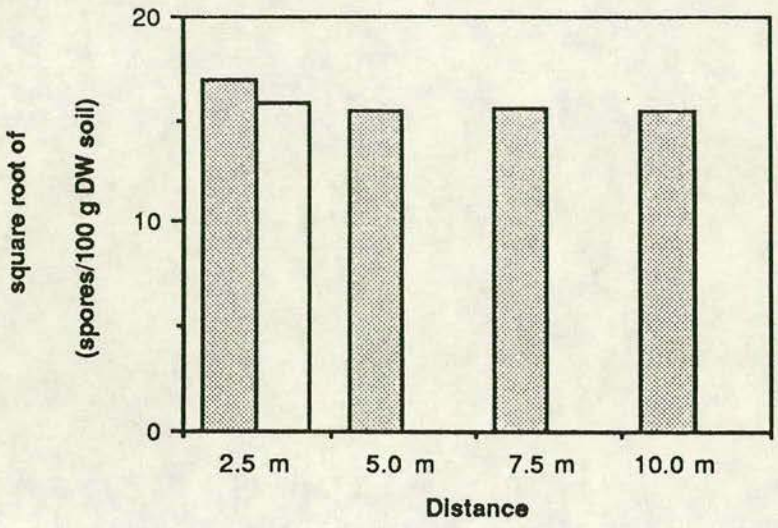


Fig. 4.5
Changes associated with distance from specimen *T. superba* trees on the mean total number of VAM fungal spores extracted from 100 g dry soils taken from four (1 ha) plots of natural forest at Mbalmayo in February 1987 (dry season). Means of 30 samples per plot. LSD indicated by error bars only when differences in means are significant - p = 0.05. Shaded bars represent distances to the north of tree while the blank bar represents distance to the south.

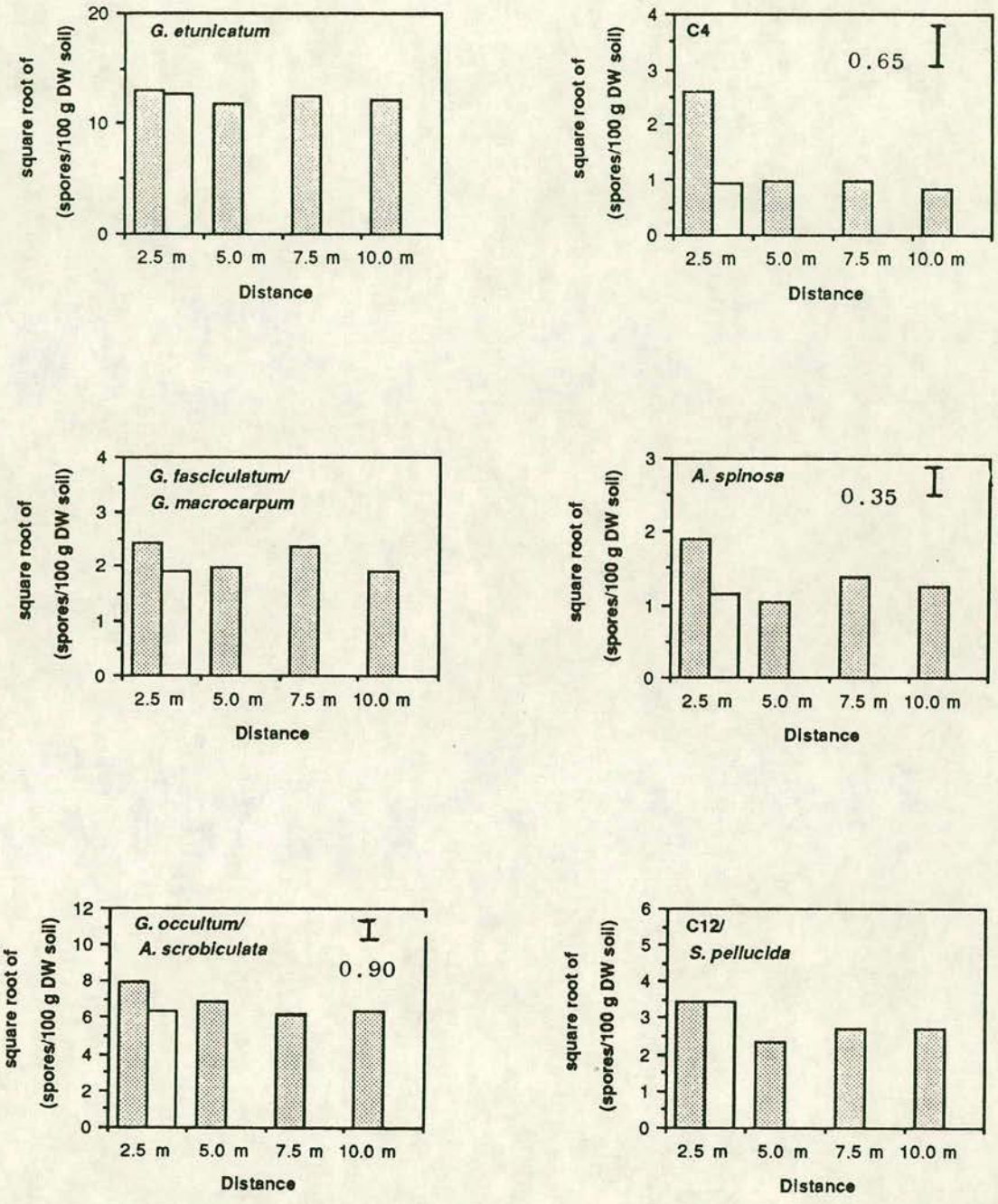
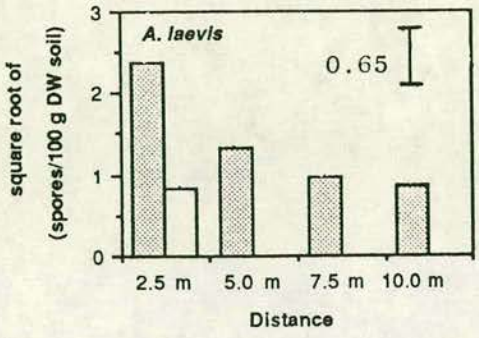
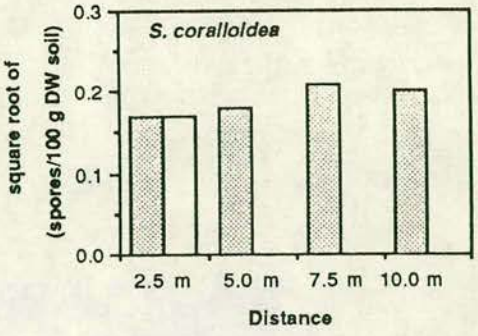
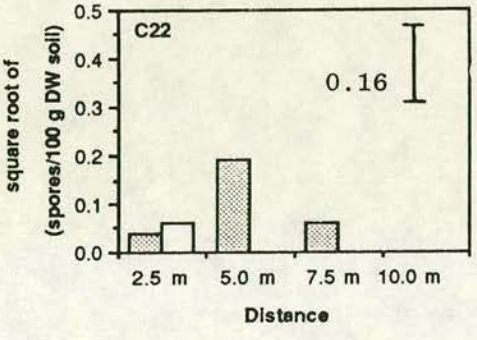
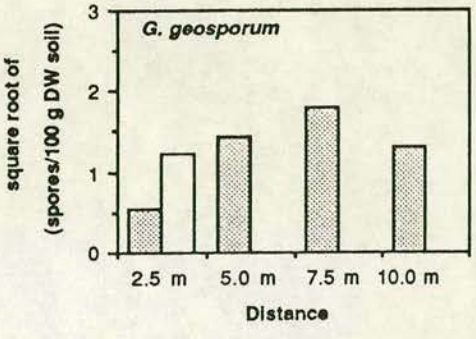


Figure 4.6
Variations among the mean spore numbers of the 12 VAM fungal types associated with different distances from specimen *T. superba* trees per 100 g dry weight soils, collected from the Mbalmayo forest before the onset of treatments in February 1987. Means of 30 samples LSD only shown where differences in means are significant at p = 0.05.









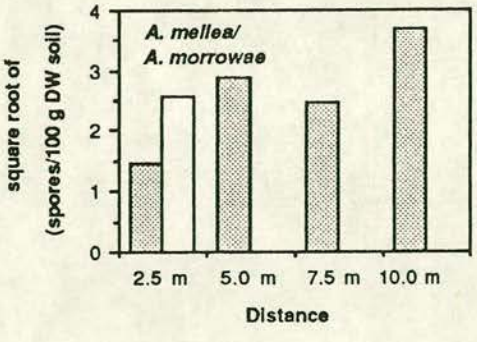


Figure 4.6 continued

4.4 INTERACTIONS: PLOT X COVER, PLOT X DISTANCE, COVER X DISTANCE AND PLOT X COVER X DISTANCE (FEBRUARY, 1987)

4.4.1 Plot x Cover

Plot x cover interactions were not significant when considering overall mean spore numbers. However, they were significant for 7 of the component fungi. The general trend observed is in line with earlier observations wherein the most fungi produced more spores along *T. superba* transects (with cover) as opposed to the herbaceous non-tree transects (without cover).

The results in Table 4.4 indicate that:

a) With *G. etunicatum* more spores were recovered from along with cover transects, in plots 1, 2 and 4 except in plot 3 where 'without cover' spore numbers exceeded those of plots 1, 2 and 4.

b) *G. occultum/A. scrobiculata* number of spores were significantly greater only along with cover transects in plot 2.

c) C12/*S. pellucida* number of spores were generally greater from with cover transects in all the plots except plot 4 which incidentally along without cover transects had the greatest number of spores of this VAM aggregate.

d) In plots 1 and 3 more spores of *A. laevis* were recovered along *T. superba* (with cover) transects; along non tree herbaceous transects (without cover) more spores of this VAM fungus were recovered in plot 4 only.

e) In all four plots *S. coralloidea* number of spores were more abundant along 'with cover' transects than along 'without cover' transects; however only along 'with cover' transects in plot 4 were numbers of spores of this fungus significantly greater.

f) C22 spores were generally retrieved along non tree herbaceous

Table 4.4 The significant interacting effects of plot (P) and cover (A) (PXA) on the mean numbers of spores of VAM fungal types extracted from 100g dry weight soils collected from the natural forest in Mbalmayo (February 1987) before the onset of treatments. Means usually of 30 samples per plot.

VAM fungal type	Cover	Plots 1	2	3	4
G. etunicatum	with	133.47	209.80	140.90	178.51
		(11.55)	(14.48)	(11.87)	(13.36)
	without	106.12	143.79	208.64	138.77
(LSD,p=0.05;2.50)		(10.30)	(11.99)	(14.44)	(11.78)
G. occultum/A. scrobiculata	with	46.24	69.39	51.41	38.07
		(6.80)	(8.33)	(7.17)	(6.17)
	without	37.95	37.09	38.19	48.16
(LSD,p=0.05;1.14)		(6.16)	(6.09)	(6.18)	(6.94)
C12/*S. pellucida*	with	10.24	12.25	9.18	5.91
		(3.20)	(3.50)	(3.03)	(2.43)
	without	3.69	2.25	8.18	24.70
(LSD,p=0.05;0.20)		(1.92)	(1.50)	(2.86)	(4.97)
A. laevis	with	1.00	0.23	2.89	0.76
		(1.00)	(0.48)	(1.70)	(0.87)
	without	0.59	0.59	0.21	2.89
(LSD,p=0.05;0.82)		(0.77)	(0.77)	(0.46)	(1.70)
S. coralloidea	with	0.59	0.59	0.21	2.89
		(0.77)	(0.77)	(0.46)	(1.70)
	without	0.11	0.10	0.00	0.05
(LSD,p=0.05;0.30)		(0.333)	(0.315)	(0.000)	(0.228)
C22	with	0.00	0.09	0.00	0.00
		(0.00)	(0.300)	(0.00)	(0.00)
	without	<0.01	<0.01	<0.02	0.00
(LSD,p=0.05;0.16)		(0.00)	(0.00)	(0.00)	(0.00)
G. geosporum	with	4.24	0.16	1.80	2.79
		(2.06)	(0.40)	(1.34)	(1.67)
	without	0.19	4.71	1.64	0.55
(LSD,p=0.05;1.26)		(0.43)	(2.17)	(1.28)	(0.74)

transects (without cover) rather than from along with cover transects, although in plot 2 spores of this fungus were retrieved along with cover transects and were actually significantly greater in numbers than those from along without cover transects.

g) Spores of *G. geosporum* were greater along 'with cover' transects in all the plots except plot 2, which had the greatest number of spores retrieved along 'without cover' transects. In plot 2, spore numbers were significantly different from those taken along similar 'without cover' transects of plots 1, and 4 and also from along 'with cover' transects of plots 2 and 3.

4.4.2 **Cover x distance**

Of all the VAM fungi, only C12/*S. pellucida* spore numbers were affected by cover x distance interactions. Nevertheless results support earlier observations which indicated that spore numbers from samples taken along 'with cover' transects are greater and peaked closest to *T. superba* trees than from 'without cover' transects which had a more or less uniform distribution of spores of C12/*S. pellucida* with distance from the marker (Table 4.5).

4.4.3 **Plot x cover x distance**

This interaction did not significantly affect any of the VAM fungi. Table 4.6 presents a summary table and shows significant main and interacting effects of plots, cover and distance from specimen *T. superba* trees.

4.5 TEMPORAL EFFECTS ON THE SPORE POPULATION DYNAMICS (FEB. 1987: AUGUST 1987: AUGUST 1988)

The untreated control plot was sampled during two contrasting seasons; a dry season (February, 1987) and two rainy seasons (August 1987 and August 1988 respectively) to enable a deduction to be made of the seasonal effects on spore population dynamics. The results indicated that the overall mean spore numbers in August 1987 were significantly

Table 4.5 Significant interacting effects of cover x distance (AxD) on the mean spore numbers of the VAM fungal types extracted from 100 g dry weight soil samples collected from the natural forest in Mbalmayo (February 19878) prior to site preparations. Means of 30 samples per plot.

VAM fungal type	Cover	Distance				
		2.5m south	2.5m north	5.0m north	7.5m north	10.0m north
C12/*S. pellucida*	With	17.47 (4.18)	16.0 (4.00)	3.92 (1.98)	4.75 (2.18)	8.24 (2.87)
(LSD.p=0.05;1.4)	Without	7.08 (2.66)	8.52 (2.99)	7.29 (2.70)	10.63 (3.26)	6.35 (2.52)

(_____) indicate square root transformed values

Table 4.6 Summary of the analysis of variance showing the mean numbers of 12 VAM fungal spore types extracted from 100g dry weight soils collected from a natural forest in Mbalmayo, before the onset of treatments. Sampled, February 1987 (dry season), with means usually of 30 samples per plot. Significant differences in VAM fungal types are shown between plots and as associated with amounts of cover and distance from the specimen *Terminalia superba* trees.

VAM fungal type	Between Plots	Amounts of Cover	Distance from *Terminalia* tree	PxA	PxD	AxD	PxAxD
G etunicatum	+	-	-	+	-	-	-
C4	+	+	+	-	-	-	-
G. fasciculatum/ G. macrocarpum	+	+	-	-	-	-	-
A. spinosa	+	-	+	-	-	-	-
G. occultum/ A. scrobiculata	-	+	+	+	-	-	-
C12/*S. pellucida*	+	-	-	+	-	+	-
A. laevis	-	-	+	+	-	-	-
S. coralloidea	+	-	-	+	-	-	-
C22	-	-	+	+	-	-	-
G. geosporum	-	-	-	+	-	-	-
A. mellea/A. morrowae	+	-	-	-	-	-	-
Sclerocystis spp.	np	np	np	np	np	np	np
Overall spore numbers	-	-	-	-	-	-	-

\+ = significant differences at p = 0.05
\- = not significantly different
np = not present

less than in February 1987 and August 1988 (Figure 4.7). The spore numbers of the majority of VAM fungal types (8) were significantly altered by temporal effects. They included *G. etunicatum*, *G. fasciculatum/G. macrocarpum*, *A. spinosa*, C12/*S. pellucida*, *A. laevis*, *S. coralloidea*, *G. geosporum* and the *Sclerocystis* spp. (Figure 4.8).

In general more spores of a majority of VAM fungi were produced in February 1987 (dry season); however, only the VAM fungi *A. spinosa*, C12/*S.pellucida* and *A. laevis* produced significantly more spores in the dry season (Feb 1987) in comparison with the two rainy seasons (August 1987 and August 1988).

G. fasciculatum/G. macrocarpum exhibited a similar trend although the decrease in spore numbers only became significant in August 1988. In contrast, *G. geosporum* and to a lesser extent *S. coralloidea* gave rise to a significant increase in spores with time (Figure 4.8). *A. mellea/A. morrowae* showed a similar trend to *G. geosporum* although not significantly.

The number of *G. etunicatum* spores decreased significantly in August 1987 (rainy season) but returned to the original level a year later in August 1988.

Lastly, spores of *Sclerocystis* spp. were only recovered in August 1988 whereas species such as *G. occultum/A. scrobiculata* remained unaffected by seasonal influences (Figure 4.8).

The cover effects on spore numbers of *A. laevis* and *A. mellea/A. morrowae* changed with time. Numbers of spores were significantly fewer in areas 'without cover' in February 1987 (dry season) than in August 1987 and August 1988 (the two rainy seasons) whereas spores of *A. laevis* were not retrieved in any soil samples collected in August 1988 (rainy season) (Table 4.7).

Distance effects on the other hand significantly affected the spore numbers of only C4 which decreased with time as well as distance from specimen (*T. superba*) trees (Table 4.8).

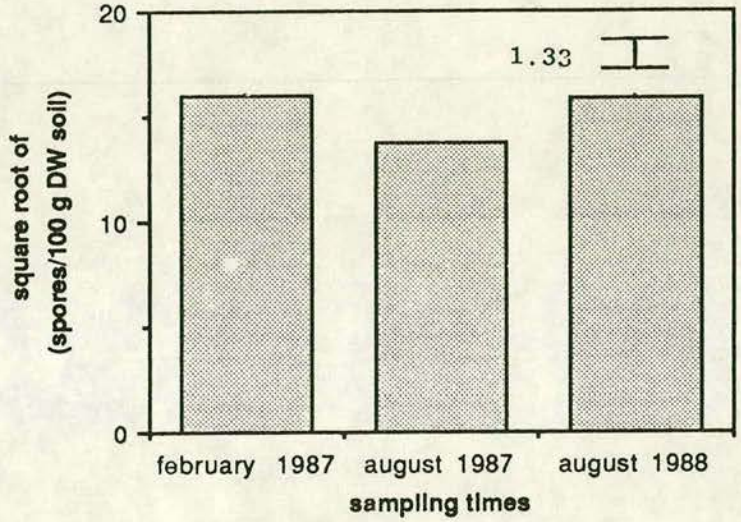


Figure 4.7
Variations in the mean total spore numbers of the VAM fungi in the control plot over time, extracted from 100 g dry weight soils collected from the natural forest in Mbalmayo. Sampled in February 1987 (dry season), August 1987 (rainy season), August 1988 (rainy season). Means of 30 samples per sampling occasion. LSD indicated by error bars only when means are significantly different at p = 0.05.

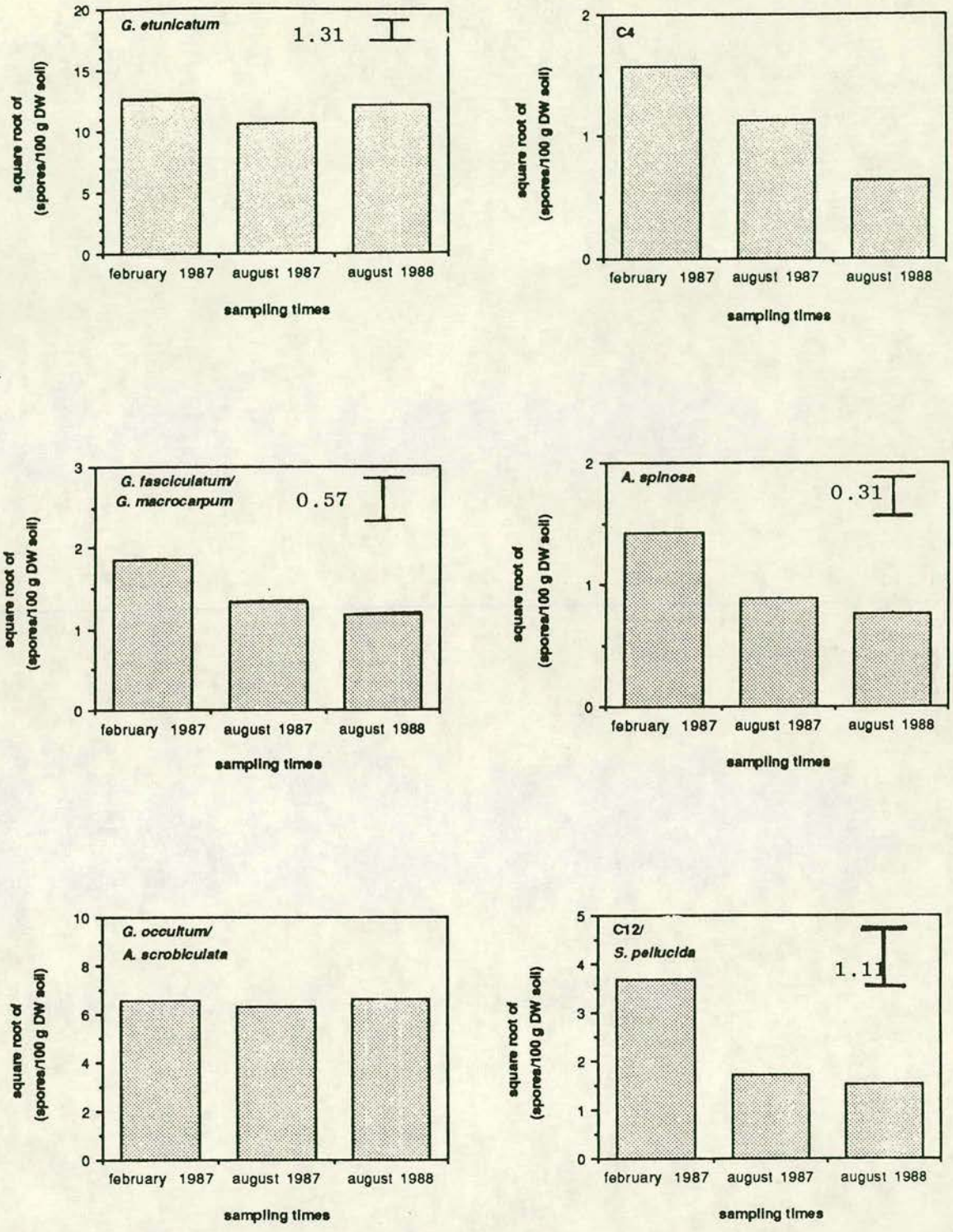
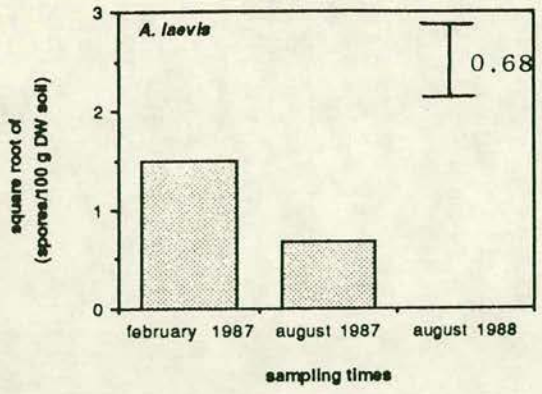
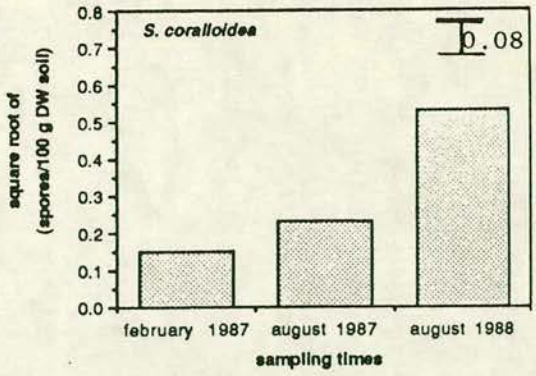
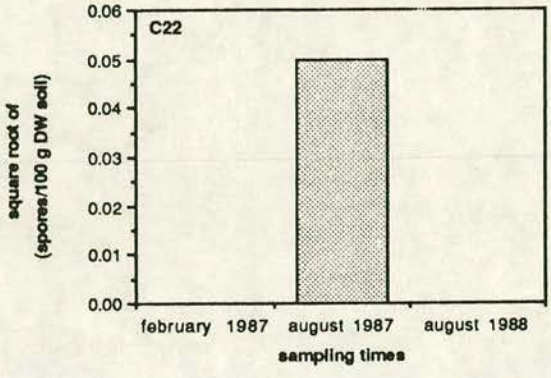
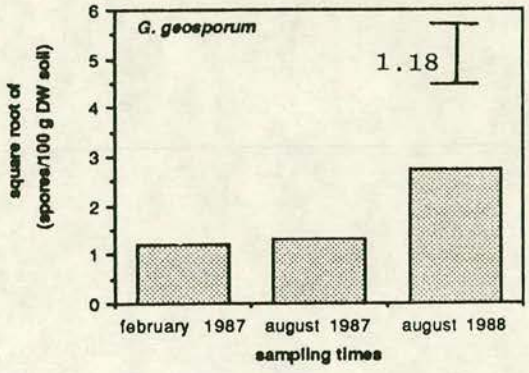


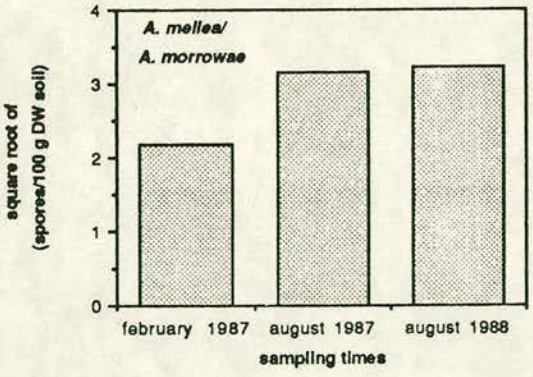
Fig. 4.8
Variations in the mean spore numbers of 12 VAM fungi in the control plot over time, extracted from 100 g dry soils collected from the natural forest at Mbalmayo. Sampled in February 1987 (dry season), August 1987 (rainy season) and August 1988 (rainy season). Means of 30 samples per sampling occasion.
LSD indicated by error bars only when differences in means are significant at p = 0.05.











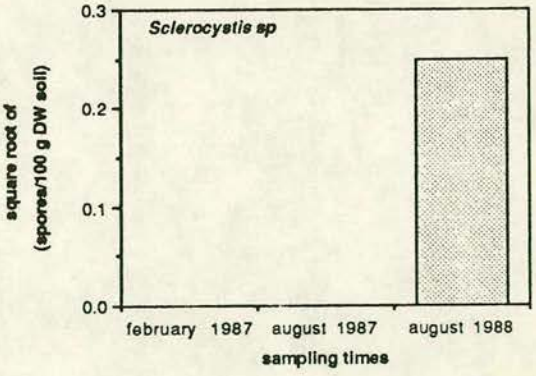


Fig. 4.8 continued

Table 4.7 Variations in the mean spore numbers of the VAM fungal types in the control plot as affected by cover extracted from 100 g dry weight soils from the Mbalmayo Forest at three diferent sampling times (Feb 1987, Aug 1987 and Aug 1988). Means of 30 samples per sampling occasion.

VAM fungal types	COVER	TIME		
		1	2	3
G. etunicatum	with without	13.36 11.78	9.87 11.41	12.02 12.29
C4	with without	1.79 1.35	1.39 0.85	0.89 0.39
G. fasciculatum/G. macrocarpum	with without	2.18 1.54	1.41 1.25	1.32 1.03
A. spinosa	with without	1.52 1.34	0.77 1.01	0.72 0.79
G. occultum/A. scrobiculata	with without	2.43 4.97	1.19 2.26	1.34 1.75
C12/*S. pellucida*	with without	2.43 4.97	1.19 2.26	1.34 1.75
A. laevis LSD,p=0.05;0.97	with without	0.88 1.70	0.99 0.00	0.00 0.00
S. coralloidea	with without	0.07 0.23	0.27 0.20	0.67 0.39
C22	with without	0.00 0.00	0.09 0.00	0.00 0.00
G. geosporum	with without	1.67 0.74	0.92 1.73	2.41 3.40
A. mellea/A. morrowae LSD,P=0.05;2.22	with without	3.74 0.61	2.60 3.71	4.29 2.18
Sclerocystis spp	with without	0.00 0.00	0.00 0.00	0.07 0.43
Total mean spore numbers	with without	16.54 15.46	12.77 14.64	15.38 15.00

square root transformed values only
LSD only shown where significant
Time 1 = sampling in February 1987
2 = sampling in August 1987
3 = sampling in August 1988

Table 4.8 Variations in the mean spore numbers of the VAM fungal types in the Control plot as affected by distance from specimen *T. superba* trees, extracted from 100 g dry weight soil samples from the Mbalamayo forest. Means of 30 samples (sampled in February 1987, August 1987 and August 1988).

VAM fungal type	Time	Distance				
		2.5 m south	2.5 m north	5.0 m north	7.5 m north	10.0m north
G. etunicatum	1	12.54	13.93	11.56	13.02	11.78
	2	11.13	11.01	10.59	10.41	10.07
	3	12.94	12.91	12.22	11.45	11.27
C4 (LSD,p=0.05 ;1.35)	1	0.98	3.57	1.74	1.14	0.40
	2	1.56	1.31	0.67	1.00	1.07
	3	0.47	0.96	0.74	0.64	0.40
G. fasciculatum/G. macrocarpum	1	1.73	2.32	2.11	2.08	1.09
	2	1.33	2.81	0.79	0.79	0.96
	3	1.54	1.02	0.83	1.11	1.29
A. spinosa	1	1.09	1.99	1.14	1.68	1.23
	2	0.94	1.02	09.6	0.62	0.90
	3	0.50	0.81	0.83	0.90	0.74
G.occultum/ A. scrobiculata	1	6.74	7.25	7.07	5.50	6.22
	2	7.17	7.61	4.99	5.69	6.12
	3	6.07	6.95	6.79	6.41	6.97
C12/*S. pellucida*	1	3.59	4.28	3.00	4.49	3.15
	2	1.62	1.52	1.92	1.74	1.84
	3	1.40	1.90	1.55	1.31	1.56
A. laevis	1	1.31	2.41	1.45	0.58	0.69
	2	0.64	0.72	0.41	0.37	0.33
	3	0.00	0.00	0.00	0.00	0.00
S. coralloidea	1	1.17	0.17	0.40	0.00	0.00
	2	0.17	0.17	0.33	0.33	0.17
	3	0.50	0.50	0.57	0.50	0.57
C22	1	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.24
	3	0.00	0.00	0.00	0.00	0.00
G. geosporum	1	0.94	0.40	1.41	1.13	2.15
	2	1.04	1.01	1.05	2.52	1.01
	3	2.23	3.39	2.22	3.39	2.54
A. mellea/A. morrowae	1	2.59	1.62	1.95	2.16	2.56
	2	2.66	4.67	1.81	3.38	3.27
	3	2.67	1.56	3.96	3.15	4.82

Slcerocy-stis spp	1 2 3	0.00 0.00 0.17	0.00 0.00 0.17	0.00 0.00 0.17	0.00 0.00 0.17	0.00 0.00 0.50
Total mean spore numbers	1 2 3	16.08 14.26 15.26	17.80 15.29 15.70	15.27 12.63 15.36	15.99 13.49 14.55	14.85 12.88 15.07

square root transformed values only
Time 1 represents sampling done in February 1987
2 " " " " August "
3 " " " " August 1988
LSD only shown where there are significant differences of means

4.6 SPORE POPULATIONS IN SOILS TAKEN FROM THE RHIZOSPHERIC ZONES OF SIX PLANT SPECIES IN THE MBALMAYO FOREST RESERVE (AUGUST 1988)

Soil samples were obtained directly from the root zones of *Triplochiton scleroxylon*, *Lovoa trichilioides*, *Khaya* sp., *Entrandrophragma cylindricum* (all of which are members of *Meliaceae)*, *Musanga cecropioides* and *Eupatorium odoratum* (Table 4.9). The results indicated that rhizospheric soils of each tree species was, as with *T. superba* dominated by *Glomus etunicatum*.

In contrast the highly invasive weed *Eupatorium odoratum* though associated with the same array of fungi as *T. superba* and the other species of trees, was dominated by *G. geosporum* and *G. occultum/A. scrobiculata* instead. *Musanga cecropioides*, a pioneer species of secondary tropical forests had much smaller numbers of spores than the other tree species and in fact species like *A. spinosa* were not recovered from the root zones of this plant. VAM species such as C12/*S. pellucida* were generally evenly distributed irrespective of the plant species sampled.

Spores of C4, *A. laevis* and C22 were not recovered from the rhizospheres of any of the plant species sampled.

4.7 ASSESSMENT OF VAM SPECIES RICHNESS AND ABUNDANCE (SIMPSON'S DIVESITY INDEX, D) AND EVENNESS (SIMPSON'S EQUITABILITY INDEX, E) (FEBRUARY 1987)

VAM species diversity (D) and equitability (E) as tested by Simpson's diversity and equitability indices, indicate species richness, commonness and rarity (Begon *et al.*, 1990). Simpson's diversity, index (D) normally has a range corresponding to the number of species in the sample whereas the equitability index (E) assumes values between 0-1.

Using Simpson's diversity index therefore, the fungal richness and abundance in the Mbalmayo Forest was observed to be low.

Plot 1 however was the most species rich (2.403) followed by plot 4 (2.284), plot 3 (2.155) and plot 2 (2.147). The analysis of variance

Table 4.9
Variations observed in the analysis of mean spore numbers of VAM fungi extracted from 100 g soil samples collected from the root zones of the plant species shown. Means of 4 (100ml) samples per plant species.~

	Plant Species							
VAM fungal types	*Triplochiton scleroxlyon*	*Lovoa trichilioides*	*Khaya* sp.	*Entandrophragma cylindricum*	*Musanga cecropioides*	*Eupatorium odoratum*	Grand mean	LSD,p=0.05
G. etunicantum	290.02 (17.03)	457.53 (21.39)	254.40 (15.95)	297.56 (17.25)	101.0 (10.05)	67.57 (9.22)	224.40 (14.98)	2.63*
G. fasciculatum/ G. macrocarpus	55.80 (7.47)	23.81 (4.88)	0.72 (0.85)	46.38 (6.81)	18.15 (4.26)	11.76 (3.43)	21.34 (4.62)	2.06
A. spinosa	0.25 (0.50)	1.80 (1.34)	1.72 (1.31)	0.25 (0.50)	1.0 (1.00)	nil	0.58 (0.76)	1.02
G. occultum/A. scrobiculata	115.78 (10.76)	93.12 (9.65)	109.20 (10.45)	63.20 (7.95)	42.90 (6.55)	311.88 (17.66)	09.62 (10.47)	2.15
C12/*S. pellucida*	0.30 (0.60)	1.00 (1.00)	0.36 (0.60)	0.25 (0.50)	0.36 (0.06)	0.06 (0.25)	0.35 (0.59)	ns
A. laevis	nil	nil	nil	nil	nil	nil	nil	
S. coralloidea	0.06 (0.25)	nil	0.36 (0.60)	0.25 (0.50)	1.54 (1.24)	0.22 (0.47)	ns	
G. geosporum	47.61 (6.90)	33.52 (5.29)	32.15 (5.67)	26.63 (5.16)	4.04 (2.01)	366.72 (18.35)	53.44 (7.31)	3.05
A. mellea/A. morrowae	nil	0.50 (0.71)	28.20 (5.31)	74.82	66.91 (8.18)	nil	14.52 (3.81)	1.58
Sclerocystis sp.	nil	nil	0.25 (0.50)	nil	nil	4.97 (2.23)	0.61 (0.78)	0.45

Analysis done on square root transformations of numbers of spores of the different VAM fungi extracted from 100 g dry weight soil samples.

* significant difference at P = 0.05

Table 4.10 Simpson diversity index (D) and Equitability index (E) showing variations in VAM species richness and abundance, and evenness respectively before the onset of treatments (February 1987) influenced by plot differences (P), amounts of cover (A) and distance from *T. superba* trees. Means of 30 samples per plot.

Plots	1	2	3	4	LSD p = 0.05
(D)	2.403	2.147	2.155	2.284	ns
(E)	0.02289	0.02559	0.02667	0.02421	ns

Cover	With	Without	LSD p=0.05
D	2.312	2.182	ns
E	0.02368	0.02601	ns

Distance	2.5m south	2.5m north	5.0m north	7.5m north	10.0m north	LSD
D	2.109	2.469	2.234	2.174	2.250	ns
E	0.02615	0.02205	0.02483	0.02596	0.02522	

however did not indicate significant differences in VAM species diversity between the plots (Table 4.10). Neither did cover, distance nor the resulting interactions significantly alter the abundance and richness of VAM fungi.

Similarly the low values of Simpson equitability index (E) obtained for all the plots indicated a highly uneven distribution of VAM fungi within the population (Table 4.10).

4.8 DISCUSSION

All the VAM fungi from this moist semi-deciduous forest at Mbalmayo appeared in the four plots sampled. In addition the total spore numbers were surprisingly evenly distributed considering the widely recorded clumped distribution of VAM fungal spores in soils (Porter, 1982; Walker *et al.*, 1982; St John and Hunt, 1983). Results of this study indicate possibilities for smoothing out such variability in spore distribution by taking large numbers of samples (as suggested by St John and Koske, 1988) and by sampling over large areas (Anderson *et al.*, 1983) as was done in this study. Such an approach increases the chances of recovering all the different VAM fungi from each sample area.

Unlike *G. etunicatum* some individual VAM fungi nevertheless showed clumped distributions such as *A. laevis*, C4, *A. spinosa*, *G. occultum/A. scrobiculata* which may be related to the association of these fungi with specific components of the native vegetation and to the location of roots within this forest as suggested by Walker *et al.* (1982) in their study of poplar stands.

For a natural tropical ecosystem such as the Mbalmayo Forest the overall mean spore number of 262 spores/100 g dry soil seems large. This is in contrast to reports by Sieverding (1989) who indicated that spore numbers of VAM fungi from natural tropical ecosystems were generally small despite the vast diversity of fungi in these ecosystems. However, the number of spores recovered from Mbalmayo compare favourably with reports from a lowland tropical forest in Singapore (Louis and Lim, 1987) and a subtropical evergreen forest in

Meghalaya (Sharma *et al.*, 1986; Mukerji and Kapoor, 1986). In contrast to natural tropical ecosystems, such as the Mbalmayo Forest, tropical agricultural systems have been shown to contain greater spore numbers but with less types of VAM fungi (Sieverding, 1989). A survey by Johnson (1977) of a temperate coniferous forest in New Zealand shows that the spore numbers from the semi-deciduous forest at Mbalmayo were remarkably fewer. Comparable spore numbers to those at Mbalmayo, however, have been reported from poplar stands in Central Iowa (Walker *et al.*, 1982) and from apple rootstocks in the United States (Miller *et al.*, 1985). High spore numbers may be the result of various factors such as the degree of infection of plant roots soil reaction and nutritional status (Hayman, 1970), host plants (Hayman *et al.*, 1978) and environmental factors (Furlan and Fortin, 1973; Schenck and Schroder, 1974; Koske, 1981).

Soils of the Mbalmayo forest, as in most areas of the tropics, are low in nutrients, particularly in available phosphates (0.015%, Lawson *et al.*, 1990). They are also acidic (pH 4.4) and clayey, this increases the leaching potentials of cations which in turn immobilize phosphates. When phosphates are immobilized plant dependence on the mycorrhizal symbiosis is enhanced and maximum root colonization and (Hayman, 1970; Louis and Lim, 1987) subsequent sporulation occurs. Menge *et al.* (1978) have shown, however, that much of the influence of soil fertility on root colonization is plant mediated and are not directly related to soil nutrient status.

Prevailing environmental conditions such as temperatures, light and moisture contents of soils may also dictate VAM fungal spore populations in the Mbalmayo forest (Furlan and Fortin, 1973; Hayman 1974; Koske, 1981). Sunshine in the tropics is very variable due to the shifting rainbelts and topography (Mohr *et al.*, 1972), which in turn lead to changes in temperature, light and moisture content. In February when this study was effected, rainfall was minimal while temperatures and light were maximal. Both temperature and light have been shown to have a significant influence on colonization and sporulation by VAM fungi under greenhouse conditions (Furlan and Fortin, 1973; Hayman, 1974). Higher temperatures resulted in increased colonization and sporulation of VAM fungi. Studying

temperature effects on VAM establishment, Schenck and Schroder, (1974) observed that sporulation and vesicle development were greatest at 35°C. Similarly, increased light intensity generally increased percentage root colonisation (Hayman, 1974). Although all these studies have been conducted under greenhouse conditions they provide useful information on what may likely be happening in the field, because with February being the hottest month in Mbalmayo, it is likely that plants especially the canopy trees would have experienced increased transpiration rates and drier soil conditions which would partially limit root growth. According to Nelsen and Safir (1982), high levels of root colonization can occur in such drought stressed plants even in highly fertile soils. Given such periods of stress therefore plants in Mbalmayo presumably sporulated more profusely. Soils sampled at this time (February 1987) from the Mbalmayo forest thus had significantly greater numbers of spores of *A. spinosa*, C12/*S. pellucida* and *G. fasciculatum/G. macrocarpum* than in August 1987 and August 1988, both rainy periods.

After the onset of rains (August 1987 and August 1988) the number of spores in the Mbalmayo forest decreased for a majority of VAM fungi, probably because spore germination had occurred in response to increased moisture. Similar observations have been made in tropical Nigeria by Redhead (1977) and Sanders and Sheikh (1983).

Changes in spore numbers over time, however, were not identical for all the VAM fungi. Species such as *G. geosporum*, *A. mellea/A. morrowae* and *Sclerocystis* spp. increased in numbers with time, whereas *G. etunicatum* and *G. occultum/A. scrobiculata* decreased in spore numbers during the first period of rains (August, 1987) but increased the following rainy season (August 1988). These differences may be accounted for by differences in the life cycles of the VAM fungi (Hepper, 1977) or the result of fluctuating antagonistic and predatory organisms like mites which feed on VAM fungal spores (Ross and Rottencutter, 1977).

The effects of cover were assessed in order to examine whether *T. superba* trees associated with the same or different fungi to surrounding herbaceous vegetation. Not unexpectedly the same array of

fungi from 'with cover' transects were recovered from 'without cover' transects, thus showing that VAM fungi were in no way specific to *T. superba* trees, but were distributed throughout the forest in association with other vegetation, including herbs and shrubs. The presence on average of 5 trees of *T. superba* per hectare makes it likely that VAM fungi observed in areas 'without cover' must have arisen from shrubs, herbs or roots of other tree species, passing across the area. Also the spore numbers of most VAM fungi (except *S. coralloidea*) were generally greater along 'with cover' transects. These observations thus support the widely held view that VAM fungi are non-host specific (Walker *et al.*, 1982; Mukerji and Kapoor, 1986; Newman and Redell, 1987) which is an advantage in this plant species rich forest at Mbalmayo.

The effect of distance on VAM fungal distribution, although not as clear cut as that described by Mason *et al.* (1983) for ectomycorrhizas (where trees were spaced out) nevertheless indicated that *T. superba* trees exert a considerable influence on spore numbers. The somewhat lack of consistency in the data is probably due to the criss-crossing of roots from various trees and herbaceous species within the forest floor. Notwithstanding, generally, spore numbers peaked at 2.5 m of *T. superba* trees but declined sharply thereafter except for the VAM fungi *S. coralloidea, G. geosporum* and *A. mellea/A. morrowae* which peaked at 7.5 m to 10.0 m from specimen trees. These results indicate spatial variations in VAM fungal distribution (Walker *et al.*, 1982) and agree on a broad basis with observations on ectomycorrhizas (Mason *et al.*, 1983) in which some species preferred associations close to the base of trees while others appeared to dominate at distances further way from tree stem.

Although VAM fungi, as mentioned above, appear to be non-host specific they do, however, exhibit host preferences (Bevege, 1971; Koske and Halvorson, 1981). Results from the Mbalmayo forest lends credence to this view as soils from the rhizosphere of six plant species showed that spore production by *G. occultum* and *G. geosporum* was greatly stimulated in the presence of *Eupatorium odoratum* and *Musanga cecropioides* respectively, both plant species that readily invade disturbed sites. This view is supported by studies carried out by

Koske and Halvorson (1981) on a barrier sand dune where spore production was enhanced in the presence of *Ammophila* species compared to other species in the dune. Similarly, Hetrick and Bloom (1983) noted that C4 grasses from a Konza prairie showed a dependency on *Glomus etunicatum*, while Anderson *et al.* (1984) reported that *G. fasciculatum* and *G. geosporum* were positively associated with vascular plants of nutrient poor soils.

Attempts were made at analysing the richness, commonness and rarity of the VAM fungi within Mbalmayo using Simpson's diversity and equitability indices which describe community composition (Begon *et al.*, 1990). The range of values of Simpson's diversity index depend on the number of species in a sample whereas the equitability index ranges form 0-1, to indicate evenness. The results gave very low diversity and equitability indices probably reflecting the uneven representation in abundance of species of the VAM population. According to Begon *et al.* (1990) a species rich but inequitable community has a lower diversity index than a less species-rich but highly equitable community. In the Mbalmayo forest the unevenness is largely accounted for by *G. etunicatum* which singularly made up 55-68% of total spore counts in all plots, whereas the others made up less than 20% of the entire spore population. Such occurrences are however not uncommon; from a lowland rainforest in Singapore (Louis and Lim, 1987) the dominance by an unidentified *Glomus* species was reported. Similarly from a subtropical evergreen forest in India, Sharma *et al.* (1986) noted a *Glomus* species accounted for 75-100% the spore population. In Nigeria and Senegal (Redhead, 1977; Diem *et al.*; 1981) species of *Gigaspora* were most dominant while in Illinois, *G. fasciculatum* associated with little bluestem formed more than 79% of the total spore counts (Dickman *et al.*, 1984). These results indicate differences in endophytes to sporulate as well as differences in host/fungal associations (Koske and Halvorson, 1981).

From the diverse array of VAM fungi observed in the research plots in the Mbalmayo Forest, it appears that as in other moist tropical ecosystems (Janos, 1980) most plant species are VA mycorrhizal. My results confirm observations that trees such as *T. superba* act as a major reservoir of VAM fungal inoculum within tropical forests. Such

information thus identifies another important role for trees in agroforestry where the farmer by encouraging trees will be able to maintain high VAM fungal diversity appropriate for the array of crops he intends to cultivate. Thus the inclusion of trees in agroforestry systems may aid in optimizing productivity of a wide range of agricultural crops. The variation in spore numbers observed in this study have been indicative of differences in host/fungal preferences, soil reaction and fertility, differences in endophyte ability to sporulate and seasonal including environmental effects. By understanding the factors affecting VAM fungi in Mbalmayo useful information can thus be made available to those involved in plantation establishment or agroforestry schemes, in order to ensure maintenance of a diverse and effective VAM flora, which is more desirable and easier than having to culture and inoculate plants with VAM fungi lost through poor management schemes.

CHAPTER 5

SPORE POPULATION DYNAMICS OF THE MBALMAYO FOREST FOLLOWING CLEARANCE AND PLANTING WITH *TERMINALIA IVORENSIS*

5.1 INTRODUCTION

Prior to the onset of pre-planting site preparation, the soil samples taken in February 1987 from Mbalmayo Forest could be regarded as replicates. By August 1987 (in the rainy season), when the second set of soil samples was taken, treatments had been applied involving different degrees of soil disturbance: plot 4 remained undisturbed to serve as control; plot 2 was completely cleared of vegetation and received maximum disturbance, while plots 1 and 3 received intermediate degrees of disturbance associated with manual and mechanical clearance respectively.

When sampled in February 1987, before site preparations a relatively uniform total spore distribution was observed across the four plots. Closer inspection of the data revealed that more spores were recovered from *Terminalia superba* transects than from the herbaceous non-tree transects. The importance of trees as reservoirs of VAM fungi was also illustrated and this revealed that spore numbers peaked mostly at 2.5 m from the base of *T. superba* trees. In this chapter changes which occurred following site preparation and planting are presented.

5.2 CHANGES IN SPORE NUMBERS WITHIN AND BETWEEN THE PLOTS FOLLOWING CLEARANCE

Three months following site preparation, spore numbers decreased considerably, even in the undisturbed control. However, the fall in spore numbers observed was least in the control plot (256 to 188 spores/100 g dry soils) with a loss of 27% and greatest in the completely cleared plot (276 to 96 spores/100 g dry soil) with a loss of 65%. The mechanically cleared plot, the soils of which were more disturbed than the manually cleared plot lost only 27% of its spore population (218 to 160 spores/100 g dry soils) as compared to the manually cleared which lost 45% of its initial spore population (262 to

145 spores/100 g dry soil).

Although significant differences were not observed between spore numbers of the four plots prior to clearance, significantly fewer spores were observed in the completely cleared plot after site preparation, when compared to the two partially cleared plots and the control, thus giving a reasonably direct relationship between percentage loss of spores and the intensity of disturbance (Fig. 5.1).

A decline in spore numbers following clearance was also observed for many of the individual VAM fungi although the numbers of only four of these (i.e. *G. etunicatum*, *G. occultum/A. scrobiculata*, C12/*S. pellucida* and *A. mellea/A. morrowae*) were significantly decreased (Figure 5.2).

In sharp contrast to the number of spores retrieved prior to clearance in February 1987, site preparations led to spores of *G. etunicatum* the most dominant fungus, being present in significantly fewer numbers in both the manually and mechanically cleared plots (plots 3 and 2 respectively) when compared with the untreated control (plot 4). The completely cleared plot which before clearance possessed more spores of *G. etunicatum* than the control plot now possessed less than half that number. In all the plots sampled in August 1987, however, *G. etunicatum* remained the dominant fungus (Table 5.9).

G. occultum/A. scrobiculata still remained the second most numerous fungal aggregate after site preparation in all the four plots. The number of spores of this VAM fungal aggregate dropped slightly in the undisturbed control (43 to 40 spores/100 g dry soil) and the manually cleared (45 to 40 spores/100 g dry soil) plots, but did so sharply in the completely cleared (52 to 26 spores/100 g dry soil) plot. In contrast to the above trends, a slight increase in number of spores of *G. occultum/A. scrobiculata* was observed in the mechanically cleared plot (42 to 46 spores/100 g dry soil) which led to the spore numbers in the control plot not differing significantly from those of the mechanically cleared plot and both plots ended up having significantly more spores than in the manually and completely cleared plots - the relationship with disturbance was not totally straightforward.

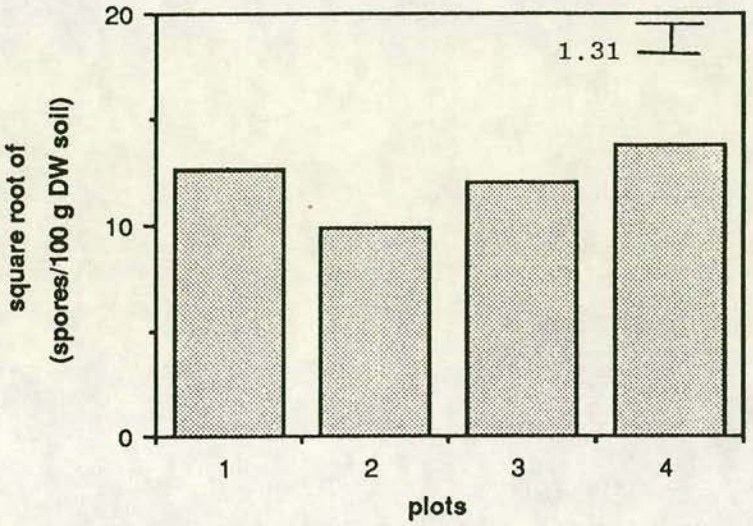


Figure 5.1
Changes in the mean total spore numbers of VAM fungi extracted from 100 g dry soil from the Mbalmayo forest after treatments (rainy season). Means of 30 samples per plot.
LSD indicated by error bars only when differences in means are significant
p = 0.05

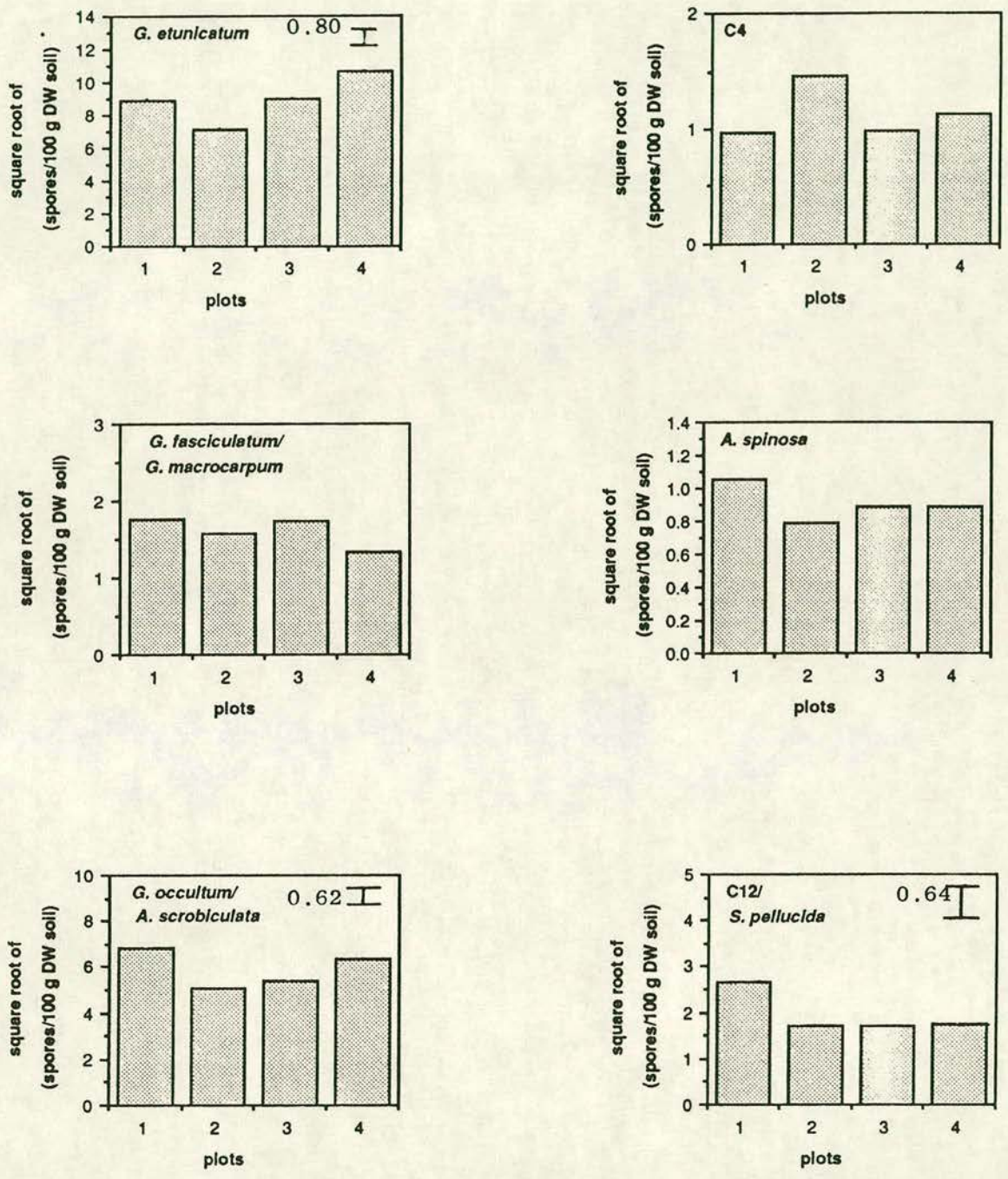
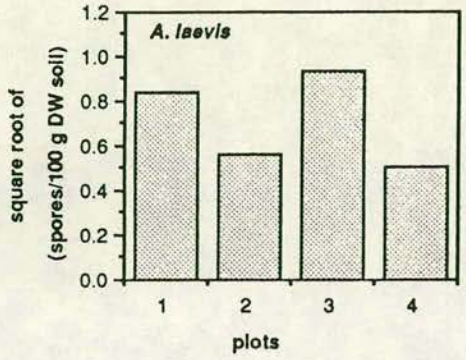
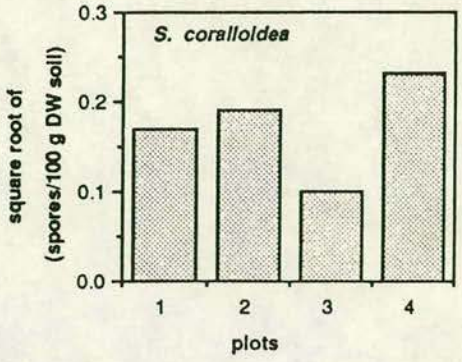
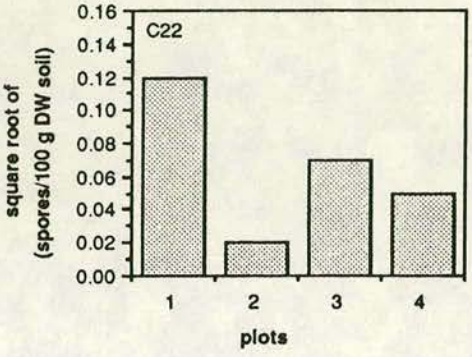
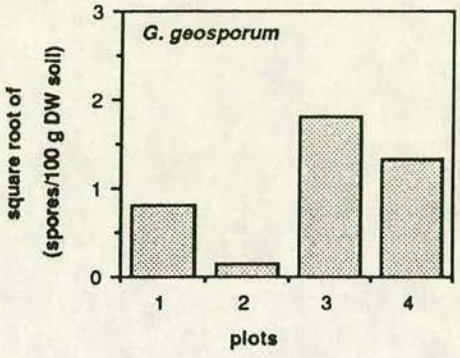


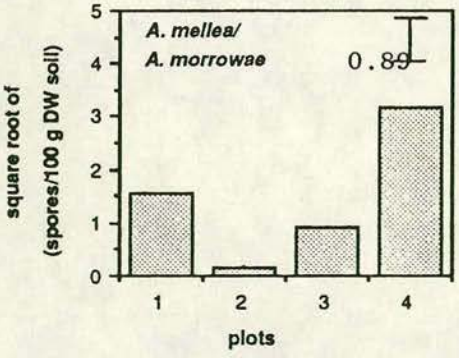
Figure 5.2
Changes between the plots of the mean spore numbers of 12 VAM fungi extracted from 100 g dry soil collected after tretments of the four (1 ha) plots at the Mbalmayo forest (August 1987), but prior to planting of young *T. ivorensis* trees. Means of 30 samples per plot.
LSD indicated by error bars when differences in means are significant at p = 0.05.











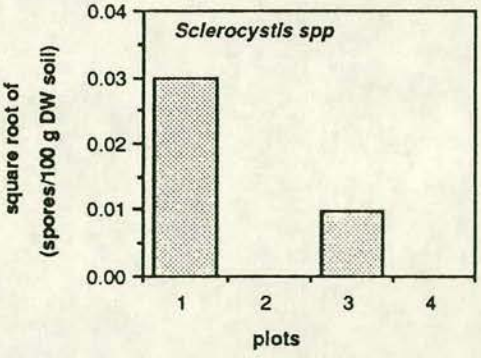


Figure 5.2 continued

Large drops in spore numbers of C12/*S. pellucida* were seen in 3 of the plots including the undisturbed control but not in the mechanically cleared plot. As a result, this led to the mechanically cleared plot possessing significantly greater numbers of spores of this species aggregate than the other three plots.

Before site preparation, the mechanically cleared plot (plot 1) had significantly greater number of spores of *A. mellea/A. morrowae* than the other 3 plots including the control. After site preparation however, a sharp drop in spore numbers of *A. mellea/A. morrowae* was observed in the mechanically cleared plot (19 to 2 spores/100 g dry soil), the completely cleared plot (6 to 0.03 spores/100 g dry soil). In contrast, in the control plot the spore numbers of this fungal aggregate increased (5 to 10 spores/100 g dry soil) and as a result it had significantly more spores than the other three plots.

Similar trends, although not significant were observed for *A. laevis*, *G. geosporum*, C22, *A. spinosa* and *S. coralloidea* (Figure 5.2).

5.3 EFFECTS OF COVER ON NUMBERS OF SPORES

In February 1987, prior to site preparation, no significant differences ($p=0.05$) were observed in the overall spore numbers retrieved from soil samples taken from transects 'passing through' *T. superba* ('with' cover) and herbaceous non-tree ('without' cover) transects. After site preparation, there was a complete change; site preparation caused a 52% drop in the overall spore numbers retrieved from soil samples taken through *T. superba* transects ('with cover') as opposed to only 31% drop from the herbaceous non-tree transects (Figure 5.3). This led to more spores now being recovered from transects through herbaceous non-tree transects.

Before and after site preparation, spore numbers of some VAM fungi were subject to significant effects of 'cover'. Prior to site preparation the number of spores of all VAM fungi except *S. coralloidea* were greater, but not necessarily significantly different along *T. superba* transects compared with the herbaceous non-tree transects. After site preparation, the VAM fungi *G. etunicatum*, *G. fasciculatum/G.*

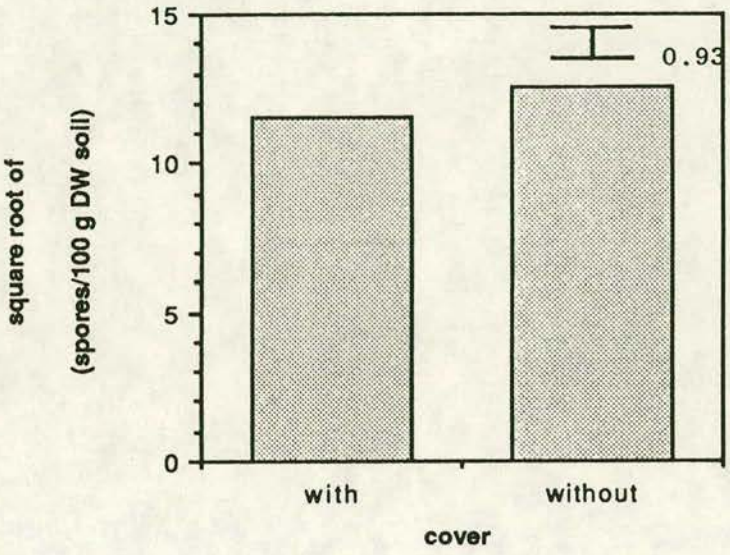


Figure 5.3
Variations resulting from amounts of cover on the mean total numbers of spores of VAM fungi extracted from 100 g dry soils collected from the plots at Mbalmayo after site clearance in August 1987 (rainy season). Means of 120 samples. LSD indicated by error bars when differences of means are significant at p = 0.05.

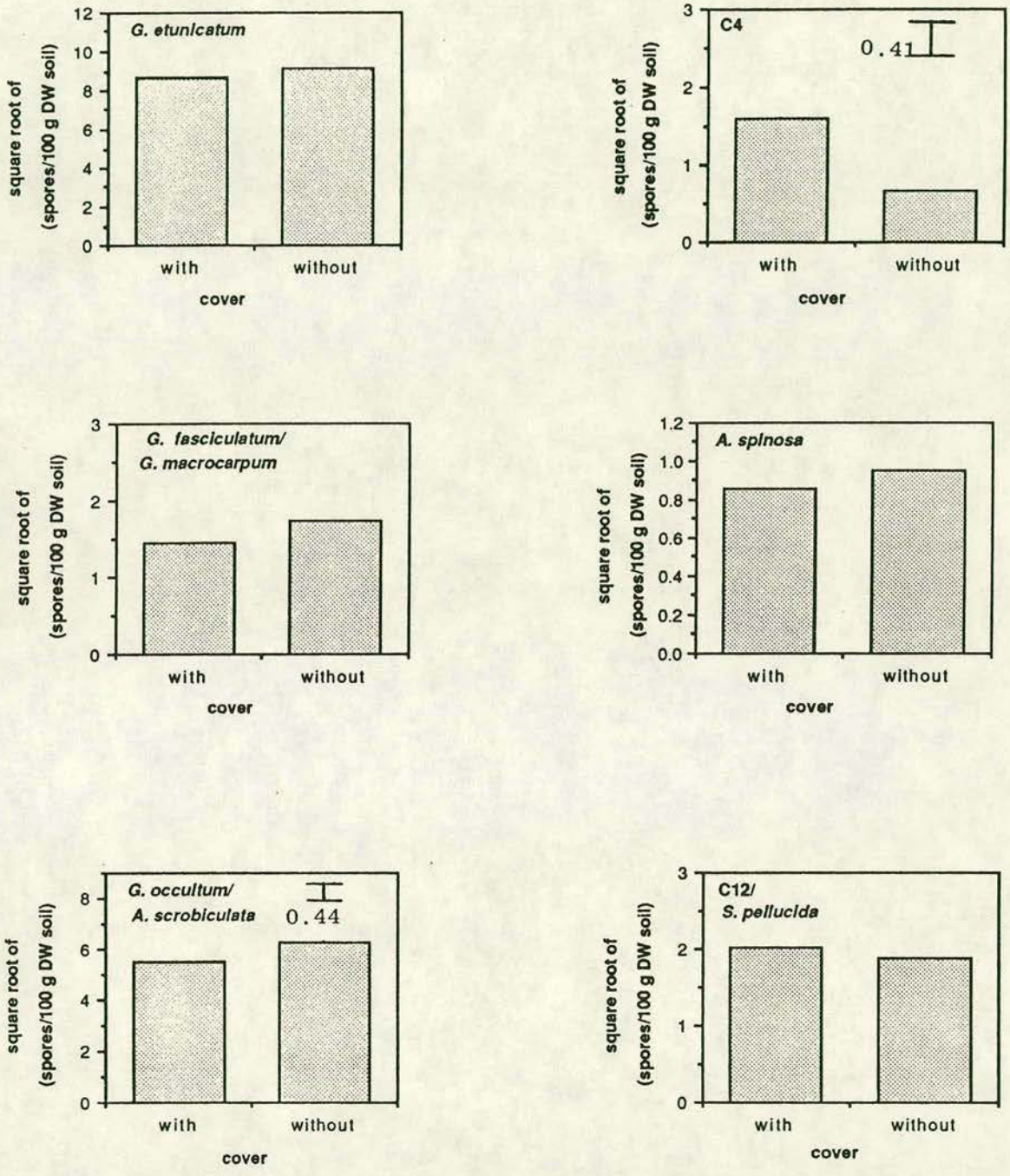
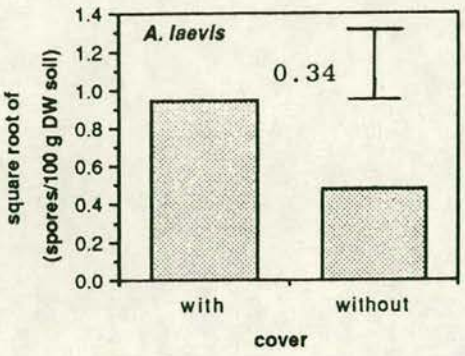
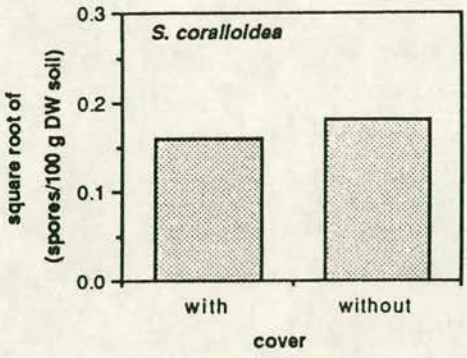
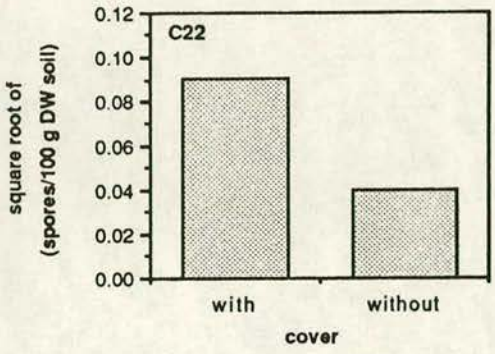
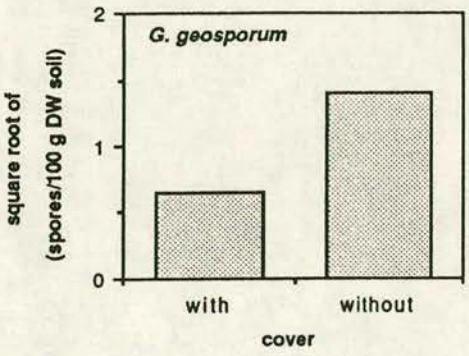


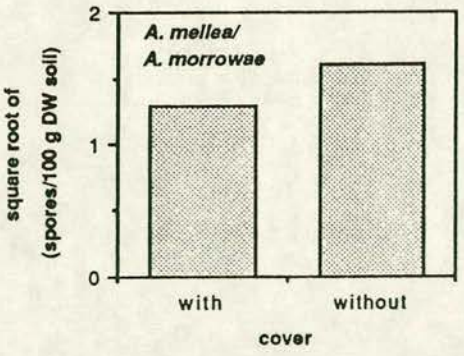
Figure 5.4
Variations in the effects of cover on the mean spore numbers of 12 VAM fungi extracted from 100 g dry soils collected after treatments of the four (1 ha) plots of the Mbalmayo forest in August 1987 (rainy season) but prior to planting of young *T. ivorensis* trees. Means of 30 samples per plot. LSD indicated by error bars only when differences of means are significant at p = 0.05.











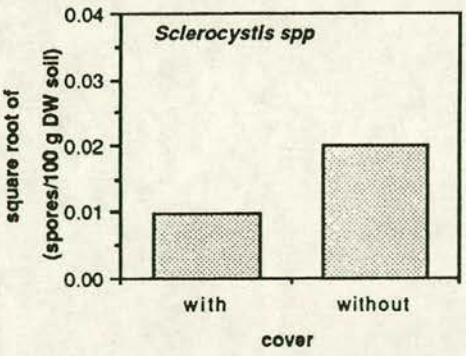


Figure 5.4 continued

macrocarpum, *A. spinosa*, *S. coralloidea*, *G. geosporum*, *A. mellea/A. morrowae*, *G. occultum/A. scrobiculata* showed a reversal of this trend whereas C12/*S. pellucida*, C22, C4 and *A. laevis* were still more abundant in samples taken along *T. superba* transects. Of the latter, only C4, *G. occultum/A. scrobiculata* and *A. laevis* were altered significantly: nonetheless more spores of *G. occultum/A. scrobiculata* were recovered from the non-tree herbaceous transects (Figure 5.4), whereas more spores of C4 and *A. laevis* were still recovered along *T. superba* transects.

5.4 EFFECTS OF DISTANCE FROM *T. SUPERBA* ON NUMBERS OF SPORES

In February 1987, the general trend shown by the effects of distance on the overall mean spore numbers was for spore numbers to be maximal at 2.5 m from trees and then decrease with increasing distance from *T. superba*. This trend however was not consistent in the herbaceous non-tree transects in which spore numbers from the markers had no particular relation close to or away from markers.

After site preparation (August, 1987) the overall mean spore numbers decreased substantially; the number of spores still decreased with increasing distance from *T. superba* (10 m) as in February 1987 (Figure 5.5).

Of the 12 VAM fungal types, 5 showed significant changes in spore numbers along transects, before site preparation; all decreasing with increasing distance from *T. superba* trees. Following site preparation this trend persisted for all VAM fungi except C22, although only *G. occultum/A. scrobiculata* differed significantly. Inspection of the data shows that spore numbers peaked at 2.5 m from *T. superba* and declined significantly at greater distances away. Unlike the situation before site preparation when a number of VAM fungi had significantly different numbers of spores at 2.5 m south and 2.5 m north of *T. superba*, this difference was not observed after site preparation (Figure 5.6).

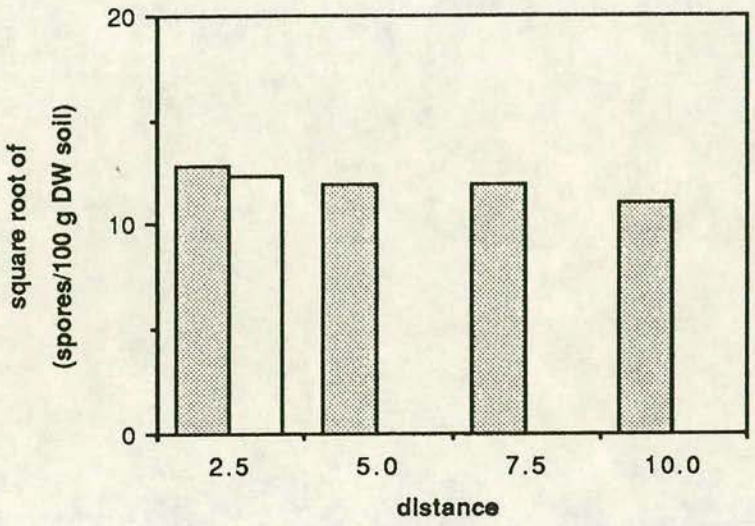


Figure 5.5
Changes associated with distance from specimen *T. superba* trees on the mean total number of VAM spores extracted from 100 g dry soils collected from the Mbalmayo plots after site clearance (August 1987). Means of samples per plot. LSD indicated by error bars only when differences in means are significant at p = 0.05. Shaded bars represent distances to the north of specimen trees while the blank bar represents distance to the south.

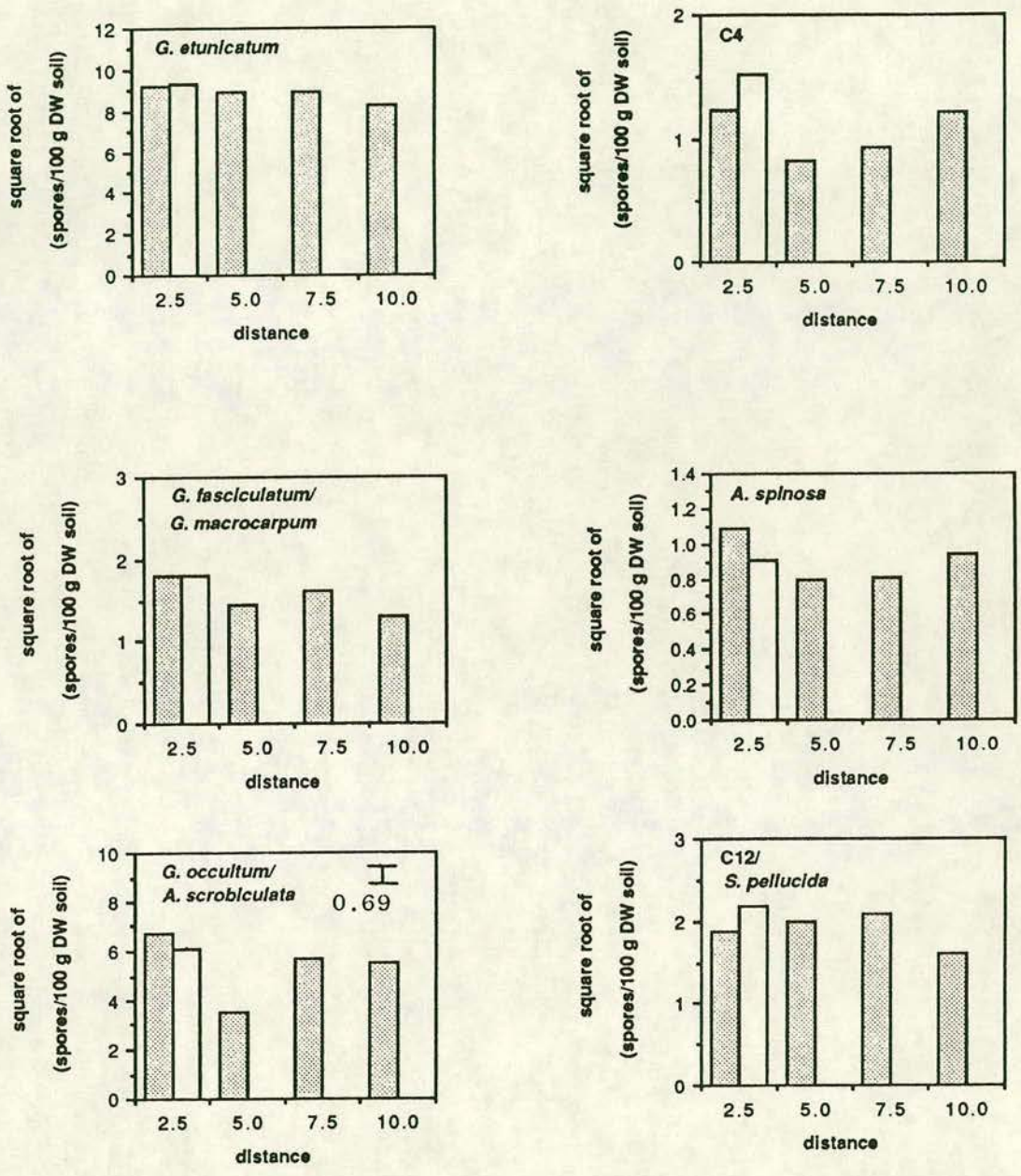
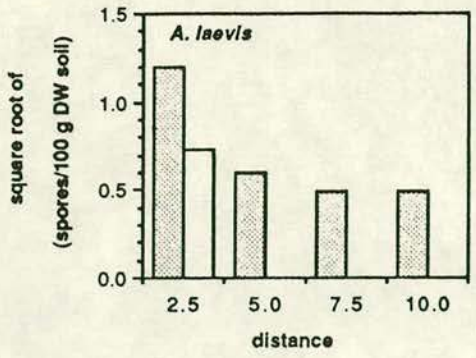
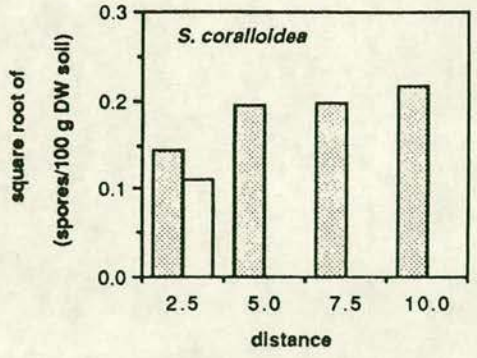
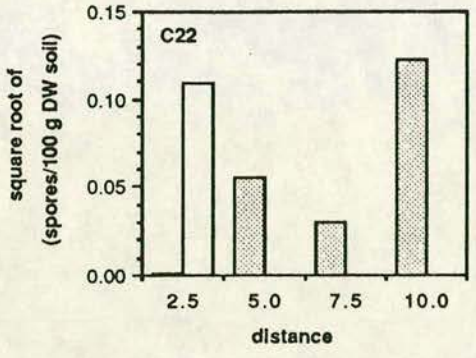
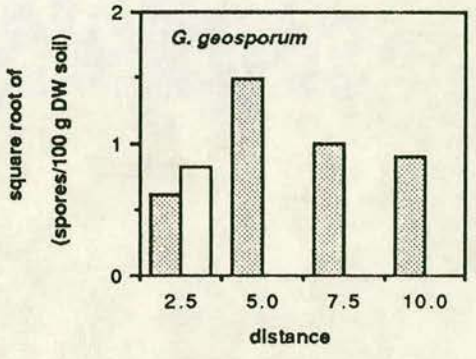


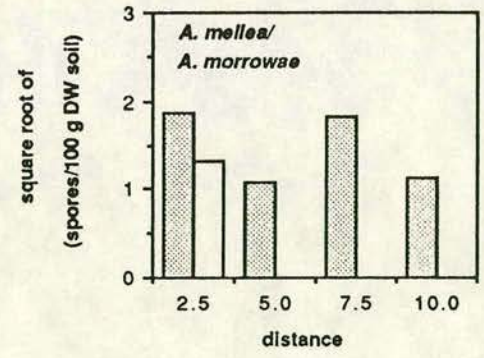
Figure 5.6
Variations in the effects of distance from specimen *T. superba* trees on the mean spore numbers of 12 VAM fungi extracted from 100 g dry soils collected after treatments of the four (1 ha) plots of the Mbalmayo forest in August 1987 (rainy season) but prior to planting of young *T. ivorensis* trees.
Means of 30 samples per plot.
LSD indicated by error bars when differences in means are significant at p = 0.05.











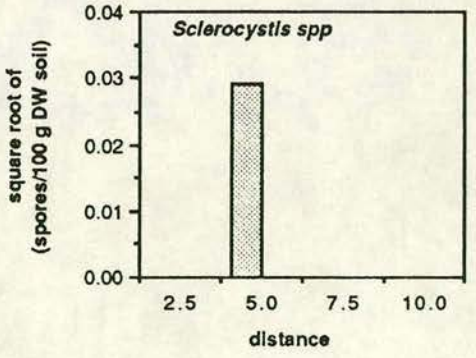


Figure 5.6 continued

5.5 INTERACTIONS: PLOT X COVER; PLOT X DISTANCE; COVER X DISTANCE AND PLOT X COVER X DISTANCE

5.5.1 Plot x cover

Seven VAM fungi were subject to plot x cover interactions before site preparation in February 1987. Following site preparation (August 1987) however, only one VAM fungus (*G. etunicatum*) was significantly affected (Table 5.1). Before site preparation, plot 2 (the completely cleared plot) and plot 4 (the undisturbed control) had greater numbers of spores of *G. etunicatum* along *T. superba* transects than occurred along similar transects in plot 3 (the manually cleared plot) and plot 1 (the mechanically cleared plot).

After site preparation, numbers of spores of *G. etunicatum* along 'with cover' transects in the control, manually and mechanically cleared plots were similar. Along the non-tree herbaceous transects ('without cover') the control plot retained more spores of *G. etunicatum* than persisted in the manually and mechanically cleared plots which in turn had greater spore numbers than the completely cleared plot (Table 5.1). In the control plot a drop of 45% was registered along *T. superba* transects, but only 6% along herbaceous non-tree transects. A similar trend was observed for the mechanically cleared and completely cleared plots with 37% and 76% drops respectively along 'with cover' transects as opposed to 30% and 63% along 'without cover' transects. A different trend was however observed in the manually cleared plot which had a 45% drop along 'with cover' transects compared to a 60% drop along 'without cover' transects.

5.5.2 Plot x distance

In February 1987, the overall mean number of spores were not significantly affected by plot x distance interactions although spore numbers mostly peaked at 2.5 m of *T. superba*. After site preparation, the control plot still displayed a distinct peak in spore numbers at 2.5 m which was not evident in either of the partially cleared plots (Table 5.2). In the completely cleared plot with all trees removed, the number of spores at 2.5 to 10 m from the stake were markedly less

Table 5.1 Significant interacting effects of plot and cover (PxA) on the means pore numbers of 1 different VAM fungal type extracted from 100 g dry weight soil samples collected from Mbalmayo forest after treatments in August 1987 (rainy season) but before the planting of young *T. ivorensis* trees. Means usually of 30 samples per plot.

VAM fungal plots	Plots	Cover	
		With +	Without -
G. etunicatum (LSD.p=0.05;1.13)	Undisturbed forest (4)	97.42 (9.87)	130.19 (11.41)
	Manually cleared (3)	77.44 (8.80)	84.64 (9.20)
	Mechanically cleared (1)	83.36 (9.13)	73.79 (8.59)
	Completely cleared (2)	49.25 (7.02)	53.14 (7.29)

Table 5.2 Variations between the plots caused by the interacting effects of plot and distance (PxD) on the mean total numbers of VAM spores extracted from 100 g dry weight soil samples, collected from the Mbalmayo forest after treatments had been applied (August 1987, rainy season) but prior to the planting of young *T. ivorensis* trees. Means usually of 30 samples per plot.

PLOT	DISTANCE				
	2.5 m south	2.5 m north	5.0 m north	7.5 m north	10 m north
Undisturbed Natural forest (control) (4)	203.00 (14.26)	234.00 (15.29)	160.00 (12.63)	182.00 (13.49)	166.00 (12.88)
Manually cleared (3)	140.00 (11.82)	142.00 (11.88)	201.00 (14.17)	125.00 (11.16)	124.00 (11.15)
Mechanically cleared (1)	142.00 (11.91)	173.00 (13.14)	142.00 (11.90)	185.00 (13.59)	163.00 (12.76)
Completely cleared (2)	128.00 (11.32)	123.00 (11.10)	85.00 (9.35)	93.00 (9.62)	50.00 (7.70)

Means based on square root transforms of spores in 100 g dry weight soil samples.
(LSD,p=0.05;2.15)

than was observed at corresponding distances from *T. superba* trees and markers along herbaceous non-tree transects in the control, manually and mechanically cleared plots respectively.

Four individual VAM fungi were significantly affected by plot x distance interactions; these were *G. fasciculatum/G. macrocarpum, G. occultum/A. scrobiculata*, C12/*S. pellucida and S. coralloidea* (Table 5.3). In the control plot, the number of spores of *G. fasciculatum/G. macrocarpum* distinctly peaked at 2.5 m of *T. superba* and stakes but dropped sharply at 5.0 m to 10.0 m. In the manually and mechanically cleared plots the same pattern was not evident with *G. fasciculatum/G. macrocarpum* with spore numbers peaking at 2.5 m of the stake, dropping significantly at 5.0 m, increasing significantly at 7.5 m and dropping again at 10.0 m (table 5.3).

In contrast the spore populations of *G. occultum/A. scrobiculata* were consistently most numerous at 2.5 m from trees/stakes in the control, manually and completely cleared plots. In the mechanically cleared plot, spore numbers were high at 2.5 m, dropped appreciably at 5.0 m but peaked significantly at 7.5 to 10.0 m.

Spore numbers of C12/*S. pellucida* were most abundant in the completely cleared plot at 2.5m north and 5.0m of stake compared to corresponding distances on other plots, but dropped significantly at 10.0 m away.

The number of spores of *S. coralloidea* in the control and mechanically cleared plots increased steadily from 2.5 m to 7.5 m but dropped at 10.0 m unlike the situation in the manually cleared plot in which spore numbers decreased with increasing distances from tree/stake. In contrast, the number of spores in the completely cleared plot decreased from 2.5 to 7.5 m but increased significantly at 10.0 m, where they were most abundant.

5.5.3 **Cover x distance**

The interaction of cover x distance only significantly affected the distribution of spores of *A. spinosa* in soil samples taken from transects through the herbaceous non-tree transects ('without cover') in which the spore number remained similar at all the distances whereas

Table 5.3 Significant interacting effects of plot and distance (PxD) on the mean spore numbers of 4 VAM fungal types extracted from 100 g dry weight soil samples from the Mbalmayo forest when sampled in August 1987 (rainy season) after treatments had been applied but prior to the planting of young *T. ivorensis* trees. Means usually of 30 samples per plot.

VAM fungal type	Plot	Distance				
		2.5m south	2.5m north	5.0m north	7.5m north	10m north
G. fasciculatum/ *G. macrocarpum*	Undisturbed (4) natural forest	2.00 (1.33)	8.00 (2.81)	0.62 (0.79)	0.62 (0.79)	0.92 (0.96)
(LSD,p=0.05;1.27)	Manually cleared (3)	3.00 (1.59)	1.46 (1.21)	4.00 (1.88)	5.00 (2.17)	3.00 (1.78)
	Mechanically cleared (1)	3.50 (1.87)	2.00 (1.40)	3.00 (1.67)	3.00 (1.73)	4.24 (2.00)
	Completely cleared (2)	6.00 (2.45)	3.00 (1.73)	2.16 (1.47)	3.10 (1.76)	0.16 (0.40)
G. occultum/ *A. scrobiculata* (LSD,p=0.05;1.38)	Undisturbed natural forest	51.41 (7.17)	58.00 (7.61)	25.00 (4.99)	32.00 (5.69)	37.00 (6.12)
	Manually cleared	31.41 (5.58)	36.00 (5.93)	29.00 (5.36)	26.00 (5.11)	27.00 (5.17)
	Mechanically cleared	37.00 (6.22)	49.00 (6.99)	37.00 (6.12)	53.00 (7.27)	54.00 (7.33)
	Completely cleared	27.00 (5.22)	39.00 (6.31)	30.00 (5.49)	22.00 (4.73)	13.00 (3.60)
C12/*S.pellucida* (LSD,p=0.05;1.43)	Undisturbed natural forest	3.00 (1.62)	2.00 (1.52)	4.00 (1.92)	3.00 (1.74)	3.00 (1.84)
	Manually cleared	5.00 (2.32)	2.00 (1.51)	2.00 (1.57)	2.00 (1.28)	3.00 (1.78)
	Mechanically cleared	9.00 (2.98)	7.00 (2.64)	8.00 (2.73)	11.00 (3.37)	3.00 (1.63)
	Completely cleared	3.24 (1.80)	23.00 (4.76)	23.00 (4.78)	9.00 (3.01)	1.00 (1.18)

Table 5.3 Continued

S. coralloidea (LSD,p=0.05;0.45)	Undisturbed (4) natural forest	0.03	0.03	0.11	0.11	0.03
		(0.167	0.167	0.333	0.333	0.167)
	Manually (3) cleared	nil	0.11	0.03	nil	nil
			(0.333)	(0.167)		
	Mechanically (1) cleared	nil	0.03	0.03	0.25	nil
			(0.167)	(0.167)	(0.50)	
	Completely (2) cleared	0.08	0.01	0.01	<0.01	0.48
		(0.276	0.095	0.109	0.044	0.695)

Analysis done on square root transforms of number of spores in 100 g dry weight soil samples (___).
LSD only shown where means are significantly different, p=0.05.

from transects through *T. superba* trees ('with cover') spore numbers were significantly greater at 2.5 m and 10.0 m than at 5.0 m and 7.5 m respectively (Table 5.4).

5.5.4 Plot x cover x distance

After site preparation, plot x cover x distance interactions were significant on the overall number of spores. Generally, spore numbers in the control plot in areas 'with' and 'without' cover were significantly greater than in the other three plots. This was followed by the total number of spores in the mechanically cleared plot but least in the completely cleared plot (Table 5.5).

Table 5.6 shows a summary of the various factors (e.g. plots cover distance and interactions) on spore numbers.

5.6 SPORE POPULATION CHANGES, A YEAR AFTER PLANTING YOUNG *T. IVORENSIS* ON THE CLEARED PLOTS

After site preparation in August 1987 a drop in spore numbers was observed in all plots including the undisturbed control. Of the three treated plots the completely cleared plot lost a greater proportion of its initial spore population than those cleared manually and mechanically.

A year after outplanting young *T. ivorensis* a large increase in overall spore numbers was observed in the completely cleared (from 96 to 472 spores/100 g dry soil), the mechanically cleared (160 to 476 spores/100 g dry soil) and manually cleared (145 to 375 spores/100 g dry soil) plots. At the p=0.05 level of significance the spore numbers in these three treated plots did not differ (Fig. 5.7).

The individual VAM fungi displayed a variety of trends and although most had increased considerably; a year after planting, only 3 VAM fungal types did so significantly. They were *A. spinosa*, *G. geosporum* and *A. mellea/A. morrowae* (Figure 5.8). After site preparation (August 1987) the number of spores of *A. spinosa* was greatest in the mechanically cleared plot. A year after planting however spores of *A. spinosa* were no longer recovered from soil samples taken in the

Table 5.4 Significant interacting effects of cover and distance (AxD) on mean spore numbers of one VAM fungal type extracted from 100 g dry weight soil samples from the Mbalmayo forest sampled in August 1987 (rainy season) after the treatments had been applied but prior to the planting of young *T. ivorensis* trees. Means of 30 samples per plot.

VAM fungal type	Cover	Distance				
		2.5 m south	2.5 m north	5.0 m north	7.5 m north	10 m north
A. spinosa	With	0.90 (0.919)	2.00 (1.318)	0.40 (0.588)	0.26 (0.514)	1.00 (0.974)
(LSD, p=0.05; 0.51)	Without	1.00 (0.900)	1.00 (0.846)	1.00 (0.998)	1.23 (1.108)	1.00 (0.905)

Table 5.5 Variations in the interacting effects of plot cover and distance (PxAxD) on the mean total number of VAM spores extracted from 100 g dry weight soil samples, collected from the Mbalmayo forest after treatments had been applied but prior to the planting of young *T. ivorensis* trees (August 1987, rainy season). Means of 30 samples per plot.

PLOT	COVER: WITH — DISTANCE					COVER: WITHOUT — DISTANCE				
	2.5 south	2.5 north	5.0 north	7.5 north	10 north	2.5 south	2.5 north	5.0 north	7.5 north	10 north
Undisturbed natural forest	193.77 (13.92)	240.25 (15.50)	154.50 (12.43)	126.56 (11.25)	115.56 (10.75)	212.87 (14.59)	227.41 (15.08)	164.61 (12.83)	247.12 (15.72)	225.3 (15.01)
Manually cleared	153.51 (12.39)	158.01 (12.57)	124.32 (11.15)	111.94 (10.58)	101.61 (10.08)	126.56 (11.25)	125.22 (11.19)	295.50 (17.19)	137.83 (11.74)	149.47 (12.23)
Mechanically cleared	129.28 (11.37)	161.04 (12.69)	153.02 (12.37)	185.5 (13.62)	147.87 (12.16)	155.25 (12.46)	184.69 (13.59)	130.65 (11.43)	183.87 (13.56)	178.49 (13.36)
Completely cleared	135.96 (11.66)	136.89 (11.70)	81 (9.00)	85.56 (9.25)	50.55 (7.11)	120.56 (10.98)	110.46 (10.51)	94.09 (9.70)	100 (10.00)	68.72 (8.29)

Statistical analysis done on square root transforms of number of spores in 100 g dry weight soil samples.
(LSD, p=0.05; 3.05)

Table 5.6 Summary of the analysis of variance of the mean total numbers of individual VAM fungal spores extracted from 100 g dry weight soils collected from the Mbalamyo forest after the plots had been cleared in August 1987 (rainy season) but prior to planting of young *T. ivorensis* plants. Means of 30 samples per plot.

VAM fungal types	Plots (P)	Amounts of *T. superba* Cover (A)	Distance from tree (D)	PxA	PxD	AxD	PxAxD
G. etunicatum	+	-	-	+	-	-	-
C4	-	+	-	-	-	-	-
G. fasciculatum/ G. macrocarpum	-	-	-	-	+	-	-
G. occultum/A. scrobiculata	+	+	+	-	+	-	-
C12/*S. pellucida*	+	-	-	-	+	-	-
A. laevis	-	+	-	-	-	-	-
S. coralloidea	-	-	-	-	+	-	-
C22	-	-	-	-	-	-	-
G. geosporum	-	-	-	-	-	-	-
A. mellea/A. morrowae	+	-	-	-	-	-	-
Sclerocystis spp.	-	-	-	-	-	-	-
Overall mean spore numbers	+	+	-	-	-	-	+

+ = Significantly different at p = 0.05
- = not significantly different

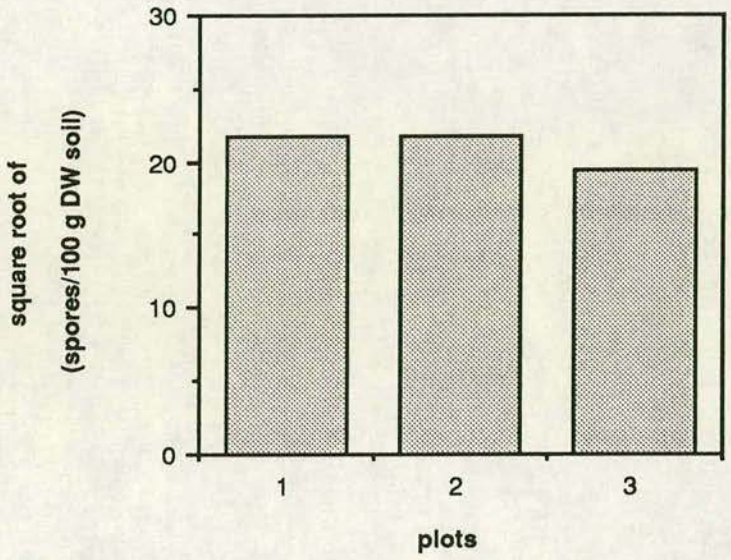


Figure 5.7 Variations between plots on the mean total numbers of spores of VAM fungi extracted from 100 g dry soils collected from the plots at Mbalmayo after site clearance and a year after planting young *T. ivorensis*. Means of 27 samples. LSD only shown when differences of means are significant at p = 0.05.

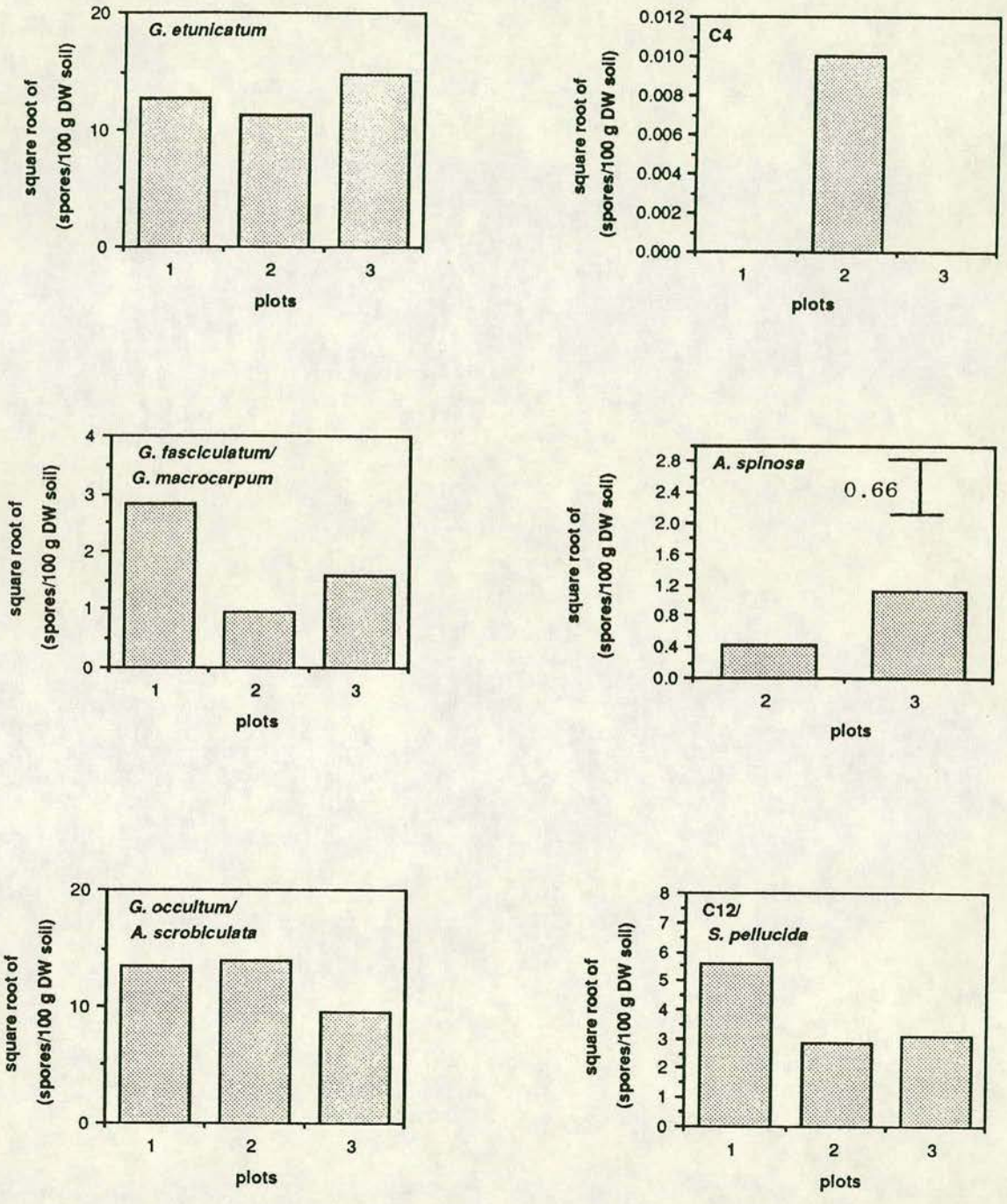
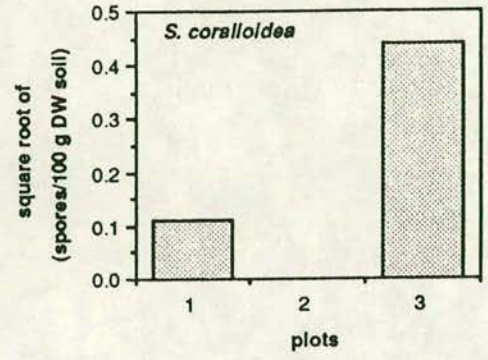
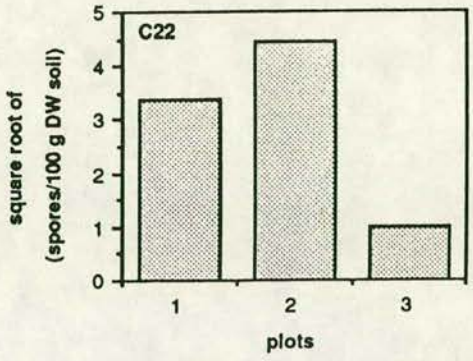
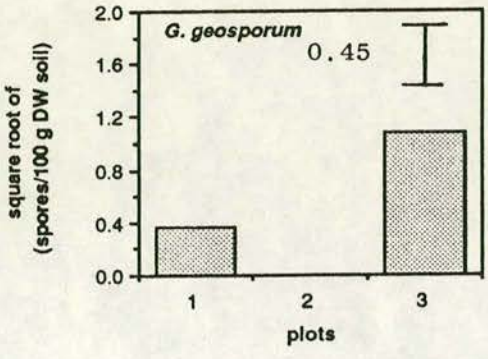
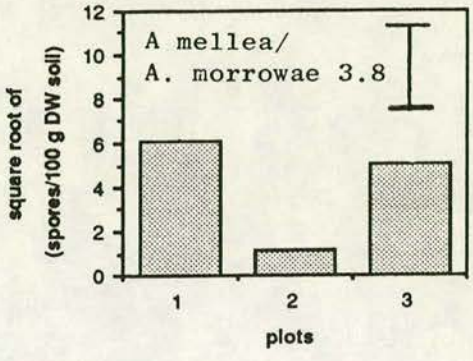


Figure 5.8
Variations between the plots in the mean numbers of spores of 12 VAM fungi extracted from 100 g dry soil collected from the three treated (1 ha) plots of the Mbalmayo forest, a year after planting young *T. ivorensis* trees (August 1988, rainy season). Means of 9 samples per plot.
LSD indicated by error bars when differences in means are significant at p = 0.05.









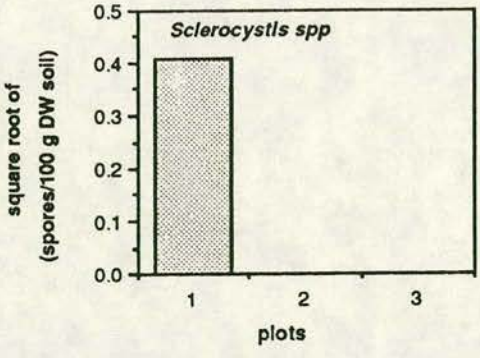


Figure 5.8 continued

mechanically treated plot (1.13 to 0 spores/100 g soils). A similar decrease was observed in the completely cleared plot where spores of *A. spinosa* fell from 0.6 to 0.2 spores/100 g dry soils. In contrast, there was an increase in the number of spores of *A. spinosa* in the manually cleared plot (0.8 to 1.3 spores/100 g dry soil) leading to this plot possessing significantly greater numbers of spores of *A. spinosa* than the other two plots (Figure 5.8).

After site preparation *G. geosporum* spore populations in the manually cleared plot were greater than in the mechanically and completely cleared plots. This trend persisted following the planting of *T. ivorensis* and although a decrease in spore numbers was observed in the manually cleared plot (3.3 to 1.2 spores/100 g dry soil) this plot had significantly more spores than the mechanically (0.64 to 0.13 spores/100 g dry soil) and completely (0.02 to 0.00 spores/100 g dry soil) cleared plots.

Although, *A. mellea/A. morrowae* spores were more abundant in the mechanically cleared plot after site preparation than in the manually and completely cleared plots planting with *T. ivorensis* led to a massive increase in spore numbers of this fungal aggregate (2.43 to 38 spores/100 g dry soil). A similar increase in spore numbers was also observed in the manually cleared plot (0.81 to 25 spores/100 g dry soil). In contrast spore numbers of this VAM fungal aggregate remained quite low in the completely cleared plot (19.03 to 1.3 spores/100 g dry soil).

One striking observation within the mechanically and completely cleared plots post planting was a change in VAM fungal dominance (Table 5.9). In the mechanically cleared plot, there had been an increase in the number of spores of *G. etunicatum* (from 79 to 162 spores/100 g dry soil). However the increase in number of spores of *G. occultum/A. scrobiculata* (46 to 184 spores/100 g dry soil) made this fungal aggregate become the dominant fungal type in this plot. In the completely cleared plot the number of spores of *G. occultum/A. scrobiculata* had also increased more dramatically (26 to 194 spores/100 g dry soil). As a result, the spore numbers of the fungal aggregate were observed to be 1.5 times greater than those of *G. etunicatum* which

had only increased from 51 to 128 spores/100 g dry soil. In sharp contrast, in the least disturbed manually cleared plot, *G. etunicatum* remained the dominant fungus with the spore numbers of this VAM fungus increasing by almost three times the number after site preparation (81 to 218 spores/100 g dry soil). *G. occultum/A. scrobiculata* spore numbers in this plot had also increased following the planting of *T. ivorensis* seedlings (30 to 92 spores/100 g dry soil) but remained 2.5 times less than the spore number of *G. etunicatum* spores (Figure 5.8).

Other VAM fungi such as C22 also increased remarkably after planting; in the manually cleared plot by 1140 times and in the completely cleared by 2000 times (0.01 to 20 spores/100 g dry soil) (Figure 5.8).

5.6.1 Effects of Distance on spore distribution, a year after planting young *T. ivorensis* trees.

Although spore numbers increased dramatically within a year of planting *T. ivorensis*, differences associated with distance were not significant. The total number of spores of all VAM fungi were still greatest at 2.5 m of *T. superba/T. ivorensis* dropping slightly at 5.0 m (Figure 5.9).

Distance effects influenced the distribution of spores of *G. occultum/A. scrobiculata* which were most numerous about 5.0 m from trees as against 2.5 m in many instances (Figure 5.10).

5.6.2 Interactions: Plot x distance

5.6.2.1 Plot x distance

After site preparation, (August, 1987) plot x distance interactions significantly affected numbers of four VAM fungal types (*G. fasciculatum/G. macrocarpum*, *G. occultum/A. scrobiculata*, C12/*S. pellucida* and *S. coralloidea*). Of these four, only the *G. fasciculatum/G. macrocarpum* aggregate remained significantly different a year after planting young *T. ivorensis* trees (Table 5.8). In addition, *G. etunicatum* similarly differed significantly after planting *T. ivorensis*.

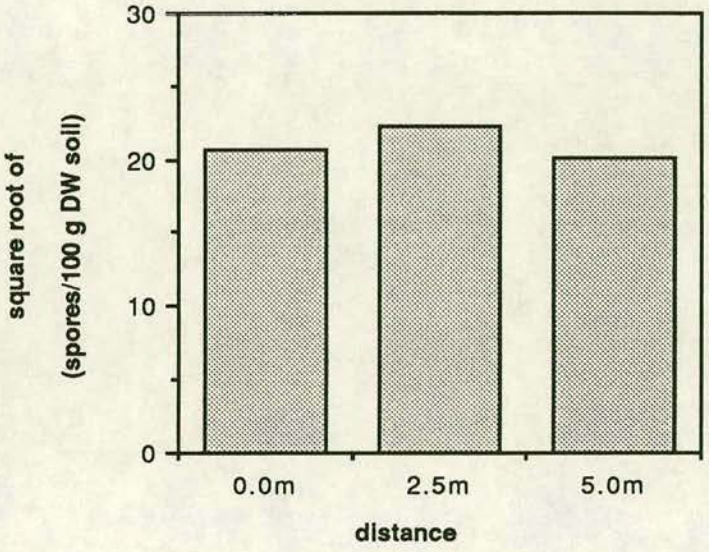


Fig. 5.9 Variations from amounts of distance on the mean total numbers of spores of VAM fungi extracted from 100 g dry soils collected from the plots at Mbalmayo, after site clearance and one year after planting young *T. ivorensis*. Means of 27 samples. LSD only shown when differences of means are significant at p = 0.05.

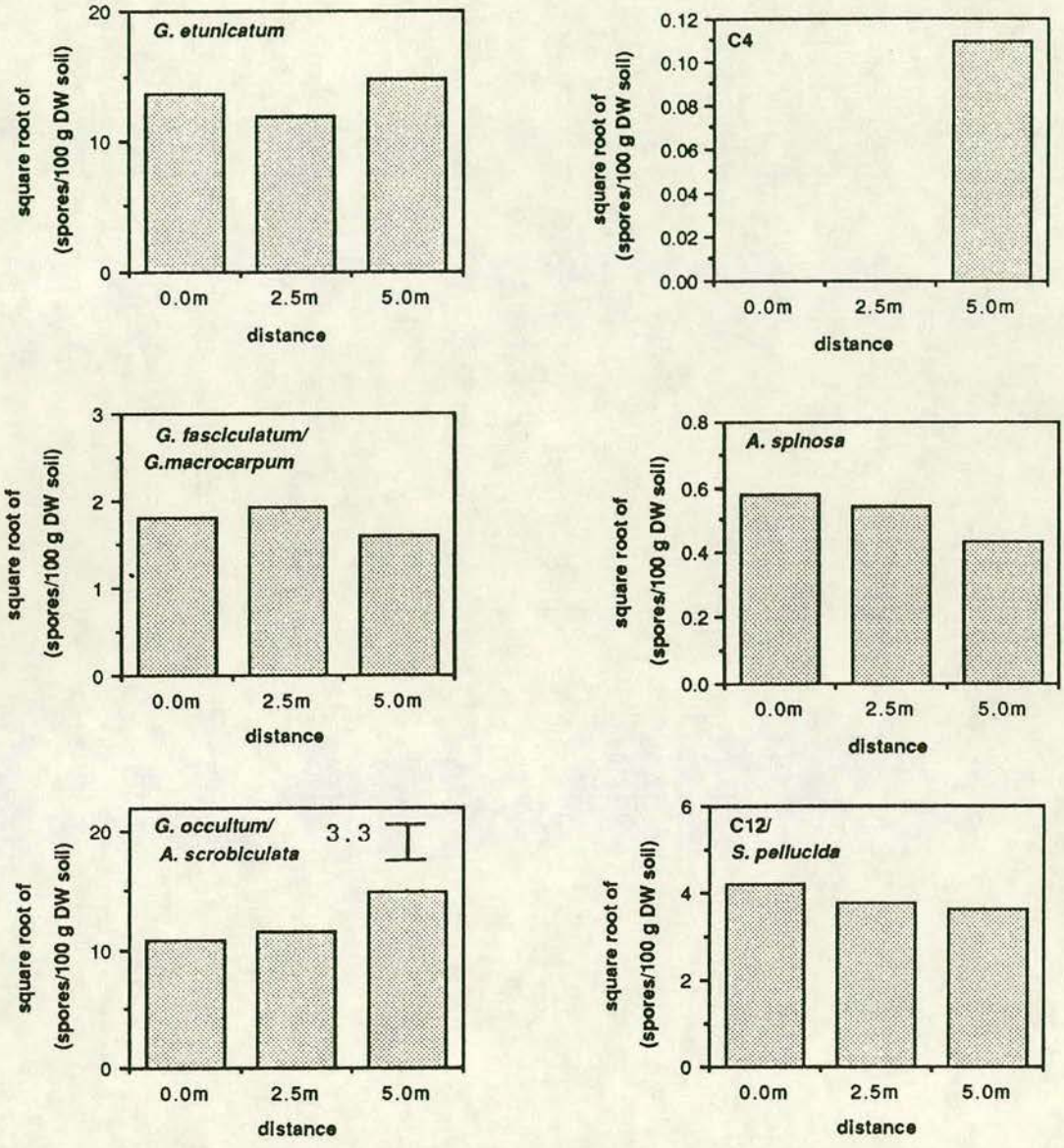
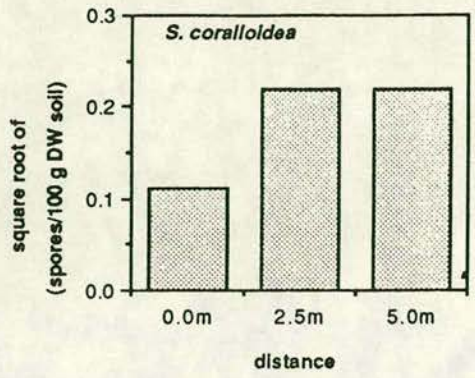
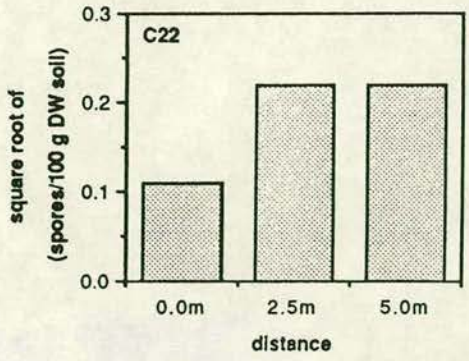
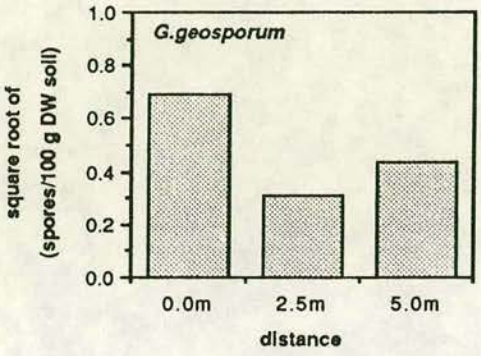
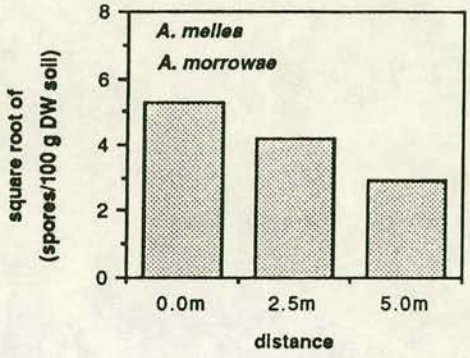


Figure 5.10
Variations resulting from effects of distance on the mean spore numbers of 12 VAM fungi extracted from 100 g dry soil collected from three treated (1 ha) plots of the Mbalmayo forest, a year after planting young *T. ivorensis* trees (August 1988, rainy season). Means of 9 samples per plot. LSD indicated by error bars when differences in means are significant at p = 0.05.









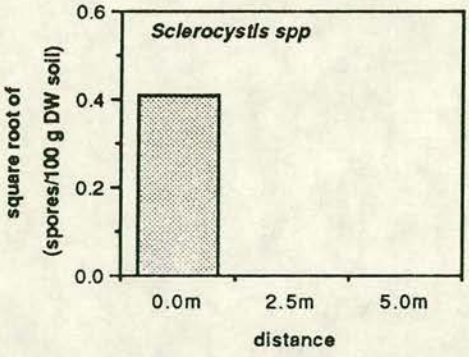


Figure 5.10 continued

Table 5.7 Changes in VAM fungal diversity and equitability, before and after treatments had been applied to the Mbalmayo Forest. Samples in February 1987 and August 1987 respectively. Means of 30 samples.

Diversity	Plots				
	4 (Control Undisturbed)	3 (Manually cleared)	1 (Mechanically cleared)	2 (Completely cleared)	LSD (p=0.05)
Feb. 1987 (before treatments)	2.284	2.155	2.403	2.147	ns
Aug. 1987 (after tretments)	2.231	2.242	2.675	2.374	0.289
Difference	-0.053	+0.087	+0.272	+0.227	
Equitability					
Feb. 1987 (before treatments)	0.0242	0.0267	0.0229	0.0256	ns
Aug 1987 (after treatments)	0.0245	0.0247	0.0204	0.0226	0.0029
Difference	+0.003	-0.0020	-0.0025	-0.0030	

Table 5.8 Significant variations in numbers of VAM spores extracted from 100 g dry weight soil samples collected from the Mbalmayo forest following treatments and a year after planting young *T. ivorensis* trees (August 1988). Means of 9 samples per plot

VAM fungal type	Plot	Distance		
		0 m	2.5 m	5.0 m
G. etunicatum (LSD.p=0.05;3.35)	Manually cleared	178.00 (13.34)	340.0 (18.45)	155.0 (12.47)
	Mechanically cleared	194.30 (13.94)	154.0 (12.40)	141.0 (11.89)
	Completely cleared	185.20 (13.61)	122.10 (11.05)	117.10 (10.82)
G. fasciculatum/ G. macrocarpum (LSD.p=0.05;1.4)	Manually cleared	1.50 (1.22)	6.0 (2.39)	1.30 (1.15)
	Mechanically cleared	15.40 (3.92)	2.40 (1.55)	9.0 (2.97)
	Completely cleared	0.11 (0.33)	4.00 (1.89)	0.50 (0.67)

Table 5.9 Proportions of the different VAM types extracted from 100 g dry weight soil samples collected from the Mbalmayo forest, after treatments but prior to planting (August 1987) and a year after planting young *T. ivorensis* (August 1988)

proportion (%) = $\frac{\text{No. of spores of the VAM fungal type}}{\text{Total number of spores extracted from 100 g dry soil}} \times 100$

		VAM FUNGAL TYPES											
TIME	PLOTS	*G. etunicatum*	*C4*	*G. fasciculatum/ G. macrocarpum*	*A. spinosa*	*G. occultum/A. scrobeulata*	*C12/S. pellucida*	*A. laevis*	*S. coralloidea*	*C22*	*G. geosporum*	*A. mellea/A. morrowae*	*Sclerocystis species*
After treatments but prior to planting	Control (4)	59.7.	1.4	1.56	0.64	21.99	2.58	0.52	0.12	0.04	2.75	8.15	nil
	Manual (3)	56.23	1.34	2.51	0.77	20.19	2.58	2.00	0.11	0.09	12.3	1.79	.02
	Mechanical (1)	60.68	0.62	2.58	0.88	29.69	6.39	3.45	0.11	0.06	0.97	3.12	nil
	Complete clearance(2)	54.78	1.68	5.09	1.07	30.81	3.69	1.88	0.20	nil	0.40	0.40	nil
After treatments and planting (August 1988)	Control	64.5	0.45	0.95	0.35	19.02	1.84	nil	0.24	nil	4.19	8.25	0.2
	Manual	59.16	nil	0.98	0.46	24.3	3.39	nil	0.12	0.78	0.75	10.0	nil
	Mechanical	34.5	nil	1.88	nil	41.97	7.13	nil	0.02	5.53	0.13	8.64	0.1
	Complete Clearance	29.52	0.02	0.39	0.12	46.39	3.29	nil	nil	18.9	nil	1.3	nil

In August 1987, spore numbers of *G. fasciculatum/G. macrocarpum* in the mechanically, manually and completely cleared plots were similar and only differed significantly from those of the undisturbed control plot which had significantly less spores at 5.0 m to 10.0 m distances of *T. superba* trees/stakes. A year after planting *T. ivorensis* seedlings there was still a peak spore abundance at 2.5 m to 5.0 m in the spore numbers in this plot, manually and completely cleared plots, but not in the mechanically cleared plot.

Whereas after preparation *G. etunicatum* however, was not affected by plot x distance interactions; a year after planting, the mean spore number had a significantly distinct peak at 2.5 m from *T. superba* trees within the manually cleared plot. However no such differences was observed in the mechanically and completely cleared plots (Table 5.6).

5.7 VAM SPECIES RICHNESS, ABUNDANCE AND EVENNESS AFTER SITE PREPARATION (AS INDICATED BY SIMPSON'S DIVERSITY (D) AND EQUITABILITY INDICES (E)).

Before site clearance there were no signficant between plot differences in VAM species richness, abundance and evenness as indicated by Simpsons, diversity (D) and equitability (E) indices (Table 5.7). Following site clearance, VAM species richness and abundance increased in the cleared plots with the greatest increase being in the mechanically cleared plot (2.403 to 2.675) making this plot significantly different from the other two cleared plots (Table 5.7). A direct relationship was observed between VAM species richness and abundance which increased with corresponding increases in the unevenness. In contrast, in the undisturbed control plot VAM species richness and abundance (diversity) decreased, with decreasing unevenness (Table 5.7). Cover and distance had no significant effects on VAM species richness, abundance and evenness.

5.8 DISCUSSION

The VAM spore populations recovered from soils collected from the Mbalmayo Forest following site clearance were considerably lower than

in the first set of samples taken from the undisturbed forest in February, 1987. Such decreases in spore numbers may be attributable to site disturbance during clearance, seasonal effects and the presence of predators.

The site clearance methods employed in this study, resulted in a close relation between percentage loss of spores and the intensity of soil disturbance; with the decreases being greatest in the completely cleared plot (65%), and intermediate in the partially cleared plots (27-45%). When natural tropical ecosystems are opened for cultivation, both long and short term effects on the soils physical, chemical and microbiological properties are realized (Smith and Sobek, 1979; McColl and Powers, 1984). In an earlier report (Lawson *et al.*, 1990), changes were observed within the research plots at Mbalmayo, where significant soil compaction was realized in the completely cleared and mechanically cleared plots, both of which employed the use of a bulldozer, as opposed to the manually cleared and undisturbed control plots. Soil compaction may have encouraged surface runoff and soil erosion in these plots which may have reduced spore numbers. Also it may be assumed that high temperatures existing in bare soils such as those of the clearfelled plot and parts of the mechanically cleared plot after host plants have been reduced or completely eliminated may dramatically affect the survival of different VA fungal structures, especially spores as suggested by Gianninazzi-Pearson and Diem (1982). Further evidence which lends credence to this view is supplied by Ahmad (1989) in a study on the effects of logging practices on VA fungal propagules in a Malaysian forest who observed that mechanical compaction, exposure and erosion significantly reduced VAM propagules (spores, hyphae infected roots) by 30-50%.

Observations from studies of the vertical distribution of VA fungal spores in soils, show that spores are concentrated on the top 20 cm with rapid decreases at increasing depths (Sutton and Barron, 1972; Redhead, 1977). St John *et al.* (1983) have similarly shown that spores are more abundant in the humus horizons which in the tropics are thin (0-5 cm) because of rapid litter decomposition. Removal of these humus and intensely rooted surface layers by the bulldozer as occurred in the completely cleared plot in this study significantly decreased

the number of spores and as a result affected the extent of mycorrhiza formation (Hall and Armstrong, 1979; Habte *et al.*, 1988; Habte 1989). It was therefore not surprising to see larger decreases in spore numbers in the completely cleared plot where the top layers of soil were removed and all plants eliminated.

Following site preparation, some VA fungal species such as *G. etunicatum* lost a larger proportion of spores than others including *G. occultum/A. scrobiculata*, in the completely and mechanically cleared plots whereas in the manually cleared plot most VAM fungi dropped by similar amounts. These results may indicate inherent differences in root distribution of trees as opposed to herbaceous vegetation and the degrees of disturbance inflicted on each plot. It is likely that *G. etunicatum* spores were more associated with superficial tree roots whereas *G. occultum/A. scrobiculata* was more associated with deep rooted herbaceous vegetation. This hypothesis undoubtedly needs further investigation.

Seasonal effects resulting in decreased VAM spore numbers were inferred from observations that most VAM fungi decreased at the onset of the rains (August, 1987) in all the plots including the undisturbed control plot. In all the plots, this decrease may have been due to an increase in spore germination in response to increased moisture and fresh root growth. Species such as C4, *G. fasciculatum/G. macrocarpum*, C12/*S. pellucida* and *A. laevis* showed this trend with spore numbers decreasing appreciably in the two rainy seasons (August 1987 and August 1988). Similarly, Redhead (1977) and Sanders and Sheikh (1983) reported an inverse relationship between rainfall (moisture) and numbers of spores in Nigeria, although some exceptions were noted (Redhead, 1977).

Increased nutrient levels in the manually and mechanically cleared plots may also have contributed to reduced incidences of VAM spores. Three months following clearance in these partially cleared plots nutrient levels soared, attributable to the input of felled detritus and the more rapid mineralisation of organic matter caused by higher levels of soil temperature and moisture (Lawson *et al.*, 1990). According to Hayman (1970) root colonization and sporulation are

increased when plants are in nutrient stress, for example where P is scarce mycorrhizas have been observed to be abundant (Gerdemann, 1968; Hayman, 1982). This may well have reduced VAM spore incidences in the partially cleared plots in the Mbalmayo Forest.

A year after planting with *Terminalia ivorensis* (August, 1988) the spore numbers had dramatically increased in all three cleared plots, especially in the mechanically and completely cleared plots. The dominant fungus *G. etunicatum* was seen to have increased by three times in the least disturbed manually cleared plot but only by 2 and 2.5 times in the mechanically and completely cleared plots. The second most numerous fungal aggregate, *G. occultum/A. scrobiculata* increased by three times in the manually cleared plot but by factors of four and seven in the mechanically cleared and completely cleared plots respectively. C22 also showed a rather dramatic increase in spore numbers; 100 times in the manually cleared plot, 1140 times in the mechanically cleared plot and 2000 times in the completely cleared plot. The dramatic increases in spore numbers are largely due to increased root densities (Abbott and Robson, 1982) brought about by the introduction of *T. ivorensis* trees and the invasion of the more disturbed mechanically and completely cleared plots by the invasive ruderal *Eupatorium odoratum* and pioneer species *Musanga cecropioides*.

More importantly the results highlight the increases in number of spores of *G. occultum/A. scrobiculata* which in the mechanically cleared and completely cleared plots lead to a change in VAM dominance from *G. etunicatum* to *G. occultum/A. scrobiculata*. In contrast, in the least disturbed manually cleared plot, the species distribution remained similar to that of the control plot where *G. etunicatum* was maintained as the dominant fungus.

The quantitative enhancement of some fungi are indicative of conducive host and environmental factors (Sieverding, 1989). Environmental conditions such as increased light and temperatures encouraged in the mechanically and completely cleared plots, favour the rapid growth of *Eupatorium odoratum* (Diederichs, 1983). Incidentally in chapter 4 of this study, the quantitatively enhanced fungal aggregate, *G. occultum/A. scrobiculata* had shown a close association with *Eupatorium*

odoratum among which it sporulated profusely. It is not surprising therefore, that *G. occultum* and *A. scrobiculata* which can also tolerate a wide range of host and environmental conditions (Sieverding, 1989) were able to sporulate profusely in the mechanically and completely cleared plots, which had been invaded by *Eupatorium odoratum*, and thus outnumber the previously dominant *G. etunicatum* fungus. The importance of such a shift in dominance from one endophyte to another is illustrated by host growth responses; this shift may be beneficial or deleterious to plant growth and survival depending on the effectiveness of the host/symbiont association. An effective fungus, according to Lopez-Aguillon and Mosse, (1987) being one that readily infects and enhances growth. Experiments assessing infection intensities on outplanted *T. ivorensis* trees might give an indication of such effectiveness (see chapter 6).

A lot remains to be learned concerning the behaviour of VAM fungi in tropical ecosystems. This study brings to light the effects of site preparation on VAM propagules particularly spores which are greatly reduced especially in the completely cleared plot. This shows how ecologically insensitive this complete clearance method is and how much more acceptable partial clearance is. Fortunately planting was effected which led to a recovery of spore populations. However, the mechanically cleared and completely cleared plots were opened up a lot; as a result the ruderal *Eupatorium odoratum* was encouraged into these plots and this appeared to have altered the composition of the VAM flora. Although the effects of this shift are thought to be deleterious to plant growth and survival (see chapter 6) a lot remains to be done to ascertain this. It is therefore imperative to learn more to ensure that management practices for improved crop (forest and agricultural) production are monitored closely to ensure that beneficial VAM associations are not jeopardized.

CHAPTER 6

ROOT ASSESSMENTS

6.1 INTRODUCTION

Plant growth and nutrient absorption by VA mycorrhizal plants largely depends on the degree of VAM infection (Harley and Smith, 1983). It was important therefore, to assess mycorrhizal development in this study in order initially to observe the mycorrhizal status of other predominant vegetation in the Mbalmayo Forest and to confirm if the vegetation including specimens of *Terminalia superba* was VA-mycorrhizal. It was also desirable to see if the rapid decrease in amounts of inocula (as determined by spore numbers only) following disturbance during site preparation (seen in Chapter 5) affected the mycorrhizal development and growth of the outplanted *T. ivorensis* trees, as has been suggested might happen by Daft and Nicolson (1969) and Sanders and Sheikh (1983) in experiments testing different amounts of inocula.

Disturbance effects on VAM (as discussed in Chapter 5) have been reported to reduce amounts of inocula with consequent effects on reducing infection intensities (Black and Tinker, 1979; Reeves *et al.*, 1979; Allen and MacMahon, 1985; Allen, 1986; Jasper *et al.*, 1987; Read and Birch, 1988 and Stahl *et al.*, 1988). Contrary to these observations, Molina *et al.* (1978) and Rose (1981) found no correlation between reduced spore numbers and infection intensities.

Most surveys of infection in lowland humid tropical ecosystems accord widespread infection of forest trees. In Nigeria, (Redhead, 1960, 1980) 44 indigenous tree and 25 exotic species were all found to be VA mycorrhizal. Of 44 tree species sampled in a dry lowland forest in Tanzania (Hogberg, 1982) 37 were VA mycorrhizal while the remainder were ectomycorrhizal. In India, Thapar and Khan (1973) found 22 forest tree species to be VA mycorrhizal. Similarly in Brazil 56 forest tree species were described as having endotrophic mycorrhizas (Thomazini, 1974). Saif (1981) surveying pioneer or colonizing species in Pakistan, observed that *Eupatorium odoratum* was highly

dependent upon VA mycorrhizas although *Trema orientalis*, a small gap-colonising tree from the Philippines was observed to be non-mycorrhizal (Tupas and Sajise, 1976).

6.2 MATERIALS AND METHODS

6.2.1 Sampling of *T. ivorensis* roots

In March 1989 (1½ years after planting), 10 *T. ivorensis* were randomly selected from each of the treated (manual, mechanical and completely cleared) plots. Using a trowel 500 ml of rhizospheric soil containing *T. ivorensis* roots were dug 25 cm from the bases of the selected trees both on north and south sides. A total of sixty samples were collected from three plots, put in labelled plastic bags and taken to the laboratory for examination, after washing off adhered soils.

A technique developed by I.T.E. (Anna James, pers. comm.) for selecting a random sample of root pieces for assessing mycorrhizal infection was adopted. The washed roots of each plant were cut up into 1 cm segments using a sharp blade, after which they were put into beakers and thoroughly mixed. The root segments were then spread on a plastic tray (35 cm x 35 cm) marked with 100 randomly selected dots; the root segment closest to each of the dots was picked out using forceps into another beaker giving a sample size of 100 root segments. The selection of 100 root segments in this manner is known to provide an adequate sample size resulting in the least standard error (Giovannetti and Mosse, 1980; Anna James, per. comm).

6.2.1.1 Clearing and staining of roots of *Terminalia ivorensis*

Roots were cleared and stained using the method of Phillips and Hayman (1970) chosen because it is less time consuming than embedding and sectioning roots and has been shown to be effective for a wide range of host plants (Giovannetti and Mosse, 1980). The root segments were put into 30 ml capacity universal bottles to which was added 10% Potassium hydroxide (10 g KOH in 100 ml H_2O); they were then autoclaved at 15 psi for 10 minutes to clear cytoplasm and nuclei. Afterwards, the KOH solution was decanted and the roots rinsed several times with tapwater

until they were clear. At this stage the root segments were immersed and bleached in freshly prepared alkaline hydrogen peroxide (3 ml ammonia to 10 ml, 30% H_2O_2 + 587 ml H_2O) at room temperature. When roots had been bleached, the hydrogen peroxide was decanted and the roots rinsed with tapwater before being immersed in 1% hydrochloric acid (10 ml of IN HCl + 350 ml H_2O) for 3-4 minutes to acidify them for effective staining. The HCl was then decanted and the roots, without being rinsed, were immersed in 0.05% Trypan Blue in 10% lactophenol (10 ml lactophenol + 90 ml H_2O + 0.05 g Trypan Blue), before being autoclaved for 10 minutes at 15 psi. Following autoclaving, root samples were left in Trypan Blue lactophenol for a further two hours (to ensure adequate staining) before decanting the Trypan Blue.

6.2.1.2 VA-mycorrhizal assessment

Initially the gridline intersect method was chosen for assessing VAM root infections because of its qualitative and quantitative advantages over other methods (see Giovannetti and Mosse, 1980). However, it soon became evident when examining roots of *T. ivorensis* that their morphology precluded the use of this technique as the deep staining of the secondary thickened areas of the endodermis and stele obscured, when viewed with a dissecting microscope, the infections in the outermost layer of cortical cells (Plate 6.1).

After considering other techniques (Giovannetti and Mosse, 1980) the slide length method was adopted. Carefully, the outer infected cortical layers were peeled from each 1 cm root segment into a petri dish containing water and marked with 25 randomly arranged dots. The segments closest to each of the dots were mounted on glass microscope slides. Infection was assessed with a high powered microscope (x 150, x 1000) and the amounts and types of infections (including vesicles, arbuscules, intercellular or intracellular hyphae) were recorded. On advice from a statistician, infection intensities were rated

1, representing 0-33% of cells of cortical layer infected.
3, " 34-66%
5, " 67-100%

Records of intensity were then averaged for a sample of 25 segments.

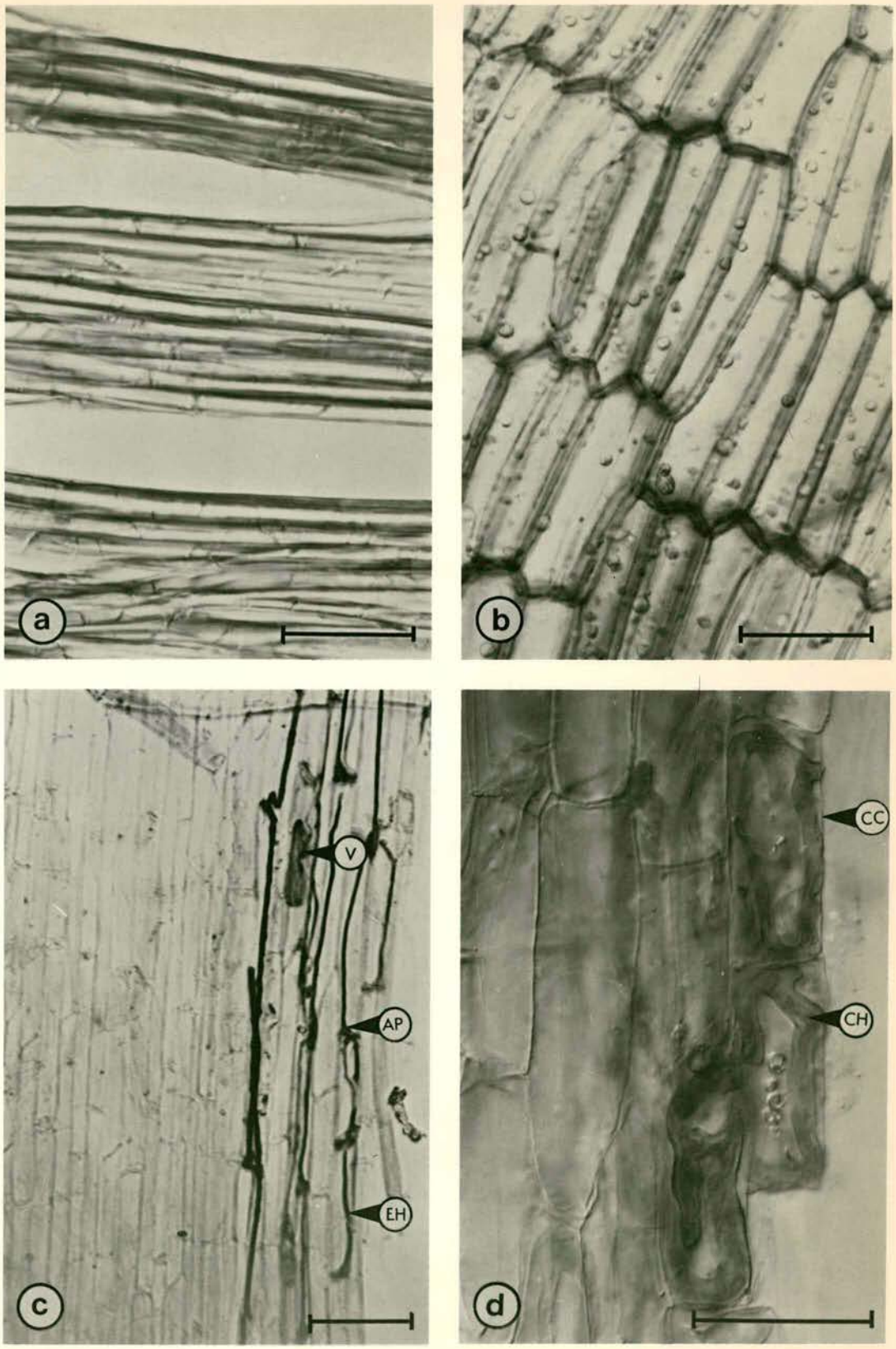
Platge 6.1

a) Secondary thickening the stele scale bar, 22.5 mm = 50 µm

b) Secondary thickening of cells of the endodermis
scale bar, 22 mm = 50 µm

c) Outermost layer of cortical cells infected by VAM fungi showing vesicles (v), appressorium (AP) and external hyphae (EH)
scale bar 17.2 mm = 50 µm

d) Infected cortical cells (CC) showing coiled hyphae (CH)
scale bar 30 mm = 50 µm


a
b
V
AP
EH
c
CC
CH
d

Statistical analyses (ANOVA) was done to identify significant differences.

6.2.2 **The mycorrhizal status of mature *T. superba* and other plant species in the Mbalmayo Forest.**

6.2.2.1 Sampling

In contrast to those of young *T. ivorensis*, the roots of mature *T. superba* were sampled 50 cm from the bases of designated trees. Samples of *T. superba* roots were taken from the manual, mechanical and control plots, and understandably, not from the completely cleared plot. In each instance a tree buttress was traced and with a pickaxe and spade, and a trench was dug 50 cm from the bases to a depth of about 30 cm deep, the limit of fine root development. 500 ml of soil containing these feeder roots were put into labelled plastic bags.

In addition to the roots of native *T. superba*, roots of the following plants were sampled; *Alchornea floribunda, Afromomum sp., Marantochloa purpurea, Arthropteris cameroonensis, Costus afer, Cyperus sp., Eupatorium odoratum, Maniophyton fulvum* and *Uapaca sp.* These species were chosen because they were common in the understories of all four plots.

A total of 5 plants of each species was investigated; rhizospheric soils containing roots were removed and bulked to form one sample per plant species. Nine of the samples were taken (one per plant species) were immediately returned to the laboratory, and washed before cutting roots into 1 cm segments.

6.2.2.2 Clearing and staining roots of *T. superba* and other naturally occurring plants

The method described in section 6.2.1.2 for roots of *T. ivorensis* was used here.

6.2.2.3 Assessment of infection

As the aim was to establish the presence or absence of VAM infections quantitative assessments of the amount of infection were not made. The slide ± method (Giovannetti and Mosse, 1980) was thus used. 25, 1 cm root segments of each plant species were mounted on glass slides as described in section 6.2.1.3, and records made of the presence or absence of infection.

6.2.3 Survival of outplanted *T. ivorensis* trees

In the manual, mechanical and completely cleared plots, a centrally located area of 50 m x 50 m was marked out and the survival of out-planted *T. ivorensis* trees monitored over 23 months (September 1987 to July 1988).

6.3 RESULTS

6.3.1 Infection of outplanted *T. ivorensis* trees

The infection intensities of roots of *T. ivorensis* out-planted into the different plots differed significantly (p=0.05). Infection was greatest in roots in the mechanical 'recru' plot (73.1%) followed by 67.1% in the manual 'recru' plot and 59.1% in the completely cleared plot.

The types of infection (i.e. vesicles, arbuscules, coiled hyphae, intra/intracellular hyphae) in the different plots also differed, albeit not significantly. (The sum of the percentage infections of different types sometimes exceed the percentages recorded in Table 6.1 because more than one type of infection was often noted on the same root segments). The percentage of root segments with coiled hyphae was greatest (46.1%) in plot 1 (the mechanically cleared plot) and least (33.4%) in plot 2 which was completely cleared. A similar trend was noted in relation to amount of external hyphae.

Table 6.1 Analysis of variance of overall infection (%) of roots of *T. ivorensis* planted in March 1989 in 3 plots which were manually, mechanically and completely cleared. Means of 25 root segments.

	PLOTS			
	Manual	Mechanical	Complete Clearance	LSD p = 0.05
Infection level	67.1%	73.1%	59.1%	8.1%

Table 6.2 Occurrence (%) of different types of infection observed in roots of *T. ivorensis* planted in March 1989 in 3 plots which were manually, mechanically and completely cleared. Means of 25 segments

	PLOTS		
Infection type	Manual	Mechanical	Complete Clearance
coiled hyphae	45.6%	46.1%	33.4%
internal hyphae	26.4%	22.2%	17.7%
external hyphae	15.9%	19.4%	13.1%
vesicles	6.9%	7.6%	8.8%
arbuscules	nil	nil	nil

LSD only shown where means are significantly different

6.3.2 Survival of outplanted *T. ivorensis*

Significant differences were observed in the survival of trees in the plots (Lawson *et al.*, 1990). Two months after planting, tree mortality was greatest in plot 2, the completely cleared plot (16%) but less in plots 1 and 3 (3% each). Subsequent losses in the completely cleared plot increased to only 19% whereas in the mechanically and manually cleared plots, losses were 10% (Figure 6.1).

6.3.3 **Mycorrhizal status of the natural vegetation of Mbalmayo Forest**

Of the ten naturally occurring species sampled in the Mbalmayo Forest, all except *Uapaca* sp were mycorrhizal (Table 6.3).

6.4 **DISCUSSION**

The lower infection intensity of the outplanted *T. ivorensis* in the clearfelled plot when compared to those of the manually and mechanically cleared plots can be explained by:-

(a) a greater reduction in spore inoculum (Chapter 5)

(b) complete removal of all host plants

(c) a change in VAM fungal dominance from *G. etunicatum* to *G. occultum/ A. scrobiculata*, the latter noted for its ineffectiveness (Dodd, pers. comm.) and

(d) a greater disturbance of extramatrical hyphae in the completely cleared plot.

The smaller concentrations of VAM fungal spores in the most severely disturbed soils of the clearfelled plot after site preparation may be attributable to the absence of alternative host plants the removal of many spores from the upper layer by the bulldozer and to a dilution effect associated with the mixing of soils in the more disturbed plots resulting in an inoculum with a lower potential for initiating new infections (Black and Tinker, 1979; Jasper *et al.*, 1987). The eventual infection intensities were thus partly related to the number of spores which after site preparation were least in plot 2 (the completely cleared plot) and most abundant in the undisturbed control, plot 4. My observations partly support data given by Sanders and

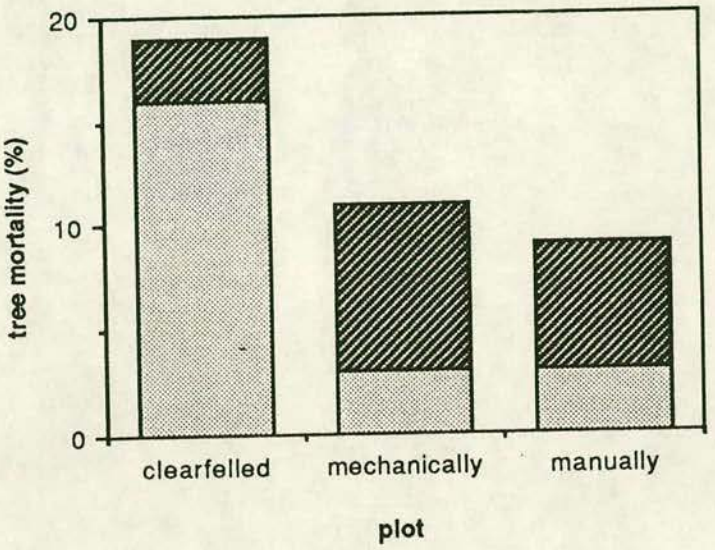


Figure 6.1
The percentage survival of planted *T. ivorensis* trees in centrally located areas of 50 x 50 m in the manually, mechanically and completely cleared plots, sampled over a period of 23 months (Sept 1987 - July 1988). After Lawson et al., (1990).

*Lighter shade denotes tree mortality by November 1987
Darker shade denotes tree mortality by July 1988

Table 6.3 The presence (+) or absence (-) of infection in some common tree/herbaceous/weedy species in the control, manual, mechanical and completely cleared plots at Mbalmayo, sampled in August 1989.

FAMILY	PLANT SPECIES	INFECTION
Asteraceae	*Eupatorium odoratum*	+
Combretaceae	*Terminalia superba*	+
Cyperaceae	*Cyperus sp*	+
Euphorbiceae	*Alchornea floribunda*	+
	Maniophyton fulvum	+
	Uapaca sp	-
Ferns	*Arthropteris cameroonsis*	+
Marantaceae	*Maranthochloa puprpurea*	+
Moraceae	*Musanga cecropioides*	+
Zingiberaceae	*Afromomum sp*	+
	Costus afer	+

Sheikh (1983) showing that a tenfold decrease in spore density (2.5 to 0.25 spores g^{-1} soil) resulted in a delay in mycorrhizal infections in maize roots and this remained lower in pots with less spore densities.

Similarly, Daft and Nicolson (1969) by varying the number of *G. macrocarpum* spores during the inoculation of tomato plants (*Lycopersicon esculentum*) found that larger spore numbers resulted in the more rapid development of infection and the earlier onset of growth responses. However, findings that infection intensities of *T. ivorensis* planted in the completely cleared plot (plot 2) remained significantly smaller for one and a half years compared to events in the other two treated plots, even though spore numbers had increased dramatically, suggest that other factors might be operating, such as the change in VAM fungal dominance (from *G. etunicatum* to *G. occultum/A. scrobiculata*). Earlier observations (see Chapter 4) showed that *G. occultum/A. scrobiculata* sporulated profusely in the presence of a noxious ruderal, *Eupatorium odoratum*, and only minimally in association with a range of tree species in Mbalmayo Forest. *Eupatorium odoratum* is VA-mycorrhizal dependent (Saif, 1981), rapidly invades disturbed sites and thrives well in conditions of increased light and temperatures; thus it is not surprising it occupied extensive areas of the clearfelled plot (plot 2) and to a lesser extent, areas of the mechanically cleared plot (plot 1).

Reports by Sieverding (1989) of tropical ecosystems, show that both *G. occultum* and *A. scrobiculata* can thrive in a broad range of environmental and soil conditions unlike the less tolerant *G. etunicatum* whose abundance greatly decreased in the clearfelled and mechanically cleared plots. The importance of shifts in dominance from one endophyte to another is reflected in host responses. According to Sieverding (1989) the effectiveness of a VA mycorrhizal fungus is related to the degree of infection. Thus the present study suggests that *G. occultum/A. scrobiculata* appears to be ineffective on the outplanted *T. ivorensis* in the clearfelled plot because infection ratings were low. Host preferences, as indicated by the close association of *G. occultum/A. scrobiculata* with *Eupatorium odoratum*, and the ineffectiveness of these VAM fungi in association with *T. ivorensis* in the clearfelled plot, have been reported from other

tropical and temperate ecosystems (Allen and Boosalis, 1983; Sieverding, 1989). Differences in effectiveness have been reported even among isolates of the same VAM fungi; interestingly, *G. occultum*, a component of the ineffective fungal aggregate (as judged by performance with *T. ivorensis*) has been reported to be ineffective on cassava plants (Howeler *et al.*, 1987). In contrast, the effects of the shift in VAM fungal dominance in the mechanically cleared plot seems to have been alleviated because this site treatment allowed less invasion by *Eupatorium odoratum*, by retaining a range of other naturally occurring plants, especially trees which sustained the dominant forest fungus *G. etunicatum* in sufficient amounts, to successfully initiate greater infection intensities.

Studies by Francis and Read (1984), Evans and Miller (1988), Fairchild and Miller (1988), Read and Birch (1988) and Stahl *et al.* (1988) have suggested that the debilitating effects of disturbance which decrease the ability of VAM fungi to infect is also related to the disruption of VAM mycelia which form functional interconnections between plants at both intra and interspecific levels. Although the effects of the different site preparations on VAM mycelia were not monitored it could be inferred from my observations that *T. ivorensis* on the less disturbed, manually and mechanically cleared plots were more heavily infected than *T. ivorensis* in the completely cleared plot. Although the severity of disturbance was not indicated in an unpublished account by Birch and Read (cited in Read and Birch, 1988) the infection units in roots of plants growing in disturbed soils were very short (< 5 mm long) compared with 5-13 mm in roots in undisturbed soils.

Reeves *et al* (1979) also noted that infection intensities were low, 1-2% on plant species introduced to disturbed soils in contrast to 77-99% infection in undisturbed soils. Using sagebrush plants, Stahl *et al.* (1988) found the infection was restricted in disturbed conditions and that VAM infections even after 2 years of plants in disturbed sites was insufficient to aid establishment, growth and survival of the sagebrush plants. The lower survival of outplanted *T. ivorensis* in the severely disturbed completely cleared plot, may also reflect decreased P uptake in the absence of sufficient infection, and the adverse influences of compaction and erosion on root penetration.

The changes observed in the balance of the different types of infection (ie vesicles, coiled hyphae, external and internal hyphae) may reflect changing soil conditions and/or the composition of infecting VAM species. Results from chapter 5 indicated a shift in VAM fungal dominance from *G. etunicatum* to *G. occultum/A. scrobiculata* in the mechanically and completely cleared plots. As *Glomus* species will likely form similar infection structures there are probably few differences between their mycorrhizas. Temperature changes are more likely to be the determinants of the different infection types observed. Schenck and Schroeder (1974) observed that maximum mycelial colonisation occurred at 28° and 34°C, arbuscule formation at 30°C and vesicle development at 35°C. Results of this nature support those of this study wherein the clearfelled plot with the highest temperatures had the greatest vesicle development. Surprisingly, arbuscular type infections were not seen in *T. ivorensis* roots examined in Cameroon possibly because these structures are relatively short-lived (life span of 4-15 days, Cox and Tinker, 1976) or because roots were not actively growing when samples were taken during the dry season.

Infections in all but one plant species in the Mbalmayo Forest (*Uapaca* sp.) confirm the ubiquity of VAM infections among tropical plants (Janos, 1980). The exception, *Uapaca* sp., may instead form sheathing ectomycorrhizas. Hogberg (1982) working in a Miombo woodland in Tanzania suggested that some species of the *Uapaca* form ectomycorrhizas while others were endomycorrhizal: representatives of the same genus (*Alchornea floribunda* and *Maniophytum fulvum*) and family as *Uapaca* sp. namely the *Euphorbiaceae*, were found to have VA-mycorrhizas. Results of this kind stress the importance of VAM assessments and make it clear that inferring the presence or absence of VAM infections from the occurrence of host species of the same genus or family could badly be in error. Infection as observed in a member of a generally non-mycorrhizal family such as the *Cyperaceae* may have resulted from a close association of the specimen in question (*Cyperus* sp.) to extensively infected VA mycorrhizal hosts known as companion species (Hirrel *et al*, 1978; Miller *et al*. 1982). However, observations that *T. superba* roots were VA-mycorrhizal supported those by Maeda (1954) who found *T. cattapa* roots belonging to the same genus to be VA-mycorrhizal.

The presence of infection in a range of plant species in Mbalmayo Forest confirms the ubiquity of VA mycorrhizal infections in tropical plants and probably indicates dependence on VA mycorrhizas. The extent of infection can be crucial for plant growth. In this study it was observed that disturbance probably affected the level of infection via a reduction in spore inoculum and host plants, a change in VAM fungal dominance from an effective forest fungus to an ineffective one and finally through the severe disruption of the VAM mycelial network. Management operations such as the establishment of large scale plantations and agroforestry schemes need be carefully considered in the light of the evidence in this thesis, namely that practices leading to severe soil disturbance may disrupt networks of VAM mycelia and adversely affect the level of infection and hence productivity of outplanted crops (agricultural and forestry).

CHAPTER 7

GENERAL DISCUSSION

7.1 EVALUATION OF EXPERIMENTAL APPROACHES AND METHODS

In this chapter, some of the problems encountered in the experimental approaches and methods are discussed. In addition, an overview of the results, their implications for the understanding of the ecology of VAM fungi in tropical moist rainforests and their practical applications in silviculture and agroforestry are debated. Lastly, proposals are made for future research.

7.1.1 Use of spores in assessing vesicular-arbuscular mycorrhizal (VAM) fungal populations

To predict the distribution and abundance of VAM fungi, knowledge of the biology of each fungus is required (Bowen, 1987). VAM fungi can be identified and quantified using spores, hyphae or infected root fragments in soils. Surveys in tropical and temperate regions have largely used spores to indicate VAM fungal distribution and abundance (Johnson, 1977; Redhead, 1977; Diem *et al.*, 1981; Koske, 1981; Koske and Halvorson, 1981; Walker *et al.*, 1982; Mukerji and Kapoor, 1986; Sharma *et al.*, 1986; Louis and Lim, 1987). Similarly, in the present survey spores were used to assess the dynamics of VAM fungal populations; an approach criticized on the bases that underestimates are likely to have been incurred because not all fungi sporulate (Read *et al.*, 1986) and not all spores are retrieved from soils with existing extraction procedures (Ianson and Allen, 1986) especially small spored fungi in soils with large amounts of organic matter (St. John *et al.*, 1983). These reservations/limitations were considered at the onset of this study and set against the problems likely to be encountered when using either hyphae (Nicolson, 1959) or infected root fragments (Kough and Linderman, 1986). It was decided that spores had the advantage of being easier to count; several techniques had been developed to increase the success of spore recovery from soils, (Porter, 1982) and most importantly the descriptions and subsequent identification of VAM fungi relied almost exclusively on spore characteristics (Walker, 1983;

Schenck and Perez, 1987; Morton, 1988). It is likely there are non-sporing forms of VA mycorrhizal fungi in the Mbalmayo rainforest and future research should investigate their existence. However, few non-sporing forms are known at present (e.g. *G. tenue*) compared to the vast majority of sporing VAM fungi. Some workers have suggested they may not be of particular significance but this point of view is debatable.

Comparison of VAM fungal distribution and abundance in Mbalmayo with those in other tropical ecosystems has been greatly hampered because the fungi have often been inadequately described and identified. A lot of time and effort was put into properly describing and identifying the fungi in the Mbalmayo rainforest. This has contributed immensely to understanding their distribution and abundance in this ecosystem. Characters such as spore colour, size, shape, hyphal attachments have helped in identifying VAM fungi although the extent of their usefulness is limited, because these characters vary and overlap both within and between species (Morton, 1988). Wall characters were more diagnostic, but difficult to decipher. Attempts made by Walker (1983), through the use of murographs and muronyms, provided a major advance towards understanding differences in wall structures while the use of reagents such as Melzers proved invaluable for delimiting some of the many types of walls (e.g. membranous and amorphous walls).

Ideally spore identification should be effected on pot cultured spores, but attempts made at establishing mixed pot cultures were discarded as pot soils were contaminated by a 'weed' fungus *G. clarum* which sporulated profusely to the exclusion of other fungi. Field collected spores in prime and unparasitized condition were used; future research however will focus on establishing pot cultures of single taxa, carefully avoiding contamination by other VAM fungi. In fact one idea attracting a lot of interest in the United States is the setting up of pot culture banks in selected laboratories with the facilities for propagating and keeping them free from contamination (e.g. the International Culture Collection (INVAM) started by the University of Florida in Gainesville under the auspices of Dr N C Schenck and Yvonne Perez).

It should be noted however that because present methods of spore

identification remain so laborious there is a tendency for many researchers, except the experts in VAM identification, to fit new 'finds' into existing taxa. The almost universal misrepresentation of *G. fasciculatum* exemplifies this (Morton, 1988). In addition, the grouping of different fungi in this study (e.g. *G. fasciculatum* and *G. macrocarpum; A. mellea* and *A. morrowae* and *G. occultum* and *A. scrobiculata*) illustrates the difficulties encountered in trying to separate closely related VAM fungal spores. With experience however, the differences became apparent and in future they would be separately quantified. Serological techniques will, when improved upon, facilitate spore identification and minimize mistakes.

Attempts to relate spore numbers to the amount of infection in *T. ivorensis* should be treated with caution. After site preparation, the percentage loss of spores in each cleared plot seemed directly related to the degree of soil disturbance. Unlike the events described by Daft and Nicolson (1969) infection intensities remained low in the completely cleared plot after spore numbers had increased a year and a half (1½ years) after site clearance. These results indicate that spore numbers do not necessarily correlate positively with infection intensities (Molina *et al*, 1978; Rose, 1981). According to Abbott and Robson (1981) when correlation between spore number and infection intensity is weak, it is an indication of competition between indigenous fungi and/or it is due to differential infection rates of different fungi. The infection rates have not been tested in this study so it remains unclear what the reasons are for the poor correlation with spore numbers. It is however suspected that in addition to Abbott and Robson's (1981) views, the low infection intensities may also be the result of (a) severe reduction in VAM fungal propagules after site clearance (especially the disruption of the hyphal network and infected root pieces known to colonise host plants more rapidly than spores, (Powell, 1976), (b) the change in VAM fungal dominance (Sieverding 1989) from *G. etunicatum* to *G. occultum/A. scrobiculata* (which may be ineffective on *T. ivorensis*) and the state of maturity of the spores (Tommerup, 1983). According to Tommerup (1983) a fungus such as *A. laevis* may have a larger number of spores in soils than other species but if these spores germinate slowly or are quiescent or dormant, then other fungi without these restrictions will

form mycorrhizas.

In future, other methods of estimating numbers of infective propagules, (spores, hyphae and infected root fragments) for example, the Most Probable Number (MPN) should be employed, so that a better relationship is established between numbers of infective propagules and infection intensities. It is realised however, that it is not easy to optimize the MPN technique as, at present, there is no established relationship between numbers of infective propagules and the expected development of mycorrhizas in different soils (Wilson and Trinick, 1982; Adelman and Morton, 1986).

7.1.2 **Sampling techniques**

Only a few systematic studies have addressed the validity of sampling methods used in assessing VAM fungal populations (Tews and Koske, 1986). From observations that VAM fungal spores do not occur randomly in soils (Porter, 1982; Walker *et al.*, 1982; St John and Hunt, 1983) and that the variance of spore counts correlates positively with the size of the sample area (Anderson *et al.*, 1983), it was clear that the accuracy of VAM spore recovery depended on the intensity of sampling and the area sampled. For these reasons, and following advice from a statistician (Mr R I Smith, pers comm) large sample areas of 100 x 100 m^2 plots were established and large numbers of samples (30 per plot) were taken and subsampled (six subsamples per sample) to decrease errors associated with means of spore numbers (Reich and Barnard, 1984). The large number of samples and the subsampling procedures adopted, and the large areas (100 x 100 m^2) explored probably resulted in the unexpected homogeneity of total spore counts and ensured the retrieval of most types of sporing VAM fungi (Walker *et al.*, 1982).

Sampling along transects in this study was adopted from previous surveys such as by Sylvia (1986) in some Florida fore-dunes, and proved successful in giving indications of the possible effects of trees and herbaceous vegetation on spore numbers. Samples taken along *T. superba* transects (with cover) had greater spore numbers as opposed to soil samples taken from those transects in herbaceous non-tree vegetation. Such a positive correlation between plant cover and spore

numbers has also been reported by Anderson *et al.*, (1984) and Miller (1987).

Distance effects on spore abundance have rarely been reported (Koske and Halvorson, 1981) but in this study distance seemed to complement cover effects wherein spore numbers peaked closest (2.5 m) to *T. superba* and with increasing distance (at 10.0 m). In cases where spore distribution was variable, the likely effects of roots of other trees or herbaceous vegetation criss-crossing the forest floor may have been responsible.

One serious criticism of the sampling design however remains namely the absence of replicate plots after the imposition of treatments. This was attributable to logistical problems including the duration of the present study. This difficulty was resolved by the preparation of replicate plots in successive years, although in this study the results presented are only for the first set of plots established in February 1987.

Approaching the study in this manner however, indicated the likely effects of disturbing soils to different degrees on spore populations as opposed to the situation in which one treatment (e.g. manual clearance) was replicated several times.

7.1.3 **Statistical analysis**

Untransformed spore counts were not appropriate for analysis of variance because the assumptions of normality of distribution, homogeneity of variance and additivity of treatment effects were not satisfied. Instead, the square root transformation recommended for counts by Snedecor and Cochran (1967) and Mead and Curnow (1983) was applied to data before the analysis of variance was effected.

Proportions and root infection percentages were similarly transformed (angular transformation) before analyses of variance. Square root transformation of spore counts data proved successful, satisfying all the assumptions of ANOVA; however some authors (Walker *et al.*, 1982; Sylvia 1986) have used log transformations to successfully satisfy the

assumptions of ANOVA.

Results of analysing spore counts sometimes showed that different treatments significantly affected even species that were relatively sparse. These were included in Figures and/or tables because species with few detectable spores may nevertheless be very 'effective' and therefore beneficial to plant growth.

Simpsons diversity and equitability indices were used to supply additional information on species richness commonness and rarity (Begon, Harper and Townsend, 1990) which are often ignored when the composition of a community is described simply in terms of numbers of species. In the Mbalmayo forest 17 VAM fungal species were recovered, a number described as rich (Sieverding 1989) from a natural tropical ecosystem. Similarly, the mean spore number (262 spores/100 g dry soils) was well above those of several other surveys (e.g. Redhead, 1977). However, the uneven distribution of spores with *G. etunicatum* making up more than 50% of the total spore population, meant that the remaining 16 VAM fungi made up less than 40% of the total spore population. Using Simpson's diversity and equitability indices the quality of information gathered could hopefully be improved upon.

7.1.4 **Assessment of related environmental variables (Light and Temperature)**

Failure to regularly assess the treated plots for differences in light intensities and temperatures, both of which directly or indirectly influence VAM fungal sporulation, was unfortunate. After applying treatments to three of the four plots, it was observed that light intensities differed; the least being in the manually cleared plot which retained most of its dominant trees and the greatest light intensities being in the completely cleared plot. Evidence by Gerdemann (1968) has shown that shading can reduce root infections and spore production; Wetselaar (1981) also noted that shading affected host responses to VA-mycorrhizas. Diederichs (1983) using *Eupatorium odoratum* observed a definite relationship between infection intensities and the efficiency of VA-mycorrhizas where growth was increased with increased light intensities. This might explain why spore production

and the infection intensities within *T. ivorensis* roots in the more shaded manually cleared plot, were always lower than in the less shaded mechanically cleared plot albeit the difference waswithout statistical significance. Observations that the completely cleared plot, with the most intense light intensities, showed increased spore production but low mycorrhizal infection on outplanted *T. ivorensis* roots are not at variance with the suggestion that the spores dominated by *G. occultum/A. scrobiculata* were largely produced with *Eupatorium odoratum* which rapidly invaded the plot and were less effective on roots of *T. ivorensis* which were sparsely infected.

Temperature effects like those of light, have been demonstrated in glasshouses, to influence root colonisation by VAM fungi. Higher temperatures generally result in greater root infections (Furlan and Fortin, 1973; Hayman, 1974) and increased sporulation (Furlan and Fortin, 1973). The value of this observation, obtained from experiments conducted in controlled glasshouses, is not so much in direct applicability to natural conditions observed in Mbalmayo, nevertheless, they provide useful indications of the environmental factors which likely influence some of the observations made. Future monitoring of these variables should increase our understanding of the consequences of site preparation methods for plantation establishment on VAM fungi.

7.2 OVERVIEW OF THE ECOLOGY OF VAM FUNGI IN THE MBALMAYO RAINFOREST

The known edaphic and climatic stresses in the tropics, as well as observations that most tropical plant root systems characteristically lack roothairs, imply that they depend implicitly for nutrition and growth on mycorrhizas especially the vesicular-arbuscular mycorrhizal type. Soils of the Mbalmayo forest particularly had these constraints, being low in available phosphorus with high aluminium toxicity (Lawson *et al.*, 1990). It is not surprising therefore that this rainforest like other tropical rainforests (Redhead, 1977; Janos, 1980) showed the existence of a diverse array of VAM fungi distributed among four genera; *Acaulospora: A. laevis, A. mellea, A. morrowae, A. scrobiculata* and *A. spinosa; Glomus: G. etunicatum, G. fasciculatum, G. geosporum, G. macrocarpum* and *G. occultum*; *Sclerocystis: S.*

pachycaulis and *S. microcarpum; Scutellispora: S. coralloidea* and *S. pellucida*. Three VAM types remain unidentified. Interestingly, the same array of fungi found associated with trees was also found among the herbaceous vegetation, illustrating the non-host specificity of VAM fungi (Newman and Reddell, 1987). The lack of specificity is of immense advantage and possibly accounts for the variety of plant species (more than 200) in the plots at Mbalmayo. It remains unclear why species in certain genera such as species of *Entrophospora* and *Gigaspora* were not retrieved. The general consensus from previous observations is that species of *Gigaspora* and *Sclerocystis* are common in tropical soils (Redhead, 1977; Mosse *et al.*, 1981; Sieverding, 1989), *Acaulospora* species are better adapted to soils of low pH, with species such as *A. laevis* having a narrow host range whereas others such as *A. scrobiculata* have a wide host range (Sieverding, 1989) and *Glomus* species are common in alkaline soils or more fertile soils (Young *et al.*, 1985; Abbott and Robson, 1989). In contrast to observations by Young *et al.*, (1985) and Abbott and Robson (1989) species of *Glomus* were abundant in the acidic soils of Mbalmayo, probably indicating the tolerance of species of *Glomus* to a wide range of soil reactions (pH). The total spore population of this semi deciduous forest ecosystem was well above those associated with natural plant communities in other tropical and temperate ecosystems (see Chapter 4). *G. etunicatum*, the dominant fungus in soils of the Mbalmayo forest, represented more than 50% of the entire spore population. Spore numbers were greater in transects set through *T. superba* (with cover) than from transects set through herbaceous non-tree vegetation. This indicates a close positive correlation between plant cover and spore numbers as had been observed by Anderson *et al.*, (1984). Distance effects, like cover, showed spore numbers peaking close to *T. superba* (2.5 m). In addition distance effects revealed the clumped distribution of spores in soils of Mbalmayo supporting views by Walker *et al.*, (1982) that VAM fungal spores are not randomly distributed in soil.

Opening tropical rainforests for the cultivation of agricultural or tree crops can seriously disrupt the otherwise resilient and stable plant-soil ecological entity. Of particular interest were the effects of different degrees of soil disturbance incurred during site

preparation for plantation establishment on VAM populations. Site clearance methods ranged from the least destructive (manual clearance method) most destructive, complete clearance. The forms of site clearance appeared to have both short and long term effects on VAM fungal spore populations operating directly, or indirectly, through resulting changes in soil physical, chemical and microbiological properties (McColl and Powers, 1984).

Following site clearance soil physical properties in the mechanically and completely cleared plots were significantly altered by the use of a heavy bulldozer (24 tons). Soils in these plots were compacted (Lawson *et al.*, 1990) thus possibly reducing water infiltration rates and thereby increasing water runoff and soil erosion. Water runoff may have washed away many spores thus reducing their overall populations in these plots after site clearance. Also, the increased exposure to sunshine caused by the reduction/elimination of canopy trees, resulted in increased soil temperature not tolerated by indigenous VAM fungi, thereby increasing the possibility of spore deaths or decreased VAM activity (Moawad, 1979). According to Ahmad (1989) studying the effects of logging practices in a Malaysian forest mechanical compaction, from the use of bulldozers, erosion and exposure contributed to a 30-50% decrease in VAM fungal propagules (i.e. spores, hyphae and infected root fragments). Interestingly, spore losses after site clearance seemed directly related to amounts of soil disturbance. It seemed likely therefore that greater spore losses, as in the completely cleared plot may have been due to removal of the humus and intensely rooted layers in which spores are concentrated (Sutton and Barron, 1972; Redhead, 1977; St John *et al.*, 1983). The reduction or complete elimination of host plants indirectly reduced spore numbers through reduced root colonisation which in turn reduced sporulation (Mukerji and Kapoor, 1986). Other factors indirectly reducing spore populations may have been the disruption of the hyphal network in soils (Jasper *et al.*, 1989) and the disruption of living root to living root connections (Janos, 1980) known to initiate root infections faster than spores (Powell, 1976). In effect, factors which delay or prevent infection inevitably reduce sporulation. The nutrient flush observed three months after site clearance (Lawson *et al.*, 1989) may have affected spore production.

Increased nutrient levels may temporarily favour less colonisation of plant roots by VAM fungi and thus subsequently reduce sporulation. According to Hayman (1970) sporulation is maxima when plants are in nutrient stress, thus where P is readily available to plants the ability of plants to become infected and sporulate may have been affected.

Seasonal effects probably affected VAM spore populations as observations in the undisturbed control plot indicate. A majority of VAM fungi sporulate profusely in dry seasons (February, 1987) probably when plants are in moisture stress, but decreased during the two periods of rain (August 1987 and August 1988) presumably as spores germinated in response to increased moisture. Similar observations were reported by Redhead (1977) and Sanders and Sheikh (1983) working in Nigeria. These observations were not replicated by a minority of VAM fungi probably reflecting differences in VAM life cycles. Some workers (Okigbo and Lal, 1979; Louis and Lim, 1987) however argue that seasonal variation in the tropics are fairly small; on the contrary, Mohr (1972) observed that seasonal variations could be marked as a result of shifting rainbelts.

The effects of other soil organisms such as mites, worms, parasitic fungi and bacteria may also have had a quantitative impact on VAM spore populations. Fungal spores provide nutrient concentrations within soils and thus are likely sources of food for mites and worms (Griffin, 1972). Other parasitic fungi and bacteria on VAM fungal spores may have caused spore deaths, thereby decreasing spore numbers (Sutton and Barron, 1972). It remains unclear however, whether fluctuations in numbers of these soil organisms lead to changes in spore numbers observed in the Mbalmayo forest.

Interestingly, trees appeared to be more sensitive to the effects of site preparation than herbaceous vegetation. More spores were lost from areas 'with' cover as opposed to areas 'without' cover (Chapter 5). Distance effects also indicated similar trends to cover wherein more spores were lost close to *T. superba* trees (2.5 m) than away from them. Also more spores of *G. etunicatum* were lost compared to those of the VAM fungal aggregate *G. occultum/A. scrobiculata*. This may

indicate differences in (a) the root morphology of *T. superba* compared to herbaceous vegetation and (b) the distribution of VAM fungi on roots which presumably associated with *G. etunicatum* nearer soil surfaces than *G. occultum/A. scrobiculata*; for this reason the former was more likely to be affected by site clearance than the latter. These root characters need to be studied more closely, for a better understanding of what may be happening.

One year after planting *T. ivorensis* seedlings on the cleared sites there was a dramatic increase in spore numbers. This was probably brought about by increased root densities (Abbott and Robson, 1981). In the mechanically and completely cleared plots the dramatic increase in spore numbers was largely accounted for by a sharp rise in *G. occultum/A. scrobiculata*. In contrast, the species distribution and pattern of VAM fungi in the undisturbed control plot, remained in the the manually cleared plot. VAM fungal species are not considered to be host-specific towards (i.e. hosts other than non-mycorrhizal and ectomycorrhizal taxa) in favourable conditions (Harley and Smith, 1983).

Although observations from the Mbalmayo forest indicate the presence of the same array of fungi as before treatments, the quantitative composition of VAM population was altered. The shift in VAM dominance from *G. etunicatum* to *G. occultum/A. scrobiculata* was observed in the mechanically and completely cleared plots. According to Sieverding (1989) such a shift in dominance indicates adaptations to environment or host preferences and this may be beneficial or deleterious to introduced crops because it is expected that the dominant fungus will be the first to infect the plant. In such a case, therefore, the effectiveness of the dominant species is crucial, (Abbott and Robson, 1981). Because the infection intensities of outplanted *T. ivorensis* in the completely cleared plot remained significantly lower than in the less disturbed manually and mechanically cleared plots, even after spore numbers had increased, it is suspected that the switch in VAM dominance may have been deleterious to the growth of *T. ivorensis* trees.

7.3 IMPLICATIONS FOR MANAGEMENT

Very little is known at present concerning the effects of site clearance on VAM fungi in tropical ecosystems. It is clear from this study that the most destructive site clearance methods lead to a diminution in VAM spores which are an integral part of VAM inocula needed to initiate symbiotic associations. The diminution of spore inoculum had far reaching effects on the survival of outplanted trees which was least in the most disturbed completely cleared plot. Over 1½ years, during which soils were repeatedly sampled, *G. etunicatum* remained the dominant fungus in the undisturbed control and manually cleared plots. The more damaging treatments of the mechanically and completely cleared plots encouraged the growth of the ruderal, *Eupatorium odoratum* and the pioneer tree species *Musanga cecropioides*. Earlier observations show that the VAM fungal aggregate *G. occultum/A. scrobiculata* sporulated more profusely when associated with *Eupatorium odoratum*, therefore in the more disturbed clearance plots which encouraged the growth of this ruderal, there was a shift in VAM dominance from the major forest fungus *G. etunicatum* to *G. occultum/A. scrobiculata* fungal aggregate. The diminution of spore inoculum and the encouragement of VAM fungi associated with ruderals may have crucial consequences for future growth of forest trees, especially as in this study the mycorrhizal flora of the herbaceous plants seemed less effective in promoting VAM infection in *T. ivorensis* in the completely cleared plot. It is important, therefore, to continue monitoring the site at Mbalmayo for several years, to follow this changing pattern and establish its relevance for the development of forest plantations.

In addition, results of this study may have implications for the role and management of VA-mycorrhizal fungi in tropical agroecosystems. For example, one of the effects of complete clearance and shifting cultivation (Slash and burn) is a considerable reduction in VAM abundance, diversity and distribution (Gibson and Hetrick,, 1988) which may be one reason why crop productivity cannot be sustained for more than a few years. In Ontario, Canada, trees grown on clearfelled sites for 10 years were as much as 20% shorter than those grown on adjacent unscalped sites (Mullin and Campbell, 1975); these results

are similar to observations made in this study where after 1½ years infection of *T. ivorensis* remained significantly lower, and the mortality higher in the clearfelled plot, than the less disturbed manually and mechanically cleared sites. Clearly, it is going to be preferable for the farmer to use agronomic practices which maintain high VAM fungal diversity especially as evidence has shown that different crops tend to select and encourage their own group of fungi (Sieverding, 1989). One such system which may provide the answer is agroforestry. Because trees in the Mbalmayo forest had a greater abundance and diversity of VAM fungi than herbaceous vegetation, they may if retained provide a wide diversity of VAM fungi able to benefit a wide range of crops thus optimizing crop productivity for the farmer in a sustainable and ecologically acceptable manner.

7.4 RECOMMENDATIONS

The results from the Mbalmayo forest show that several gaps in knowledge exist, ie the explanation of what is happening to VAM fungi following site clearance and planting. For example there is a need to establish the effectiveness of the array of VAM fungi recovered in Mbalmayo.

By establishing single taxa pot cultures of VAM fungi from Mbalmayo, their effectiveness may be evaluated either singly or in varying combinations on a range of host plants in glasshouse conditions in order to establish which best promotes growth. This information will answer some pertinent questions which remain unanswered such as; did the change in VAM fungal dominance from *G. etunicatum* to *G. occultum/A. scrobiculata* in the severely disturbed soils of the clearfelled plot, mean a change to a less effective fungal aggregate? Was this change (probably to a less effective fungal aggregate) in part responsible for the low infection intensities and high mortality of outplanted *T. ivorensis* in this plot, thus illustrating preferential association of VAM fungi to certain host plants as observed by Sieverding (1989). According to Sieverding (1989) having knowledge about the effectiveness of the dominant species can enable predictions of the effectiveness of the total population: and hopefully corrections to the quantitative composition of fungal species could be made by adjusting methods of

site clearance.

Seasonal effects which have been shown to affect VAM spore populations in temperate ecosystems (Koske and Halvorson, 1981; Walker *et al.*, 1982; Giovannetti, 1985) need to be more closely monitored in tropical ecosystems. Conflicting observations have been made with some workers claiming there is little or no seasonal variation in the tropics (Okigbo and Lal, 1979; Louis and Lim, 1987) whereas others such as Mohr *et al.*, (1972) claim seasonal effects in the tropics are marked due to shifting rainbelts. Redhead (1977) in a survey of some sites in Nigeria reported seasonal variations in spore populations in some sites but not in others.

The quantitative impact of other soil borne microbes such as mites, worms and parasitic bacteria and fungi which feed on VAM fungal spores should be assessed. Knowledge of the population dynamics of these predators will explain their quantitative impact on VAM spore populations as it is assumed that, when they are numerous, VAM spore populations may consequently decline.

Knowledge of the life cycles of individual VAM fungi may also help explain why some spore types did not seem affected by increased moisture during rains. Germination experiments have shown that although spores may be morphologically mature, they may not be physiologically mature and hence may be quiescent or dormant and may not respond to increased moisture (Tommerup, 1983). Germination tests can be carried out under laboratory conditions to ascertain the stage of maturity of spores of the different VAM fungi from Mbalmayo.

Environmental variables such as light and temperatures also need to be monitored regularly as they directly or indirectly affect spore populations. Lastly site clearance methods such as the manual and mechanical methods should be considered for use in large scale plantation establishment schemes because they do less damage to soils physical, chemical and microbiological properties. More importantly, they preserve the indigenous VAM fungi which enhance nutrient uptake from nutrient poor soils and thus increase crop productivity. In instances where the more drastic complete clearance method is chosen, it may be necessary to re-introduce VAM fungi by inoculation.

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