THE PRESENCE AND ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN COASTAL SAND DUNE SYSTEMS

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ABSTRACT

Arbuscular mycorrhizas (AM) are mutually beneficial symbiotic associations between the roots of plants and certain Zygomycetous fungi. The role of AM fungi in coastal sand dunes has been explored in many parts of the world, though little work has been conducted in South African dune systems. This study aimed to investigate the presence and extent of mycorrhizal colonisation of a coastal sand dune in South Africa. The roots of five plant species (*Scaevola plumieri, Arctotheca populifolia, Ipomoea pes-caprae, Ehrharta villosa* and *Chrysanthemoides monilifera*) were sampled along a foredune profile at Old Woman's River in the Eastern Cape. These roots were assessed for the percentage mycorrhizal colonisation they supported. Spores extracted from the rhizosphere sand of each plant species were counted and identified to genus level. Results were related to seasonality and the position of the plants along the profile. All plant species were found to be mycorrhizal. Percentage colonisation ranged from 0-92%, depending on plant species and season. Mycorrhizal colonisation was generally highest in the winter months, and especially so in *I. pes-caprae* and *E. villosa*. The extent of various mycorrhizal structures in root tissue varied between plant species. Spore numbers ranged from 0-48 spores $100g^{-1}$ sand with highest numbers occurring in winter. *S. plumieri* and *A. populifolia* were associated with greatest spore abundance. Four fungal genera (*Glomus, Acaulospora, Scutellospora* and *Gigaspora*) were identified. Distribution of these genera showed seasonal variations between plant species. A bioassay, using *Sorghum*, was conducted to test the inoculum potentials of sand from the *Scaevola* hummock and the *Ipomoea/Ehrharta* dune. Highest percentage colonisation occurred in plants grown in the *Scaevola* sand, which also had the lowest root and shoot measurements. The bioassay confirmed that AM propagules are present and viable, even in the mobile sand of the foredune.

This study showed that mycorrhizal colonisation and spore numbers varied seasonally, but that the extent of this was dependent on plant species. The position of plants along the foredune profile tended to be less important than plant species. It is thought that the growth cycle and rooting

system of each plant species determines seasonal cycles and abundance of AM fungi. Variation within fungal populations probably also impacts on this. Knowledge of the presence and distribution of AM fungi in this system paves the way for more detailed studies which need to examine the role of these endophytes in South African sand dunes.

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CHAPTER 1

INTRODUCTION

1.1 COMMUNITY STRUCTURE AND DYNAMICS

Ecologists have long been concerned with the biological and physical forces influencing the structure and dynamics of communities. However the importance of single factors in ecology are easily confused by the complex interactions that occur among species. Communities and ecosystems consist of species at different trophic levels that interact simultaneously as competitors, predators, pathogens and mutualists (Clay, 1990). The morphological similarities of plants in many communities and their requirements for the same limited resources ensure some niche overlap. The ability of many species to co-exist (and in doing so, to affect plant biodiversity) can be explained by competitive interactions (Aarssen, 1983), resource partitioning (Tilman, 1982), colonisation into new areas, and interactions between different groups of organisms within a community (Bever *et al.*, 1996). The effects of viruses, bacteria, fungi, and animals, be they detrimental or beneficial, may profoundly affect the competitive dynamics of plant communities (Clay, 1990). Colonisation of plants by mutualistic fungi such as mycorrhizas appears to be a prerequisite for survival and species persistence, as well as plant biodiversity and ecosystem variability, in many communities (van der Heijden *et al.*, 1998a and 1998b).

1.2 MYCORRHIZAS

The growing evidence that mycorrhizal fungi have numerous physiological effects on individual plants has prompted a great deal of speculation and research on their importance in community processes such as competition. Mycorrhizas are mutually beneficial associations between soil fungi and the roots of plants.

The term symbiosis was first used by de Bary (1879 in Isaac, 1992) to describe the co-existence, or 'living together' of two or more different organisms. The term included mutualistic associations, and de Bary defined it as benefiting one organism to the detriment of the others. He also pointed out that there was a gradation from harmful parasites to organisms which benefited and supported each other. Frank (1877 in Smith and Read, 1997) did not use the term symbiosis to imply parasitism, but the co-existence of dissimilar organisms. Hence the word symbiosis is generally taken to mean a partnership between two unlike organisms living intimately together, which is usually of mutual benefit (Ainsworth, 1973). Over the years, however, many authors have returned to the definition given by de Bary, which separates mutualistic symbioses as those in which both partners benefit (Smith and Read, 1997). Law and Lewis (1983) defined mutualistic symbiosis as an association that increases the fitness of individuals. This will be the definition used in this study, although there remains some discussion as to what constitutes "fitness" (Isaac, 1992). Increased fitness may constitute enhanced survival, nutrient acquisition, reproduction and growth for the component organisms in mutualistic associations. However in terms of cost and benefit to the individual, some of these factors are difficult to assess, and may actually be lost in the association. For example, growth rates for the *associated* organisms may be lower than those of the component *individuals* under similar conditions (Isaac, 1992). Mycorrhizas are now recognised as "being common and significant representatives of the mutualistic end of the symbiotic spectrum" (Smith and Read, 1997).

The fungal partner in the mycorrhizal symbiosis may be from the Basidiomycetes, Ascomycetes or Zygomycetes, depending on the type of mycorrhiza formed. Mycorrhizal fungi are able to form symbioses with the roots of approximately 90% of terrestrial plant species that have been examined (e.g. Harley and Smith, 1983, Kendrick, 1992; Brundrett *et al.*, 1996), so their inclusion in experiments may explain patterns and mechanisms of competition that were formerly unknown.

Mycorrhizas may be endotrophic or ectotrophic. Endotrophic fungi live within the host plant root, forming hyphae which run within and between the host cells. These hyphae connect directly to the hyphal network in the soil (Harley, 1971). Ectotrophic mycorrhizas form a hyphal sheath or mantle that completely encloses the host root. The fungus only penetrates between the host cortical cells (Harley, 1971). These types of mycorrhizas exist in various forms:

- Arbuscular Mycorrhizas (AM): these are endomycorrhizal associations involving species of Zygomycetous fungi, which produce aseptate hyphae, and form internal structures for nutrient and carbohydrate exchange between fungus and plant (arbuscules), and for carbon storage (vesicles) (Allen and Allen, 1990; Smith and Read, 1997).
- Ectomycorrhizas: these are formed mostly by Basidiomycetes, though other fungi may be involved. The fungus forms a mantle around the root, and a Hartig net between root cortical cells (Brundrett *et al.*, 1996). Little or no intracellular penetration occurs (Smith and Read, 1997). This type of association is found in gymnosperms, particularly in forest trees, and some angiosperms (Harley, 1971).
- Orchid mycorrhizas: endotrophic fungi of orchidaceous plants. The fungus, generally a Basidiomycete, produces hyphal coils within the roots or stems of the host (Harley and Smith, 1983).
- Ericoid mycorrhizas: endotrophic associations of Ascomycetes (and sometimes Basidiomycetes) with plants in the order Ericales. Hyphal coils form in the outer cells of narrow plant root hairs (Harley and Smith, 1983).
- Ectendo-, monotropoid and arbutoid mycorrhizas are similar to ectomycorrhizas, but with specialised anatomical features. Ectendomycorrhizas occur in gymnosperms and angiosperms; arbutoid types in trees, shrubs and some partially achlorophyllous herbs, and monotropoid types in the Monotropaceae, all of which are achlorophyllous and herbaceous. (Harley and Smith, 1983; Brundrett *et al.*, 1996; Smith and Read, 1997).

The arbuscular mycorrhizas are of interest in this study.

1.2.1 THE HISTORY OF MYCORRHIZAL RESEARCH

Prior to 1880, mycorrhizas were observed by several botanists due to the occurrence of their "hairy structures" within roots (Rayner, 1927). Meyen (1829) and Nageli (1842) both recognised that these were fungi, but did not conclude correctly as to the significance or origin of these. In his study of orchids Reissek (1847) provided the first accurate accounts of the association, concluding that they were definitely fungal, and occurred in many monocots and dicots, but most particularly in orchids. From 1860 onwards biologists were becoming increasingly interested in problems of plant nutrition, and much work was done in this field (Rayner, 1927). In 1879 de Bary coined the term "symbiosis", and from 1880-1890 microscope observations became increasingly more accurate as techniques and optical apparatus improved. In 1885 Frank published a paper which formed the basis for his theory of beneficial symbiosis. He regarded the association of roots and mycelium as a morphologically distinct organ, to which he gave the name "Mycorrhiza" (literally meaning "fungus root"). During the following two years, Frank classified mycorrhizas into endotrophic and ectotrophic forms (Rayner, 1927; Isaac, 1992). In 1900, Stahl began a fresh attempt to examine the whole field of mycorrhizas, and for a long time this was the most comprehensive study of the associations (Rayner, 1927). Stahl recognised that plants exhibit different degrees of mycotrophy (i.e. fungal feeding), and he categorised plants as being nonmycotrophic, facultatively mycotrophic, or obligately mycotrophic. Gallaud (1905 in Rayner, 1927) corroborated some of these conclusions, and made careful observations of the special organs produced by endophytes. He confirmed the presence of vesicles and their role as storage structures, and coined the term "arbuscules", which were regarded as haustorial branch systems functioning as absorptive organs for the endophyte. Peyronel (1923 in Smith and Read, 1997) showed that the endophyte hyphae could be traced to sporocarps of species of the Endogonaceae within the surrounding soil. This was the first accurate identification of the fungus, and was confirmed in 1953 by Mosse, who showed that mycorrhizal strawberry plants were colonised by a species of *Endogone* (since transferred to *Glomus*). This was the start of the modern era in mycorrhizal research (Smith and Read, 1997).

Several researchers built upon the early observations and demonstrated the symbiosis that occurred between endogonaceous (now glomalean) fungi and many types of plants (e.g. Baylis, 1959, 1967, 1969; Mosse, 1956, 1973; Gerdemann, 1964, 1968; Nicolson, 1959, 1960). Gerdemann and Trappe (1974) developed a formal Linnean classification of the group. Since then there has been a multitude of research conducted on the topic of arbuscular mycorrhizas, from their taxonomy (Walker, 1983; Walker and Trappe, 1993; Morton and Benny, 1990) to physiology (Hayman, 1983), host range (Harley and Harley, 1987), ecological function (Pedersen and Sylvia, 1996; van der Heijden *et al.*, 1998a, 1998b), and recently molecular interactions (Simon *et al.*, 1992a,b; 1993).

Arbuscular mycorrhizas "are one of the few plant-fungus associations with a fossil record and may even have facilitated the origin of land flora" (Simon *et al.*, 1993). Fossils from the 400 million year old Rhynie Chert have provided evidence of arbuscular mycorrhizas existing along with the early land plants (Taylor *et al.*, 1995; Hass *et al.*, 1994). Fossil specimens of the early land plant *Aglaophyton major* have been found in association with an endomycorrhiza resembling *Glomus*. This has been placed into a new fossil genus *Glomites* which is characterised by aseptate hyphae occurring intraradically, some of which produce spores and others arbuscules. The arbuscules are morphologically identical to those of living AM fungi (Taylor *et al.*, 1995). The antiquity of this association has been corroborated by the similarity of fossil spores to those of living *Glomus* (Pirozynski and Dalpé, 1989), and sequence divergence data based on a molecular clock model. The latter supports a monophyletic origin of AM, most likely from a *Glomus*-like ancestor in the Palaeozoic era (415 Myr), around the time when the first land plants appeared (Simon *et al.*, 1993; Simon, 1996).

1.2.2 THE SYMBIOSIS

AM have been labelled "universal" (Nicolson, 1967), and occur in almost all plant families worldwide. They are, however, absent in some groups of plants, including members of the

Brassicaceae, Chaenopodiaceae, Juncaceae, Caryophyllaceae and Proteaceae (Smith and Read, 1997). The mycorrhizas have several components: host root, intraradical mycelium and fungal structures within the root cells, extraradical mycelium including the soil hyphal network, and spores (Merryweather and Fitter, 1998b; Smith and Read, 1997).

The benefit to the fungus is a supply of carbon from the host and a protected niche, where the microbial antagonisms of the soil are unable to affect it. Experiments on a range of woody and herbaceous plants have shown that mycorrhizal roots receive 4-20% more photosynthate than non-mycorrhizal roots (Hayman, 1983; Jakobsen and Rosendahl, 1990; Eissenstat *et al.*, 1993). St. John and Coleman (1983) state that even though the cost to the plant is high the benefits of the mycorrhizal association, in the form of improved mineral nutrition, typically increase rather than decrease plant growth. Read (1983) demonstrated that mycorrhizas cause physiological changes in many plant species. These include increased rates of growth and seed production, increased water and nutrient uptake, increased drought stress tolerance, changes in hormonal balance, decreased leakage of electrolytes from cells of diseased plants, absorption of phosphate from lower concentrations (Hayman, 1983) and a number of morphological and anatomical changes. It follows that for mycorrhizas to change the competitive balance between neighbouring plants, they must have different physiological effects on those plants.

The fungal carbon sink goes towards lipids, proteins, carbon dioxide production etc. Penetration of the fungal partner does not seem to be affected by cuticle thickness or disruptions on the root (i.e. hyphae do not tend to enter via wounds), or the emergence of lateral roots or root hairs. The latter generally become inoculated from hyphae in the soil, or runners from older roots. AM fungi generally form hyphal runners over roots, which then form appressoria and new entry points which supplement the spread of colonisation within the roots (Hayman, 1983). The host may influence entry of the fungus by mechanisms such as the production of exudates. For example, toxic exudates from the roots of mustard have been attributed to the non-mycorrhizal status of this

species (Iqbal and Qureshi, 1976). The mechanisms which prevent colonisation of non-mycorrhizal plant species are still unclear, though it is thought that certain host plants may be able to exert a genetic influence over the colonisation process (Smith and Read, 1997).

The balance between the host and the fungus is further influenced by soil fertility, particularly P levels. Field responses to mycorrhizal inoculation may be extremely unpredictable due to the influence of environmental factors, therefore extrapolations from greenhouse trials must be made with care. There is a need to look more at the reasons for endophyte differences and specificities with different host plants and soils. As Hayman (1983) emphasises, this is a finely tuned system which is much influenced by soil fertility and therefore involves a three-way interaction between the plant, fungal endophyte and soil.

Environmental factors such as temperature and light are important, and may cause fungi to grow best in the environments in which they were isolated. These factors may particularly affect the initiation of infection. It may be that lower light reduces photosynthesis, and subsequently reduces the carbohydrates available to the fungus. This would reduce the energy available to the fungus for the uptake of P and nutrients from soil, and to increase the number of arbuscules the fungus is able to produce (Hayman, 1983; Smith and Read, 1997). Terrestrial biomes are often classified by measurements of temperature and precipitation, but a more important consideration for mycorrhizal response than temperature is soil nutrients. Total rather than available nutrients are used because mycorrhizal fungi have enzymes that solubilise nutrients from the pool of total nutrients that are not otherwise available to plants. AM fungi are known to produce alkaline phosphatases for P uptake (Allen *et al.*, 1981), and may increase the uptake of N as well (Ames *et al.*, 1984).

Lewis (1973) stated that while plants vary in their dependence on the fungi, the latter are generally obligate mutualists. Molina and Trappe (1982) found that the fungi exhibit little specificity in their plant associations. This is significant to competition in that neighbouring plants may often be

colonised with the same fungal species or group of species (Read *et al.*, 1985), and different plant species are known to have different physiological responses to the same fungal species (e.g. Allen *et al.*, 1984). Mosse (1973) suggested that the symbiotic effectiveness of AM fungi depends on their specificity for soils and host plants, ability to stimulate plant growth, rate of colonisation and competitive ability. She stated that "virtually any AM plant species can be infected by virtually any AM fungal species, but the degree of VA mycorrhizal inoculation and its physiological effects can differ with different host-endophyte combinations". This means that some plants are more susceptible to mycorrhizal colonisation than others, and this may vary between closely related species. Allen and Allen (1990) write that plants with different degrees of mycotrophy form a continuum from the least to the most responsive to mycorrhizal fungi. This continuum has been noted along successional sequences, where species in the early seral stages were mostly non-mycorrhizal, followed by facultative and obligate species (Janos, 1980). Lack of mycotrophy is perhaps to be expected in early succession, as disruption of the soil, either natural or man-made, reduces or eliminates mycorrhizal inoculum. The greatest potential for competitive interactions with mycorrhizas may be in seral communities where species with different degrees of mycotrophy exist as neighbours (Allen and Allen, 1990).

1.3 SAND DUNE ECOSYSTEMS

Coastal dunes form where there is an adequate sand supply and enough wind energy to move the sand (Lubke, 1998a). Dunes are closely linked to sandy beaches, which obtain their sand from waves and longshore ocean currents, and many large dune systems are located down drift of large rivers (Lubke and McLachlan, 1998). The accumulation of sand produces various dune types, which are not only affected by wind and waves, but also by plant species and their methods of colonising the sand (Lubke, 1998c). Various definitions of dune types have been published by different authors (Hesp, 1984, 1991; Tinley, 1985; Lubke, 1998c; Olson and van der Maarel, 1989; Avis, 1992), but those used in the present study will be described in Chapter 2.

The coastal dune ecosystem is subject to a multitude of stresses in the form of salt spray, sand burial, swash inundation, dryness, high light intensity, wind exposure, soil salinity, and nutrient deficiency. The level of these stresses is highest in the beach-foredune environment, and generally decreases with distance from the coast in fully vegetated coastal dune systems. Plant succession and species richness generally tend to follow these landward-decreasing trends. Microenvironments and local habitats are generally lowest on foredunes, moderate on blowouts and parabolics, and highest on transgressive dunefields (Hesp, 1991). Plant adaptations to stresses in this environment include salt resistance, positive growth response to burial, nitrogen fixation, and variations of lifecycle, germination strategies and plant morphology (Hesp, 1991). During the past 40 years, mycorrhizas have also been shown to be a major plant adaptation to environmental stresses on dunes.

Arbuscular mycorrhizas have now been identified in maritime and lacustrine dunes in numerous locations, including the continental United States (Bergen and Koske, 1984; Koske, 1987; Koske and Halvorson, 1981; Koske and Tews, 1987; Sylvia, 1986; Koske and Gemma, 1997), Canada (Koske *et al.*, 1975), Scotland (Nicolson, 1959, 1960; Nicolson and Johnston, 1979), Italy (Giovannetti, 1985; Giovannetti and Nicolson, 1983), and Australia (Jehne and Thompson, 1981; Koske, 1975). Sand dunes provide a very interesting habitat for the study of AM associations due to the fact that they represent a pioneer environment as a result of high levels of evaporation, low levels of available nutrients, and high levels of sand movements (Puppi and Riess, 1987). Mycorrhizas are thought to increase nutrient and water uptake, drought tolerance and sand aggregation in this environment.

Nicholson pioneered work on sand dune habitats in 1959 with his investigation of mycorrhizal Gramineae. His primary objective was to "collect information regarding the distribution and intensity of such mycorrhizal infections in different species and communities, and if possible to relate these to the ecology of the higher plants". Nicolson found that although differences could be

measured in mature habitats, it was difficult to identify the factors causing infection and distribution of mycorrhizas. Sand dune communities were chosen because they had a simpler community structure with a primary succession from a pioneer phase to a climax or subclimax community. Salisbury (1952) had investigated the ecology of sand dune systems, and it was known that grasses played an important role in the colonisation of these. Nicolson's 1959 paper dealt with morphological observations of the mycorrhizas, and addressed problems in the methodology. He also suggested that there might be a nutritive relationship between the mycorrhiza and the host plant, relating particularly to the different phases of the endophyte, and to hyphal connections with external mycelium.

In a subsequent paper, Nicolson (1960) related the mycorrhizal status of plants to their age and localisation. He found that heavier infections correlated to increasing plant age and greater dune stabilisation. The incidence of mycorrhizas also seemed to depend somewhat on soil properties, such as organic matter content and possibly microbiological activity; there were no relationships with any other edaphic factors measured (pH, Ca, carbonate). In addition the author examined the possible origins of infection in the pioneer embryo-dune phase, and suggested that fungi are soil-borne, spreading on plant debris to driftline sand in advance of colonising grasses. These two papers by Nicolson became classics, and formed the basis of many other studies of mycorrhizas in dune plants.

Much of the research undertaken on mycorrhizas in sand dunes since then has involved the identification and quantification of percentage AM colonisation and spores with respect to the factors listed below, in order to assess the effect this has on plant vigour and competition in the dune environment.

1.3.1 Phosphorus Nutrition

Sand dunes are considered to be low nutrient environments (Ranwell, 1972). Seawater contains most of the elements needed for plant growth, except nitrogen, phosphorus and potassium (N, P, K), and most of these are available to plants via salt spray, particularly in the foredune environment (Rozema *et al.*, 1985; Lubke, 1998b). However, sand dune soils are not saline (Ranwell, 1972), and plants are seldom exposed to very high salinities or full strength sea water (Koske *et al.*, 1996). No large reserves of nutrients occur in dune soils, and supply by salt spray probably accounts for the bulk of several nutrients used by plants (Van der Valk, 1974). The availability of these nutrients to plants depends upon the distance of plants from the shore and the degree to which nutrients can be obtained by direct foliar uptake or from the soil (Hesp, 1991). Sand dune soils appear to be particularly favourable for the establishment of AM because of their low phosphorus content (Koske and Halvorson, 1981, Ranwell, 1972). Extraradical hyphae of mycorrhizas are able to tap available P from beyond the P depletion zone of roots (Hattingh *et al.*, 1973) much faster than ions are able to diffuse through soil (Nye and Tinker, 1977). In addition, less carbon expenditure is required from the plant for the production of hyphae than for roots (Tinker, 1975) and their smaller diameter allows them to access a greater volume of soil. Mycorrhizas are also able to access sources of P in soil that are not otherwise available to plants (Smith and Read, 1997). In a study of fungal hyphae in sand dunes, Jehne and Thompson (1981) found that there was a strong correlation between the presence of sesquioxide coatings of P around sand grains and the presence of fungal hyphae. They suggest that fungal hyphae are involved in the uptake of nutrients from the sand grain coatings, and that the association of the hyphae with the roots of colonising plant seedlings confers additional scavenging potential on the seedlings.

1.3.2 Sand Aggregation

Once an AM fungus is established in a root, growth of an extensive external mycelium can begin (Smith and Read, 1997). This often functions as an important source of inoculum for continued colonisation of the same root system, and facilitates the uptake of nutrients from the soil. AM fungi

generally produce simple branched hyphae or hyphal strands in the soil (Tisdall, 1994), some of which are able to grow at least 90cm from the root surface (Camel *et al.*, 1991). The finest of these hyphae are able to enter soil pores where they become involved in the uptake of organic matter (Nicolson, 1959; St. John *et al.*, 1983). The mechanical entanglement of soil by hyphae is important in the formation of soil aggregates. There is also evidence that extracellular polysaccharides, produced by the fungal hyphae act as a cementing agent on aggregates (Tisdall, 1991) by binding microaggragates of soil (< 250μm diameter) into stable macroaggregates (> 250μm diameter) (Edwards and Bremner, 1967; Tisdall, 1994). However the sources of carbon compounds involved in the production of these polysaccharides are not known (Haynes and Swift, 1990).

The external mycelium is very difficult to study and various methods have been used to quantify it. Many early researchers did this by measuring the size and weight of aggregates, or estimating the extent of hyphae by the weight of soil adhering to the roots (Sutton and Sheppard, 1976; Sanders *et al.*, 1977; Forster, 1979; Forster and Nicolson, 1981a,b). This was often difficult and inaccurate, and recent studies have explored new methods. Several DNA-based techniques have been used to identify different fungal species and their variation in roots and soil; these include specific PCR primers, cloned probes or RAPD-PCR polymorphisms (Simon *et al.*, 1992a,b; Wyss and Bonfante-Fasolo, 1993; Clapp *et al.*, 1995; Sanders *et al.*, 1995). Immunofluorescence methods have also been used (Wright *et al.*, 1987; Sanders *et al.*, 1992). Wright *et al.* (1996), using immunofluorescence, discovered that actively growing hyphae produce a protein (since identified as a glycoprotein (Wright *et al.*, 1998)) which they have called glomalin. A comparison between concentration of glomalin and aggregate stability showed a strong relationship for soils from many different geographical regions (Wright and Upadhyaya, 1998), and further research using hyphal traps confirmed that the soil protein was the same as AM fungus hyphal protein (Wright and Upadhyaya, 1999).

Rose (1988) speculated that fungal hyphae may be important for the release of nutrients associated with the cementing agents of sand aggregates and for the survival of early pioneer plants in sand dunes. This was supported by Forster and Nicolson (1981a and 1981b), who found that aggregation increased as dunes became stabilised and higher plant succession developed. They stated that the number and weight of aggregates were correlated. Smaller aggregates were colonised by fewer fungal species than larger ones. There was also a seasonal variation in aggregation in the foredunes, with low levels in summer, increasing during winter. This may be due to the fact that these areas are unstable, experience extremes of temperature and may be exposed to high salinity. It is suggested that over the course of the seasons, aggregates are either building up into larger ones or are breaking down again. AM fungi thus contribute to the stabilisation of sand dunes by binding sand grains into larger aggregates and improving soil structure (Forster, 1979; Forster and Nicolson, 1981a, 1981b; Koske *et al.*, 1975; Sutton and Sheppard, 1976; Rose, 1988). Improved soil structure, in turn, influences plant succession on the dunes, which occurs as they age (Nicolson, 1960; Olson, 1958; Ranwell, 1972).

1.3.3 Seasonality and Succession

Most of the dune studies previously mentioned have related their results to the successional sequence of the dunes studied and/or seasonal effects on both plants and fungi. Several studies have shown that the degree of mycorrhizal colonisation is greater in more stable areas of the dune (e.g. Nicolson, 1960; Nicolson and Johnston, 1979). The degree of mycotrophy of plants has been shown to change with seral stage. Plants that are less dependent on mycorrhizal fungi are generally most abundant during early succession (Allen and Allen, 1990), since obligately mycotrophic plant species may be prevented from colonising a site if fungal levels are low or absent (Janos, 1980; Koske and Gemma, 1990). Conversely, nonmycotrophic and facultatively mycotrophic species may be excluded by high inoculum potentials (Francis and Read, 1994). Facultatively mycotrophic species may have a role in maintaining the inoculum potential of soil and facilitating secondary succession (Janos, 1980). Foredune sites generally have a low species diversity and are associated

with rapidly accreting sand, causing burial of plants and nutrient deficient soils with low organic matter (Ranwell, 1972). In later successional sequences, hyphal networks are generally more abundant, and these account for many of the initial contacts with new plant roots and seedling roots (Francis and Read, 1994). Later successional stages in dunes also tend to have improved soil conditions (Rose, 1988), and an accompanying higher plant diversity (Koske and Gemma, 1997). However AM fungi often arrive early, along with the first plant colonisers in primary successional sites (Gemma and Koske, 1989, 1992; Koske and Gemma, 1990), and the formation of the AM fungal community is linked to aboveground changes in vegetation (Koske and Gemma, 1997).

Variations in AM populations and colonisation have often been linked to season and/or developmental stage of the host plant (Giovannetti, 1985). Different mycorrhizal effects have been observed between annual and perennial plants. Studies on agricultural plants with annual growth patterns showed that maximum spore abundance was associated with the end of the host's growing season or reproductive cycle (Sutton and Barron, 1972; Sylvia, 1986). A similar result was recorded by Giovannetti (1985) in sand dunes. Other studies on dune plants did not show this pattern (Sylvia, 1986; Gemma *et al.*, 1989). Factors such as temperature and light influence percentage colonisation (Smith and Read, 1997), and these may be dependent on seasonal climatic changes. However, many studies on dune plants have indicated that variations in mycorrhizal colonisation and spore numbers are more dependent on the lifecycle and growth form of the plant species (and fungal species) than on overall seasonality (e.g. Puppi and Reiss, 1987; Gemma *et al.*, 1989; Corkidi and Rincón, 1997a).

1.3.4 Propagule Viability and Dispersal

Fungal propagules include spores, infected root fragments and hyphae. The thick-walled fungal spores are fairly resistant long-term survival structures which are dispersed by wind and water (Koske and Gemma, 1990). Spores and sporocarps have also been found to survive passage through the digestive tracts of various mammals, birds and invertebrates (e.g. Reddell *et al.*, 1997;

McGee and Baczocha, 1994) which may have some importance in terms of local dispersion (Smith and Read, 1997). In sand dunes, spores are not readily dispersed to uncolonised areas of the dunes and so may only be of local importance (Koske and Halvorson, 1981). This means that plant species associated with high spore densities "may function as slowly spreading centres of large spore populations in the dunes" (Koske and Halvorson, 1981). This is often supported by a correlation between spore density and vegetation cover. Puppi and Riess (1987) also suggested that the spread of infection and the dispersal of mycorrhizal endophytes is problematic in dunes. They reported that plants do not form a continuum in the mobile zone of a dune, but constitute 'vegetational islands' as sand is continuously accumulated or removed. Thus an effective mycorrhizal endophyte should be able to survive in the soil if moved with the sand, and spread rapidly into any roots it encounters, so producing extensive infection even from individual entry points. The authors state that the development of infection is strongly time-dependent and reliant upon efficient mechanisms of dispersal and survival of fungal propagules.

Spores in field populations may be of different ages and in states of dormancy (Tommerup, 1983; Gemma and Koske, 1988), or are often parasitised (Brundrett *et al.*, 1996). Some remain viable in the soil for longer periods than others (Morton, *http://invam.caf.wvu.edu/articles/propagules.htm*). In sand dune environments, seawater may be a means of spore dispersal. Studies by Nicolson (1960) and Koske and Gemma (1990) indicate that propagules are often present in dune soil before the arrival of colonising plants. It appears that they are dispersed to these sites by seawater, in the same way that primary plant colonisers may be (Koske and Gemma, 1990). Koske and Gemma (1990) reported that spores maintained their inoculum potential after a seven day submergence in sea water. Koske *et al.* (1996) demonstrated that the germination ability of spores of *Gigaspora gigantea* showed a slight but significant linear decrease with the length of time immersed in sea water, declining to 61% after 21 days. These data still need to be applied to field conditions. Brown and Bledsoe (1996) reported that percentage AM colonisation in a saltmarsh halophyte may be suppressed by osmotic stresses associated with seasonal extremes in salinity. It is not known

whether spore isolates from dunes and other coastal regions are more salt tolerant than those from other habitats (Koske *et al.*, 1996), although some studies have shown that increasing levels of sodium chloride (NaCl) strongly inhibit the germination of non-coastal species of AM fungi (Hirrel, 1981; Juniper and Abbott, 1993). AM propagules have also shown different responses to cycles of wetting and drying (Braunberger *et al.*, 1996), which affected the viability of some types of spores, and it is thought that AM fungi have different strategies for coping with wetting and drying. In addition spore and hyphal viability are affected by soil disturbance, which has been shown to reduce mycorrhizal formation (McGonigle *et al.*, 1990; McGonigle and Miller, 1996).

1.3.5 Diversity of Soil Microorganisms

Biotic activity occurs mainly in the rhizosphere, where most of the food source is concentrated. Within this environment mycorrhizas interact with a range of other micro-organisms. These interactions take different forms and may be inhibitory or stimulatory, competitive or mutualistic (Fitter and Garbaye, 1994). Several studies using pot cultures have shown that the microbial community around mycorrhizal roots is strongly modified compared with non-mycorrhizal roots (Ames *et al.*, 1984; Meyer and Linderman, 1986; Citernesi *et al.*, 1996). The soil biodiversity may be increased by the production of such specific habitats, and this in turn maintains soil quality and sustainability (Kennedy and Smith, 1995). In addition, AM fungi have been shown to reduce the incidence and effects of plant root diseases caused by pathogens (Azcón-Aguilar and Barea, 1992). Filion *et al.* (1999) examined the effect of a crude extract of soluble substances produced by *Glomus intraradices* hyphae on conidial germination of two non-pathogenic fungi and on the growth of two soil bacteria. They found that the effects differed depending on the organism, ranging from a strong stimulation to a significant inhibition of growth. They suggest that the stimulation, by AM fungi, of micro-organisms that have positive effects on growth might indirectly interfere with pathogens, and thereby exert an influence on the suppression of disease. Andrade *et al.* (1998) reported that root and fungal components of mycorrhizas enhance the stability of water-stable soil aggregates individually and additively, and have an indirect effect on numbers of soil

micro-organisms "by providing a favourable and protective habitat through the creation of habitable pore space in the water-stable soil aggregates". The interaction of some rhizobacteria and AM fungi have been shown to promote plant growth, though the effects of the bacteria on the fungi may be positive or negative (Garbaye, 1991; Andrade *et al.*, 1995). AM fungi generally have a synergistic interaction with nitrogen-fixing bacteria (Fitter and Garbaye, 1994) and "improved N fixation in mycorrhizal plants appears to be the result of relief from P stress and possibly uptake of some essential micronutrients, which result in both a general improvement in growth and indirect effects upon the N-fixing system" (Smith and Read, 1997).

Antagonistic microflora have been shown to suppress AM spore germination in some soils (Wilson *et al.*, 1989), but enhance it and encourage hyphal growth in others (Azcón-Aguilar *et al.*, 1986; Linderman and Paulitz, 1990). Nematodes in roots may disrupt root tissue and so impede the spread of AM hyphae (O'Bannon and Nemec, 1979). The external mycelium is vulnerable to grazing by various soil fauna which may negatively impact on the development of the mycelium (Fitter and Sanders, 1992), or may act as a stimulus to fungal growth (Hanlon, 1981).

In sand dune systems various studies have been conducted on soil organisms. Webley *et al.* (1952) conducted one of the first studies on dune microflora, relating the occurrence of various bacteria and fungi to plant succession. Forster and Nicolson (1981a) reported the beneficial effects of interactions between plants and micro-organisms on the aggregation of sand from an embryo dune, and the importance of these to the stabilisation of dune sand. A study by Rose (1988) indicated that soil microfloral patterns were related to vegetation, physical dune characteristics and seasonal moisture patterns. Van der Putten and Troelstra (1990) found that harmful soil organisms occurred most abundantly in more stable areas of the dunes than in beach sand, and suggested that sand burial allowed *Ammophila arenaria* to escape the effects of these. However, Little and Maun (1996) reported that AM fungi protected *Ammophila* populations from plant-parasitic nematodes, and that this was more important than the effect of sand burial. In a later paper Little *et al.* (1997)

suggest that the soil community is a "manifestation of edaphic conditions" which influence both the plant and the soil communities, and that a clear separation of these effects cannot always be achieved.

Another aspect of the diversity within the soil community is the different species of mycorrhizal fungi which co-exist. Different species or isolates of AM fungi show variations in their ability to colonise different plant hosts (Smith and Read, 1997), which indicates that the composition of the AM fungal community is a determinant of plant community structure and the functioning of the ecosystem as a whole (van der Heijden *et al.*, 1998a,b).

1.3.6 Sand Dune Rehabilitation

Many researchers have used all the abovementioned factors together to demonstrate the importance of mycorrhizas to dune stabilisation (Bergen and Koske, 1984; Koske and Polson, 1984; Gemma and Koske, 1989; Koske and Gemma, 1997). Healthy dunes are vital to coastal protection against storms (Kaufman and Pilkey, 1979; Steiner, 1967). Increasing demands upon coastal sand dunes for recreational purposes and for residential and commercial uses have meant that a knowledge of dune dynamics has become very important for the wise use and management of these resources (Koske and Polson, 1984). Evidence from studies on sand dunes indicates that arbuscular mycorrhizas are essential for the growth and survival of many dune colonising plants and for the stabilisation of the dunes themselves. Many dune stabilisation projects have made use of *Ammophila breviligulata* (Bergen and Koske, 1984; Gemma and Koske, 1988) or *Ammophila arenaria* (Van der Putten and Troelestra, 1990) for this purpose due to their ability to quickly colonise dune soil. It has been reported that some of the early invading communities of *A. breviligulata* in revegetated dune stands are facultatively mycotrophic. These are able to establish in areas with a minimal hyphal network, so paving the way for secondary plant colonisers once mycorrhizas become established in the sand (Koske and Gemma, 1992). In other cases, AM fungi have been co-dispersed with the *Ammophila* planting stock, enabling a rapid increase in the AM component of the soil (Koske and Gemma,

1990; Gemma and Koske, 1992). The success of *A. breviligulata* in dune restoration has been attributed to these factors (Koske and Gemma, 1997). *A. arenaria* was introduced to South Africa for the purpose of stabilising dunes and was thought to be invasive, but this does not seem to be the case (Hertling, 1997). Nevertheless, indigenous dune plants are being studied with a view to replacing *Ammophila*. It may be that many of the plant species reported to be unsuitable for dune stabilisation and rehabilitation have not been able to establish due to a lack of propagules or the correct mycorrhizal component in the soil. Koske and Polson (1984) suggest that such studies need to be re-evaluated, since restoration of the aboveground plant community cannot be achieved without restoration of the belowground components of a site (Miller, 1985). This is particularly important in the primary succession of sand dunes (Koske and Gemma, 1997).

1.4 DIRECTIONS IN DUNE RESEARCH

Much of the dune research cited here was conducted during the 1970's through the 1980's and involved field studies, supported with information from greenhouse experiments. Field studies of mycorrhizal associations are important for isolating indigenous fungal populations; they also provide valuable additional information about the ecology of an area and the community dynamics in play. From the progression of literature on this topic, it is evident that these studies built up a knowledge base and allowed comparisons to be made between different dune systems. It appears that the next step after recording the occurrence and abundance of mycorrhizas was to try to explain how and why they functioned, what their taxonomic components were, and how they affected ecosystem functioning as a whole. In addition, many of the authors had come to the conclusion that a combination of environmental, soil, climatic, host and fungal factors were affecting the results of field trials, and that it was very difficult to separate these effects. Giovannetti (1985) wrote that "a recurrent problem when dealing with natural environments (is that it is) very difficult to distinguish a seasonal effect from effects related to the stage of development of the host, intermittent root growth or simply meteorological causes and other environmental

conditions". And, as Marx and Schenck (1983) wrote: "basic studies seem to attract the interest of more scientists, results are obtained faster, and the research is usually done in the comforts of the laboratory or green house," while in field studies, "the frustration threshold is high, current interest is low, and the work environment is frequently unpleasant and unpredictable". As a result, many recent studies seem to have moved towards more focused field and greenhouse experiments which strive to *explain* the presence and role of mycorrhizas in dunes, rather than *describe* them. In addition, fewer studies pertaining to mycorrhizas in dunes are being undertaken, as the emphasis on research has moved to the exploitation of the AM symbiosis in agriculture and the revegetation of disturbed areas such as mine strips rather than examining their role in natural systems (Koske and Polson, 1984). In South Africa, however, dune mycorrhizal studies are still in their exploratory descriptive phase. We need to record and measure the mycorrhizal component of plants and soils and compare the results to other dune studies before we can hypothesise on how mycorrhizas affect these ecosystems.

1.5 SOUTH AFRICAN STUDIES

Few detailed studies have been undertaken on the distribution of indigenous AM populations in South Africa. In addition, most of the research involving AM fungi has been centred on grassland systems (Coetzee, 1982; Allsopp and Stock, 1994; Allsopp, 1998) with little attention being paid to coastal dune regions. Naidoo (1987) undertook a preliminary investigation of arbuscular mycorrhizas in a natural Natal coastal sand dune ecosystem. She found that AM occurred in all six species of plants that she studied. These were *Scaevola plumieri, Gazania rigens, Eugenia capensis, Chrysanthemoides monilifera, Carpobrotus dimidiatus* and *Passerina rigida*, although most work was conducted on the first four species. The mycorrhizal associations with these plants were confirmed using light and electron microscopy. Large variations in infection levels within a sample and amongst species were observed. However, it was evident that there was an increase in the fungal component of mycorrhizas from the foredune to the dune scrub area. Spores belonging

to four genera of mycorrhizas were isolated from the dune soil. These included *Acaulospora, Gigaspora, Glomus* and *Sclerocystis.* In this habitat there was a correlation between the number of spores recovered, and the infection levels of each species, with the exception of *Gazania rigens.* Naidoo (1987) also conducted greenhouse trials to assess the inoculum potential of vesicular-arbuscular mycorrhizal fungal propagules in the dune soil and to demonstrate improved plant growth due to mycorrhizal fungal colonisation of root tissue. These results were inconsistent, and the author concluded that although very widespread, there was insufficient data to elucidate the role of AM fungi in this coastal dune system.

1.6 OBJECTIVES

This study was conducted on a natural foredune at a site on the Eastern Cape coast of South Africa. The objectives of the work were to:

- assess whether AM structures were associated with the chosen plant roots, and if so to what extent,
- isolate and identify (to genus level) fungal spores from the root zones of the selected plants,
- correlate both colonisation and spore numbers to seasonality and the position of plants along the foredune profile,
- assess whether different fungal genera showed a preference for particular plant species, and
- quantify the inoculum potential of the soil by means of bioassays.

CHAPTER 2

STUDY SITE

2.1 LOCALITY

Old Woman's River (27° 8'E, 33° 1'S) is situated on the Eastern Cape coast of South Africa, approximately 30km north-east of Port Alfred and 1.5km north-east of the Great Fish River mouth. The Eastern Cape is a transition region where a number of floras and vegetation types converge due to the transitional nature of the climate, geomorphology and geology (Lubke and van Wijk, 1998). The coastline from Port Alfred to the Fish River consists of shifting sands with very little vegetation cover (Lubke, 1983). Dunes in the region are generally orientated transversely to the wind direction, which is either from the south-west or the east at different periods or seasons of the year (Lubke, 1998c). Dunes shapes are either transverse or crescentic (barachnoid); the former have straight or curved ridges with axes at right angles to the wind, whilst the latter are crescent-shaped with linked axes at right angles to the wind (Avis, 1992). Invading vegetation modifies the slope and shape of the dunes (Hesp, 1984) which extend from the high tide line to the start of the climax coastal vegetation (Lubke and McLachlan, 1998).

2.2 CLIMATE

The Eastern Cape has an unpredictable and variable climate, and is largely a transition zone of climate types (Stone *et al*, 1998). By the Koppen system of climate classification, the coastal part of this region is subtropical, with monthly temperatures of between 10 and 22.2°C, though they may reach heights of 40°C, particularly during summer 'berg wind' conditions, and lows of close to 0°C in some areas. Monthly rainfall of at least 60mm occurs, though the seasonality of rainfall is less pronounced here than in other parts of the country (Stone *et al*, 1998; Lubke, 1998a,c). Along the former Ciskei coast from the Fish River Mouth to Kaysers Beach (the region incorporating Old Woman's River) rainfall occurs mainly during spring and autumn, and to a lesser degree in summer.

(Lubke, 1998a). Prevailing winds are westerly and south-westerly in winter and easterly and south-westerly in summer, though strong winds are prevalent at any time of year. The number of calm days recorded per year is around 9% (Lubke, 1998a,c).

2.3 VEGETATION

At Old Woman's River sampling of the predominant plant species was conducted along a foredune profile. The five main plant species were *Scaevola plumieri* (L.) Vahl, *Arctotheca populifolia* (Berg.) T. Norl., *Ipomoea pes-caprae* (L.) R.Br. subsp. *brasiliensis* (L.) Van Ooststr., *Ehrharta villosa* Schult.f. var. *maxima* (Stapf) and *Chrysanthemoides monilifera* (L.) T.Norl. subsp. *rotundifolia* (DC.) T.Norl.

The region closest to the beach was a hummock foredune that was steep and high on the seaward side (3m) and had a rounded shape (Plate 1A). It was colonised by *Scaevola plumieri*, a characteristic component of dune hummocks (Avis, 1992). *Arctotheca populifolia* occurred in an area adjacent to this as the only component of an incipient foredune (Plate 1B). This dune type was defined by Avis (1992) as one with fairly large hummocks, but less rounded and not steep. A transverse dune (as characterised by Lubke and McLachlan, 1998) formed by drift sands behind the *Scaevola* hummock was colonised by *Ipomoea pes-caprae* and *Ehrharta villosa*. Behind this *Chrysanthemoides monilifera* formed the start of a scrub area (Plate 1C; Figure 2.1)

Scaevola plumieri (Goodeniaceae) is a straggling shrub up to 1m tall with elongated stems which ramify through the drifting sand and help to bind the dunes. Leaves are large, succulent and yellowish-green. White fan-shaped flowers occur in the axils of the leaves. Fruit are spherical and yellow, becoming purple when ripe (Lubke and van Wijk, 1998).

PLATE 1

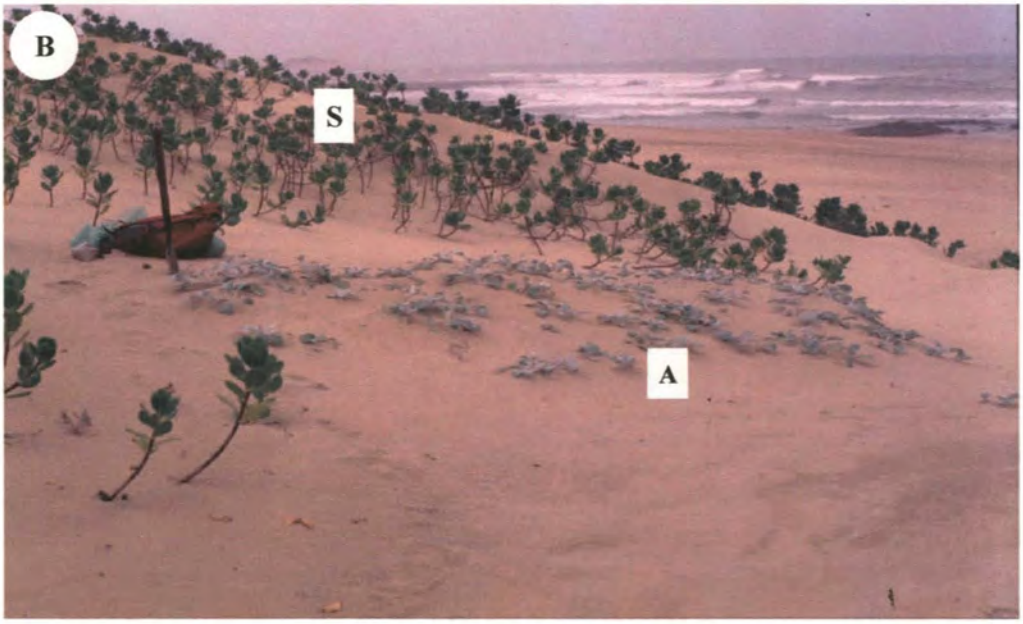
PLATE 1: STUDY SITE

Figure A: Photograph of the *Scaevola plumieri* foredune hummock sampled at Old Woman's River.

Figure B: The other side of the *Scaevola* hummock. A patch of *Arctotheca populifolia* (A) can be seen forming a smaller hummock adjacent to the *Scaevola* plants (S).

Figure C: The *Ipomoea/Ehrharta* dune (I/E). Long stolons of *Ipomoea* are visible on the surface of the sand (arrow). Behind this region, a dense cover of *Chrysanthemoides monilifera* (C) can be seen.

PLATE 1



Arctotheca populifolia (Compositae) is a large thick-stemmed herb which also stabilises dunes with the formation of hummocks. Leaves are densely covered with white woolly hairs and the yellow flowerheads are composed of large disc and ray florets (Lubke and van Wijk, 1998).

Ipomoea pes-caprae (Convolvulaceae) is a creeper with extensive red stems. Leaves have two large lobes. Large purple flowers are trumpet shaped and fruit appear as spherical brown capsules with hairy brown seeds. This species is a common and abundant pioneer on foredunes (Lubke and van Wijk, 1998).

Ehrharta villosa (Poaceae) is a robust perennial with creeping rhizomes, forming single culms or small tufts, to 1500mm tall. Leaves are bluish-green, small and wiry. The inflorescence is an open raceme of hairy spikelets. Flowering occurs sporadically from September to March. It is common on rear dunes, blow-out areas and the margin of coastal bush (Gibbs Russell *et al*, 1990; Lubke and van Wijk, 1998).

Chrysanthemoides monilifera (Compositae) occurs as a small shrub or tree 1-6m tall. The oval leaves are arranged alternately and are leathery, greyish-green and glossy. Young leaves are covered with white cobweb-like hairs. Flowers are bright yellow. Oval to spherical fruit occur as fleshy purple berries. The species is a common pioneer along the margin of the coastal scrub (Lubke and van Wijk, 1998).

The foredune profile sampled stretched from the base of the *Scaevola* hummock, nearest the beach, to the *Chrysanthemoides* community at the start of the dune scrub behind the foredune. This was a distance of approximately 36 meters (Figure 2.1).

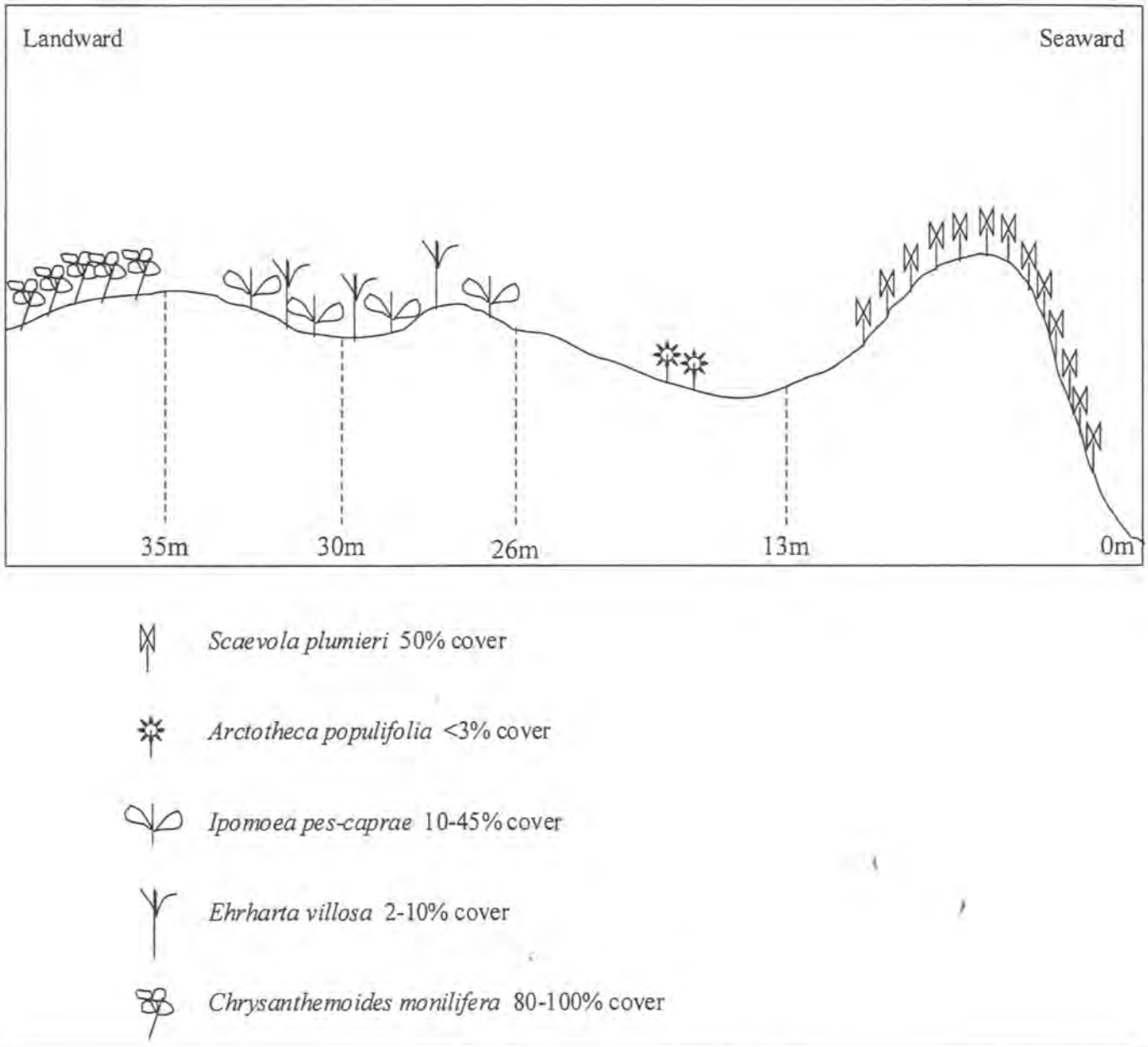


Figure 2.1 Diagrammatic representation of the foredune profile at Old Woman's River (not drawn to scale). Distance from the foot of the foredune hummock inland is indicated in meters. Percentage cover for each plant species was recorded at each sampling time and the range of these is included.

CHAPTER 3

MATERIALS AND METHODS

FIELD STUDIES

3.1 FIELD SAMPLING

Vegetation was sampled along the 36m transect which extended from the foot of the foredune (on the seaward side) to the start of the dune thicket, which has subsequently become overrun with *Acacia longifolia.* All species occurring along the profile were sampled at each visit, approximately every two months. The position of plants along the profile was noted, as well as the approximate percentage cover of each species (Figure 2.1). The profile chosen was very simple in that few species occurred, and each species community occupied a discrete zone or site along the profile (see Chapter 2). *Scaevola plumieri* and *Arctotheca populifolia* occurred as primary colonisers at the front of the foredune area; *Ipomoea pes-caprae* and *Ehrharta villosa* occurred as secondary colonisers behind these, adjacent to a blow-out zone, and *Chrysanthemoides monilifera* occurred at the start of the scrub. All plant species identifications were verified and assigned to families at the Selmar Schönland Herbarium, Grahamstown (GRA) South Africa.

3.1.1 CLIMATIC DATA

Rainfall and temperature data were obtained from the South African Weather Bureau as mean daily temperature and total daily rainfall. These were converted to mean monthly temperature and total monthly rainfall.

3.1.2 COLLECTION AND STORAGE OF ROOT SAMPLES

Much of the work conducted on sand dunes has involved sampling from what is generally termed the "root zone" of plants (e.g. Koske, 1988; Gemma *et al.*, 1989). Koske and Gemma (1997) characterised this as the zone deeper than 20cm below the surface of the dune, since few roots tend to occur above this, probably as a result of the mobility of the sand and the effect of sand burial. For these reasons the upper 20-25 cm of soil, in which few roots occur, was removed and discarded prior to the collection of all samples. Roots were then taken from the regions below this. Between 50cm and 100cm of root material was collected for each plant species. Care was taken to collect as many of the fine lateral roots as possible along with the main root systems, so as not to bias measurements (Brundrett *et al.*, 1996). Roots were not collected if they were entangled with the roots of other species in order to avoid incorrect assessments. Samples were taken of at least three different individuals of each plant species to ensure variability. Whole seedling roots were collected in addition to these when possible. Seedlings of some species, e.g. *Ehrharta villosa* and *Ipomoea pes-caprae*, were rarely seen, whilst those of other species, e.g. *Scaevola plumieri*, occurred seasonally. Root collections were undertaken every two months, between October 1997 and December 1998, in order to record any seasonal fluctuations in colonisation levels. In addition, results were correlated to the position of the plant species along the foredune profile. During one sampling trip, in February 1998, *Scaevola* roots were also collected at a depth of one meter in order to determine whether arbuscular mycorrhizal colonisation varied with root depth.

Root samples were placed into labelled vials containing distilled water in order to wash the sand from them. They were generally processed the following day. However, if processing was to be delayed, they were transferred to vials containing 50% ethanol. This served to preserve the roots (Koske and Gemma, 1989). Ethanol was chosen as the fixative over FAA (formalin:acetic acid: alcohol) due to the caustic nature of the latter (Proctor and Hughes, 1978).

3.1.3 COLLECTION AND STORAGE OF SAND SAMPLES

Three sand samples were collected along with the root specimens for every plant species. This meant that one sand sample was taken from the root zone of each individual plant selected for root sampling. This ensured that replicates were spatially separated. This was important as results were to be correlated to the position from which samples were taken along the foredune profile, and also to ensure heterogeneity of the replicates. Approximately one litre of sand was collected each time, and placed into labelled polythene bags. The samples were stored in a cold room at 5°C until spores were extracted.

3.2 LABORATORY TECHNIQUES

3.2.1 ROOT STAINING PROCEDURES

Endomycorrhizal roots look superficially similar to non-mycorrhizal roots, and AM structures are often obscured by natural pigments and cell contents within fresh roots (Brundrett *et al.*, 1996). It is therefore necessary to clear and stain roots in order to define any internal fungal structures. This can be accomplished without much staining of the plant material.

Roots were cleared and stained according to the method of Koske and Gemma (1989). This technique, at the time, was a modification of the commonly used techniques described by Phillips and Hayman (1970) and Kormanik and McGraw (1982). Koske and Gemma (1989) sought to reduce the toxic and offensive compounds used in these earlier methods, whilst lowering the cost, number and amount of chemicals required. The resulting method involved the same procedures as its predecessors but utilised safer compounds, without reducing the resolution of the staining.

Fresh or preserved roots from the study site were washed thoroughly with distilled water and then cleared. Clearing serves to remove cytoplasm and any coloured material from plant cells (Smith

and Dickson, 1997). Approximately 3g (f.wt) of roots were placed into $55cm^3$ test tubes and covered with 2.5% potassium hydroxide (KOH). The test tubes were heated in a water bath at 90°C for 20-30 mins. Roots were then rinsed in several changes of water. Any roots that were still dark, or that had not cleared completely after this stage, were bleached in a freshly prepared solution of alkaline hydrogen peroxide (H_2O_2). This was made up using 3ml of 20% NH_4OH in 30ml of 3% H_2O_2. Generally it was necessary to bleach *S. plumieri* and *C. monilifera* roots for up to 30 minutes, and *I. pes-caprae* and *A. populifolia* roots for up to 10 minutes. *E. villosa* roots never required bleaching. After bleaching the roots were rinsed carefully in several changes of water. The roots were then acidified by soaking them in 1% HCl (pH *ca* 0.65) overnight. Acidification ensures that the dye is retained effectively in the root tissue, and a rise to pH 0.8 - 1.4 indicates that a sufficient volume of acid was used (Koske and Gemma, 1989). For this reason roots were not rinsed before staining.

Staining was performed using an acidic glycerol solution (500ml glycerol, 450ml H_2O, 50ml 1% HCl) containing 0.05% trypan blue. Roots were covered in this and heated in a 90°C water bath for 15-30 minutes. Trypan blue is a commonly used stain and ideal for routine observation, since only bright-field light microscopy is required (Smith and Dickson, 1997). The trypan blue solution was poured off, and the roots destained in acidic glycerol without trypan blue. Destaining was done at room temperature. Stained root samples were stored in acidic glycerol, in the dark (Koske and Gemma, 1989).

3.2.2 ASSESSMENT OF MYCORRHIZAL COLONISATION

Stained roots were cut into approximately 1cm lengths and mounted on glass microscope slides in polyvinyl alcohol lacto-glycerol (PVLG) for semi-permanent mounts (Omar *et al.*, 1979). To ensure adequate replication only sections of the same root were mounted on each microscope slide, and at least three slides were made for each sample examined. Fifteen to twenty centimetres of root were examined for each plant specimen.

McGonigle *et al.* (1990) stated that visual determinations of "the proportion of root length colonised by AM fungi can be divided into three groups: 1) subjective estimation, 2) calculation of the percentage of root segments or microscope fields of view that contain colonisation, and 3) grid-line intersect methods". These do not include techniques using chemical estimation of chitin (e.g. Hepper, 1977, Bethenfalvay *et al.*, 1981). Giovannetti and Mosse (1980) compared various techniques for measuring AM colonisation, and concluded that all techniques probably overestimate true values. They suggested that the least laborious, but most subjective technique would be a visual estimation. However, this method is only suitable when precise values are not required. Some studies (e.g. Daft and Nicolson, 1972; Read *et al.*, 1976 and Biermann and Linderman, 1981) calculate the number of colonised root segments, divided by the total number of root segments examined. A variation of this is to calculate the number of microscope fields of view containing any colonised roots, divided by the total number of fields of view (Baylis, 1967; Dodd and Jeffries, 1986). Giovannetti and Mosse (1980) considered the grid-line intersect method to be the best compromise of all methods. In this method, a dissecting microscope is used to observe randomly dispersed roots in a 9cm petri dish containing grid lines. Intersections between grid lines and roots are quantified and designated as either colonised or non-mycorrhizal (Newman, 1966; Tennant, 1975; Giovannetti and Mosse, 1980). Modifications of this method are thought to be most frequently used for mycorrhizal assessment (Brundrett *et al.*, 1996). However a criticism of this technique is that it can only give a relative measure of colonisation, since it is often not possible to tell if roots are mycorrhizal at all intersections (McGonigle *et al.*, 1990). A grid-line intersect method using a compound microscope was suggested by Ambler and Young (1977). McGonigle *et al.* (1990) modified this method in an attempt to measure colonisation objectively by recording specifically whether arbuscules were present at each intersection, since these are the structures unique to AM fungi. This was called the magnified intersections method. This was used in the present study since it was felt that this was the least subjective method.

A Zeiss compound microscope was used to inspect the intersection between roots and a microscope eyepiece crosshair at a magnification of 200x (Figure 3.1). Roots were measured at right angles to the eyepiece crosshair. At each intersection the following scorings were made: negative (no fungal material), arbuscules, vesicles and hyphae only. The method was modified to include intraradical spores, hyphal coils and the occurrence of appressoria. A further modification was that recordings of mycorrhizal structures were converted to percentages of the total number of intersections observed. For each plant species, one hundred intersects were recorded from each sampling time.

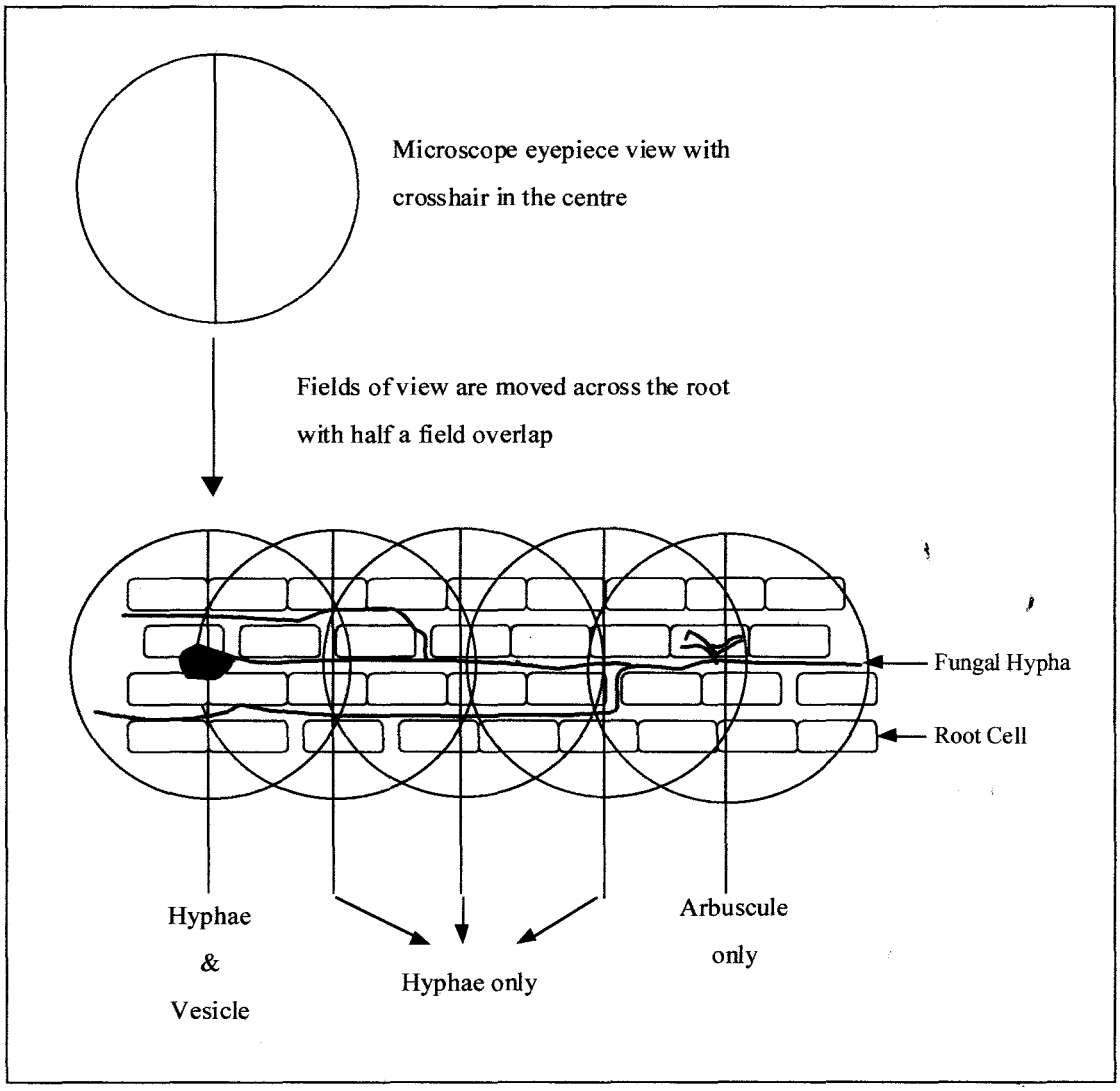


Figure 3.1 Assessment of AM colonisation using the magnified intersections method. Redrawn from Brundrett *et al*, 1996.

3.2.3 EXTRACTION OF SPORES

Wet sieving and decanting, followed by density gradient centrifugation are the most commonly used methods for spore isolation (Gerdemann and Nicolson, 1963; Daniels and Skipper, 1982; Walker *et al.*, 1982; Tommerup, 1992). Wet sieving and decanting is a simple method which uses sieves of various sizes to separate spores and other similar-sized particles from sand and clay (Daniels and Skipper, 1982). This was thus an ideal method for removing much of the dune sand from samples.

The method was adapted from Gerdemann and Nicolson (1963). A 100g subsample of sand was taken from each sample bag, placed in a 1.5L beaker and mixed thoroughly with 1L of water using a glass rod. The suspension was allowed to partially settle for 10s, and the supernatant was decanted through nested sieves with mesh sizes of 250μm, 180μm and 45μm respectively. The sediment was resuspended and washed through the sieves again. This was repeated three times, using 1L of water each time. The debris from the 250μm sieve was discarded after examination for large spores and sporocarps. The residue in the 180μm and 45μm sieves was gently rinsed under running water and poured, separately, into 50ml centrifuge tubes. This was done for each sand sample bag, resulting in three replicates from each site or plant community.

3.2.4 PURIFICATION OF SPORES

Density gradient centrifugation is an effective purification technique in that either small or large quantities of soil can be processed rapidly and efficiently with most of the debris being removed from the spores (Daniels and Skipper, 1982). Ohms (1957), Ross and Harper (1970) and Mertz *et al.* (1979) suggested the use of sucrose gradients. This method was attempted for this study, using an adaptation from Gerdemann (1961), in which 10ml of a 20% sucrose solution was layered over 10ml of a 60% sucrose solution in a 50ml centrifuge tube. To this was added a similar layer of water and finally a suspension of sievings. This was centrifuged for 5 minutes at 3000 rpm. The

spores were expected to collect in the middle layer. However, this proved largely unsuccessful due to the varying particle sizes within the dune sand.

A second method was tried using kaolin (Allsopp, pers comm; Brundrett *et al.*, 1996). Sievings and water were stirred with 2g fine kaolin in 50ml centrifuge tubes. These were centrifuged for 5 mins at 1800 rpm. The water was poured off and the kaolin pellet was mixed thoroughly with 20ml of a 50% sucrose solution. This was centrifuged for 1 minute at 1800 rpm and poured over rapidly-draining filter paper. However few, if any, spores were collected by this method.

The third and most successful method tried was an adaptation of that by Walker *et al.* (1982). This involved sucrose centrifugation which did not require gradients. The spore suspensions, in water, were centrifuged at 1800 rpm for 5 minutes to remove any floating organic debris. The supernatant was carefully removed by pouring over 9cm Whatman No.1 filter paper discs, to examine it for spores, since it was suspected that some might be lost if the supernatant was discarded. The remaining pellet in the centrifuge tubes was mixed with 40ml of a 60% sucrose solution. This was centrifuged for a further 5 minutes at 1800 rpm. The supernatant, containing spores, was poured onto the 45µm sieve, and this was rinsed with running water to wash off the sucrose. The remaining residue was washed off the 45µm sieve onto a 9cm diameter Whatman #1 filter paper disc in a Buchner funnel, whilst pulling a vacuum in order to drain off the liquid. Each filter paper was placed into a labelled petri dish. Prior to purification, approximately $1cm^2$ grids were drawn onto each filter paper.

In each of the above methods, an MSE bench-top centrifuge with swinging bucket rotor was used for the centrifugation process.

3.2.5 ENUMERATION OF SPORES

Each filter paper was examined under a Zeiss dissecting microscope at a magnification of 40x. Spores were counted by adding up the number within each grid on the filter paper, and counts were expressed per 100g of sand. All mycorrhizal spores which appeared to be healthy (not desiccated, broken or parasitised) were counted. Representative samples of the different spore types counted were removed and mounted in PVLG for semi-permanent mounts (Omar *et al.*, 1979) as well as PVLG with Melzer's Reagent (1:1 v/v PVLG/Melzer's) (Morton., 1991, 1992). Melzer's Reagent causes the walls of certain spore genera to change colour, and is therefore useful as an identification tool. It was originally developed by Melzer (1924) to view ornamentations on spores of *Russula* species (Morton, 1988). In each of these mountants, some spores were left intact whilst others were gently squashed with the cover slip in order to break them and expose the wall layers.

3.2.6 IDENTIFICATION OF SPORES

Spores were identified to genus level according to the criteria of Schenck and Perez (1990) and Morton (1988 and 1997). Several other books, papers and websites were used, including Walker (1983), Morton and Benny (1990), Brundrett *et al.* (1996), the INVAM website: *http://invam.caf.wvu.edu*, and Walker and Vestberg (1998). Before commencing, each objective of the microscope was calibrated using an eyepiece graticule and stage micrometer. As many spores as were available were examined, with numbers ranging from one to 41 for each spore type. The characteristics used for identification included spore colour, shape and size, wall structure and ornamentation, hyphal attachment and occlusion. Identification of field-collected spores is often not all that accurate, due to the fact that characters may change with age (e.g. colour, number of spore walls), and age is not a known factor in field collections. Characteristics in field collected spores, may also be altered due to microbial activity or passage through an animal's digestive system (Brundrett *et al.*, 1996). For these reasons, spores were identified only to genus level. In some instances tentative species identifications were made.

All the spore characteristics that were used for identification are described below.

Spore colour

Morton (1988) described the range in spore colours, which is dependent on how light interacts with the specimen. Glomalean spore colours range from cyan to yellow to magenta (CYM), the darkest of which may appear to be black. Colours were classified using a modified version of the INVAM colour chart (Morton, 1992) published in Brundrett *et al.* (1996). This chart defines colour as a CYM percentage. Colour was viewed both under a dissecting microscope, using reflected light, and under a compound microscope after spores had been mounted and crushed, using transmitted light. Colour is a widely used character in spore identification, but it is not necessarily stable. Colour can change according to the age of the spore, and some species may lose their outer layers as they age (Brundrett *et al.*, 1996; Walker and Vestberg, 1998). However, the spore wall colours of several genera change when stained with Melzer's Reagent, and this is therefore regarded as an identifiable charactersitic (Morton, 1997).

Spore size

Walker and Vestberg (1998) and Morton (1988) raise some doubt as to the validity of spore dimensions as a stable characteristic, since these can be so variable within a population. However, significant differences in spore sizes can help to differentiate between genera and species (Brundrett *et al.*, 1996). Glomalean fungi vary in size from 10μm to 800μm (Morton, 1988). Spore length was measured from the point of hyphal attachment along that axis, and included all spore wall components. Spore width was measured as the widest axis at right angles to the length (Walker and Vestberg, 1998). Measurements were taken from below any ornamentation present. Once all measurements for each genus had been recorded, sizes were given as a range of minimum to maximum values, with average measurements in parentheses.

Spore shape

Most glomalean fungi are spherical (globose), although some maybe oval, oblong or occasionally other shapes (Morton, 1988). However, spore shape may be considered a dubious character for the

same reasons as spore size. Therefore, for the purpose of this study, spore shape was described as being ellipsoid or globose to subglobose (Morton, 1997).

Wall Structure

This is regarded as the most important taxonomic character in spores. All comparative morphological evidence indicates that almost all species level differences occur exclusively in properties of the spore wall (Morton, 1997). Various types, groups and layers of the spore wall may occur. Walker (1983) describes wall groups as "an aggregation of walls that are either adherent, or that remain close together when a spore is crushed". Wall types have conventionally been classified according to the descriptions of Walker (1983). Further amendments to these were made by Walker and Sanders (1986), Morton (1988), and Schenck and Perez (1990). Morton (1990) redefined the wall types based on developmental patterns and processes. For the purposes of spore wall descriptions in this study, these new developmental concepts were used, and correlated with the former character concepts when necessary. Wall layers are defined as follows:

- The spore wall originates from the subtending hyphal wall. Only one spore wall is synthesised and may consist of several layers, which often occur above the flexible inner walls. The spore wall may have one or more outer layers that slough from the spore surface during growth or after maturity. Such layers are described by their properties while intact (e.g. mucilaginous, granular etc.) (Morton, 1997).
- The Unitary layer is a single rigid wall, with no discernible sublayers. Also known as the Unit Wall.
- The Laminate layer or wall is a permanent structure with a variable number of sublayers which often adhere to one another, but which may become separated. This layer gives structural integrity to the spore (Morton, 1997). The number of layers often increases with spore age (Walker, 1983).

- The Germinal wall is the innermost sublayer of the laminate layer, which often has a slightly different ultrastructural composition to the other walls (Morton, 1997). It appears prior to germ tube formation (Walker, 1983).
- The Flexible Inner Wall (FIW) originates independently of, and subsequently to, synthesis of the spore wall. It is described by its thickness, plasticity and reaction to Melzer's reagent (Morton, 1997). Each flexible inner wall usually has two layers of common origin which subsequently differentiate. These walls used to be classified separately as either membranous (thin, flexible, often wrinkled) (Walker, 1983), coriaceous (robust, thick, tough, flexible) (Walker, 1986) or amorphous (formless, elastic, of variable thickness, stains dark red-purple in Melzer's) (Morton, 1986). This is no longer the case, although generally the thinner the FIW, the greater its flexibility and the weaker its reaction to Melzer's (Morton, 1997).

In the new classification wall groups are referred to as the spore wall with component layers, and the flexible inner walls with component layers (Morton, 1990).

Ornamentation

Unitary and laminate walls may be ornamented. Many types of ornamentations occur and may include pits, rounded projections, knobs, crowded spines, tubercles, hemispherical warts and alveolate reticulations (Morton, 1988; Brundrett *et al.*, 1996). Spores were examined for ornamentations under the Zeiss compound microscope, and an Olympus BX 50 light microscope with Nomarski interference. Measurements were made up to and excluding the spore wall.

Hyphal attachment

Most AM fungi bear spores on or in a single subtending hypha (Morton, 1988), and the type of hyphal attachment is important in distinguishing between genera. Morton (1988) details the number, colour, shape and widths of hyphae which may subtend a spore. Of these, the shape of the

hypha seems to be the most useful taxonomic tool. Spores identified had their hyphal attachments characterised on the basis of the following points:

- The shape of hyphae is often quite variable among species of *Glomus.* In *Gigaspora* and *Scutellospora* the sporogenous hypha is always bulbous (with minor variation), and in *Acaulospora* and *Entrophospora* most species form sessile spores and therefore lack subtending hyphae, though some species may form a pedicel (shortened branch of a subtending hypha) (Morton, 1997).
- Hyphal width is measured at the point of attachment between spore and hypha. This measurement can be quite variable however, and is not an important identification tool (Morton, 1988).
- The number of hyphae is variable within species and is therefore not taxonomically important. However, most species with more than one subtending hypha appear to be in *Glomus,* and occasionally *Scutellospora* and *Gigaspora* (Morton, 1988).

Occlusion

An occlusion may be a plug, septum, wall thickening or flexible inner wall, which serves to block the channel (or pore) connecting the spore contents to the cytoplasm of the vegetative hypha. This is necessary in order to partition spore contents, thus allowing differentiation and physiological integrity (Morton, 1988). Occluding structures vary between individuals, both inter- and intraspecifically, but the complete absence of any type of occlusion has only been noted for three species of *Glomus* (Morton, 1988). Some spores within species of *Gigaspora* and *Scutellospora* may have an open pore, but this is rare and more often the occlusion is difficult to see since it may have the same appearance as the laminate layer of the spore wall (Morton, 1997). Spores were examined for the presence and nature of occlusions according to these factors.

Auxiliary Cells

Also referred to as auxiliary bodies or auxiliary vesicles, these are structures associated with soil hyphae (Brundrett *et al.*, 1996) and do not constitute part of spore identifications. They are included in this section since they are often extracted from soil along with spores, and have varying morphological characters, which differ between fungal genera. Auxiliary cells occur in clusters of 2-12 at the ends of branching AM hyphae in the soil. They are thin-walled cells, thought to function as storage structures (Smith and Read, 1997), and occur only in the suborder Gigasporineae: *Gigaspora* and *Scutellospora* (Morton and Benny, 1990). Within *Gigaspora*, they form spiny or papillate structures, which are difficult to distinguish between species. Within *Scultellospora* they vary from smooth to knobby (Morton, 1997).

3.3 DESCRIPTIONS OF FUNGAL GENERA

AM fungal spores were identified to generic level on the basis of descriptions published by Walker (1983), Walker and Sanders (1986), Morton (1988), Schenck and Perez (1990), and Morton (1990, 1997). An abridged description of AM genera based on these sources is given below.

***Glomus* (Tul. & Tul.) Gerde. & Trappe**

Spores are borne terminally on one more hyphae, and vary in phenotypic properties and number of layers of the spore wall. Immature spores may stain weakly in Melzer's reagent. Spore contents are partitioned from the subtending hypha by various mechanisms, and germination generally takes the form of a germ tube emerging through the lumen of the subtending hypha (Morton, 1988). Spores generally form on a narrow or flared hypha (Schenck and Pérez, 1990).

***Acaulospora* (Gerde. & Trappe) Berch**

Spores are borne laterally on the neck of a sporiferous saccule (Morton, 1988), becoming sessile after detachment from this. Spore walls may be ornamented. Walls are often complex and may

comprise one or more inner wall layers, which may stain red or purple in reaction to Melzer's reagent. Spore germination arises from a germination shield (Morton, 1988).

***Gigaspora* (Gerde. & Trappe) Walker & Sanders**

Spores are borne terminally and individually on bulbous sporogenous cells (Morton, 1997) which have spore contents sealed off from them by an amorphous plug. The laminate layer is enclosed by a permanent outer layer. Wall layers are never darker than yellow in healthy spores (Morton, 1988), and flexible inner walls are never present. Ornamentations never occur. Germination arises from warty protuberances in the laminate layer of the spore wall. Auxiliary cells are generally echinulate or finely papillate (Walker and Sanders, 1986). Spores in the Gigasporaceae are generally obviously larger than those in the Glomaceae (Morton, 1988).

***Scutellospora* Walker & Sanders**

As with *Gigaspora*, spores are borne individually on a bulbous sporogenous cell, which is sealed off from the spore by an amorphous plug. A permanent outer layer, which may be smooth or ornamented, encloses the laminate layer of the spore wall. Spores are relatively larger than those in the Glomaceae. One to three bilayered flexible inner walls are always present and may stain red or purple with exposure to Melzer's reagent (Morton, 1988). A germination shield is always associated with the innermost flexible inner wall; this gives rise to germ tubes, which initiate germination. Auxiliary cells are smooth to knobby or broadly papillate (Walker and Sanders, 1986).

3.4 BIOASSAYS

Mycorrhizal associations are often studied by means of growth experiments to provide some measure of control over environmental conditions (Brundrett *et al.*, 1996). Spores, mycorrhiza-colonised root fragments and mycelia in the soil are able to function as mycorrhizal propagules (Read *et al.*, 1976; Brundrett *et al.*, 1991). Garrett (1956) defined inoculum potential as an

organism's energy for growth at the surface of its host, and stated that it is consequence of the numbers of active propagules of that organism and their nutritional status. Thus, the mycorrhizal inoculum potential of soils can be measured by growing host plants in soil cores to measure the occurrence of mycorrhizal colonisation in the host plants (Brundrett *et al.*, 1991, Abbott and Robson, 1991). In this study, soil cores were not used. Instead, sand from root zones of the same plant species, in the same region of the dune, was pooled. This was because the dune has no distinct soil profile, so disturbance to the soil structure was minimal in the loose sand. Bioassays were thus conducted, using this sand to measure the mycorrhizal inoculum potential within the foredune.

3.4.1 SAND TREATMENTS

Sand was collected from the *S. plumieri* foredune hummock and the *E. villosa / I. pes-caprae* dune, along the study site transect line, in July 1998. These were chosen since they were two very different foredune sites, one colonised by a single plant species, and the other by two (although *Ipomoea* was more dominant in this zone at the time). In each zone, several sand samples were taken and then pooled (i.e. all foredune samples pooled, and all dune slack samples pooled) in order to adequately represent the heterogeneity of each site (Brundrett *et al.*, 1996; Morton, 1997). Approximately 50L of sand was collected from each region. Half of the *Scaevola* and *Ipomoea* sand was placed into 2kg heat resistant bags, and autoclaved in a bench-top pressure cooker for 20 minutes at 220°C 15psi / kilopascals. This was repeated twice for each bag of sand in order to kill any mycorrhizal propagules. Thus, for the greenhouse experiments three sand treatments were used: 1) sand collected from the *S. plumieri* foredune, 2) sand collected from the *I. pes-caprae/E. villosa* dune, and 3) sterilised dune sand.

3.4.2 GREENHOUSE EXPERIMENTS

Sorghum was used as a host plant, since it has been shown to be mycorrhizal dependent (Brundrett *et al.*, 1996). As with most grasses, it has a fine fibrous root system which is able to infiltrate the

growth medium fairly rapidly, resulting in higher mycorrhizal formation (Struble and Skipper, 1988; Simpson and Daft, 1990). *Sorghum* seeds (cultivar NK 283) were rinsed thoroughly with water to remove any fungicides from their coats, and placed into a large conical flask containing water for 3-4 days. The water was aerated using a fish tank aerator. Once the seeds began to germinate, they were removed for planting.

Seeds were sterilised by placing them into 3.5% sodium hypochlorite for 15 minutes, and were then rinsed in sterile distilled water and planted into 2L pots. A flexible mesh was placed into the base of each pot to prevent loss of the fine dune sand. Five seeds were planted in each pot, and the treatments were kept separately from each other and from other plants in the greenhouse to avoid contamination. Plants were watered every two days from a sterilised watering can with a shower nozzle on the spout so as to avoid splashing and cross-contamination. Once a week from the second week onward, plants were watered with Long Ashton's nutrient solution (Hewitt, 1966) with the phosphorus content reduced to 10ppm. Plants were harvested after five weeks.

3.4.3 MEASUREMENTS

The plants were carefully removed from the pots to avoid any damage to the roots. The roots were untangled, straightened out and washed, and the root and shoot lengths were measured (cm). Shoots were placed (without weighing) into brown paper bags for drying. Drying was executed in a soils oven at 21°C for 2 weeks (Okalebo *et al.*, 1993).

Once as much sand as possible had been washed from the roots, they were weighed (f.wt). Half of each root system was then preserved in 50% ethanol until they could be stained for an assessment of mycorrhizal colonisation. The other half was weighed (f.wt) and placed into brown paper bags for drying. Once completely dried out, roots and shoots were weighed. Fresh weights from the complete root systems were converted to dry weights, using the formula:

$$\text{Total DW} = \text{Total FW} \times (\text{DW of Root}/\text{FW of Root})$$

Roots from the three replicate pots for each treatment were stained, and the five plants from each of those pots were assessed for the degree of mycorrhizal colonisation, using the methods described in Section 3.2.1 and 3.2.2. Particular note was made of the presence of hyphal entry points on the roots, since the assay was of short duration and it was not expected that extensive colonisation would have occurred yet.

3.5 PHOTOGRAPHY

Colour photographs and slides were taken at the study site at Old Woman's River to show the dune profile and plant communities. Colour slides and prints were taken of mounted root samples and spores, using an Olympus BX 50 light microscope with Nomarski interference, at the electron microscope unit, Rhodes University. A dark blue filter was used when photographing roots containing AM structures and a light blue filter was used when photographing spores in order to heighten contrasts. *Sorghum* plants were photographed in the greenhouse, and pictures were also taken of the plants once they had been harvested to show the appearance to roots and shoots.

3.6 STATISTICAL ANALYSIS OF DATA

Total percentage AM colonisation and spore numbers were summarised by calculating means and standard deviations. Differences between plant species and months were analysed using one-way analysis of variance (ANOVA), and multiple range analysis at 95% LSD. Root colonisation and spore count data are not normally distributed (Sylvia, 1986; St. John and Koske, 1988; Koske and Gemma, 1997) and were therefore transformed prior to analysis in order to stabilise the variance (Fowler and Cohen, 1990). Root colonisation values occurred as percentages, which are proportions. Arcsine transformation is appropriate for such data (Fowler and Cohen, 1990). Arcsine transformation involved firstly dividing observations (x) by 100, secondly obtaining the

square root of x, and finally calculating the inverse sine of x (i.e. the angle whose sine equals this value) (Fowler and Cohen, 1990). Spore count data was transformed by adding 1 to the abundance value (x+1) due to the occurrence of zero counts in the data, and taking the square root of the sum (Little and Hills, 1978; Fowler and Cohen, 1990; Koske and Gemma, 1997). Relationships between percentage root colonisation and spore numbers were determined using simple linear regressions. Depth data for *S. plumieri* was analysed using a two-sample t-test. For the bioassay root and shoot data, means and standard deviations were obtained. Differences between treatments were analysed with one-way ANOVA, and multiple range analysis at 95% LSD. The relationships between root and shoot data were determined using simple linear regressions. Since root and shoot data were not counts, but normally distributed measurements, data did not have to undergo transformations. A confidence level of 95% was used to determine statistical significance for all analyses. The computer programs used to perform statistical functions included Microsoft Excel 97 (Microsoft Corp., 1985-1996), Statistica 5.5 (StatSoft Inc., 1984-1999) and Statgraphics 7.0 (Statistical Graphics Corp., 1993).

CHAPTER 4

RESULTS

4.1 CLIMATIC DATA

Figure 4.1 shows total monthly rainfall and mean monthly temperature data for the Old Woman's River area for the period November 97 to December 98. Temperatures were highest in February and rainfall reached a maximum in August. Lowest rainfall was in December 97 and June 98, with lowest temperatures between June and August. Mean monthly temperatures ranged from 15-21°C, and total monthly rainfall from 7.5-53mm.

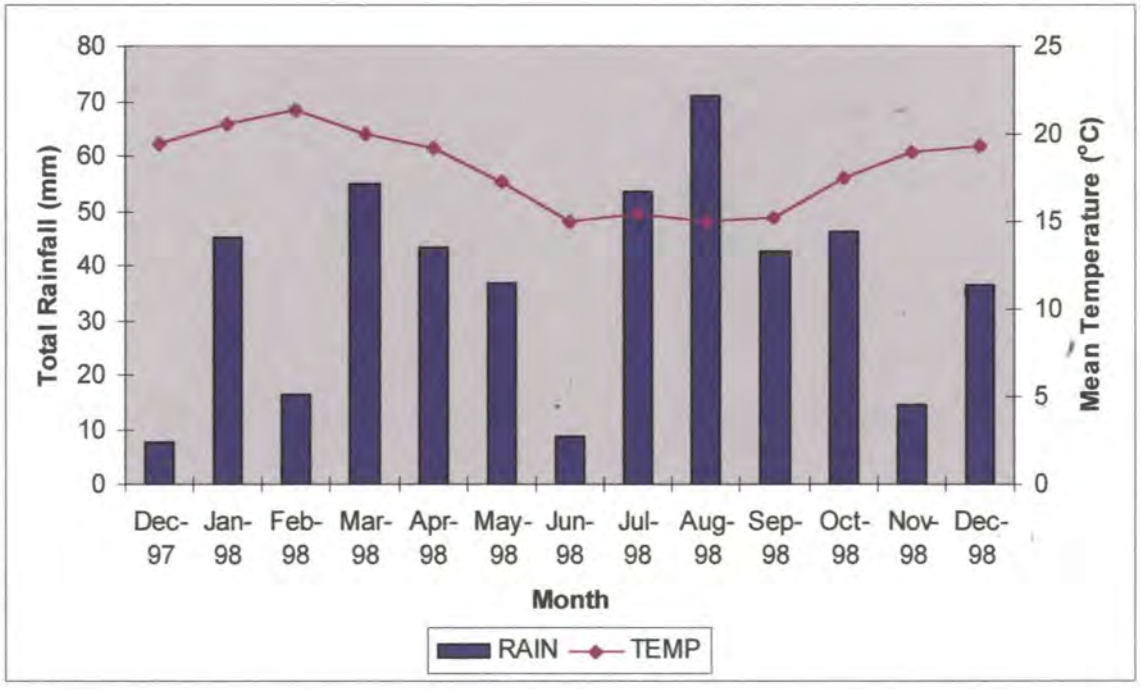


Figure 4.1 Total monthly rainfall (mm) and mean monthly temperature (°C) in the Old Woman's River area for the duration of the study period (South African Weather Bureau).

LABORATORY STUDIES

4.2 ASSESSMENT OF MYCORRHIZAL COLONISATION

Percentage colonisation ranged from 0% to 95.2% during the six sampling periods, and differed according to plant species and season. Figure 4.2 shows the mean percentage colonisation for each dune plant species over the six sampling times.

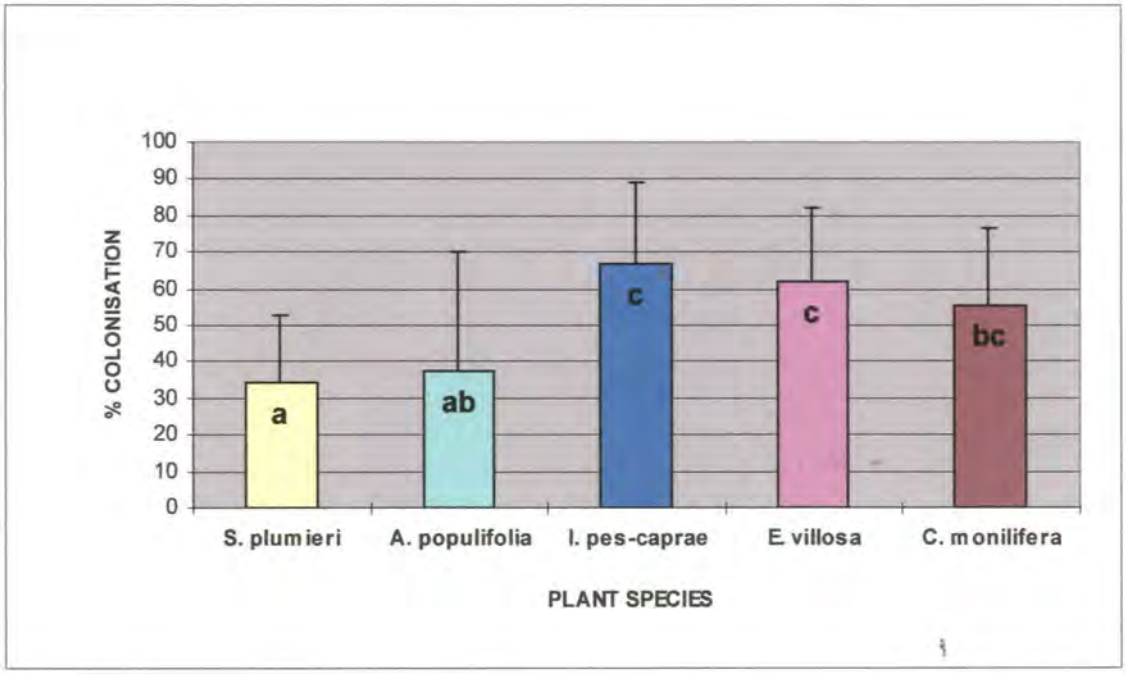


Figure 4.2 Mean annual percentage AM colonisation for each plant species (n=6). Lines on bars show (+) standard deviation. Bars sharing the same letter are not significantly different.

From Figure 4.2 it can be seen that *S. plumieri* and *A. populifolia* showed the lowest mean annual colonisation, though the latter had the highest standard deviation (32.85%). *I. pes-caprae* showed the highest mean annual percentage colonisations, followed closely by *E. villosa* and *C. monilifera.* One-way Analysis of Variance (ANOVA) indicated that, at $F_{4\ 118}=4.91$; $p \leq 0.001$, *S. plumieri* and *A. populifolia* have significantly lower annual colonisation levels. High standard deviations indicate

that seasonal trends are masked. Seedling roots were not included in the analysis due to the lack of a sufficient number of replicates.

4.2.1 SEASONALITY

An ANOVA of seasonal changes in colonisation in *S. plumieri* indicated a significant difference ($F_{5,17}$=7.19; p≤0.001). A multiple range analysis (MRA) showed that these differences were between February (lowest, at 4.3%) and December 98 (highest, at 61.4%) when compared with the other months sampled (Figure 4.3a).

A. populifolia plants showed lowest colonisation values, of 2.4% and 0%, in December 97 (mid-summer) and September 98 (spring) respectively (Figure 4.3b). Colonisation reached a maximum value of 83.6% in December 98. ANOVA showed that AM colonisation levels fluctuated significantly over the course of the year in this plant species ($F_{5,19}$=12.56; p≤0.0001).

I. pes-caprae consistently showed mean colonisation levels above 30%. Colonisation ranged from 35.2% in December 97 (mid-summer) to 95% in May (winter). Colonisation in December 97 was significantly lower than in all other months (Figure 4.3c) at a significance level of 0.001% ($F_{5,22}$=12.71).

E. villosa showed opposite trends to *I. pes-caprae,* decreasing to its lowest colonisation levels in winter (May; 38.8%), and reaching its highest levels in July (late winter), and then decreasing again in the summer months (Figure 4.3d). Significant differences ($F_{5,19}$=2,75; p≤0.049), occurred only between the months of December 97 and July, and May and July. Colonisation levels were always greater than 35%.

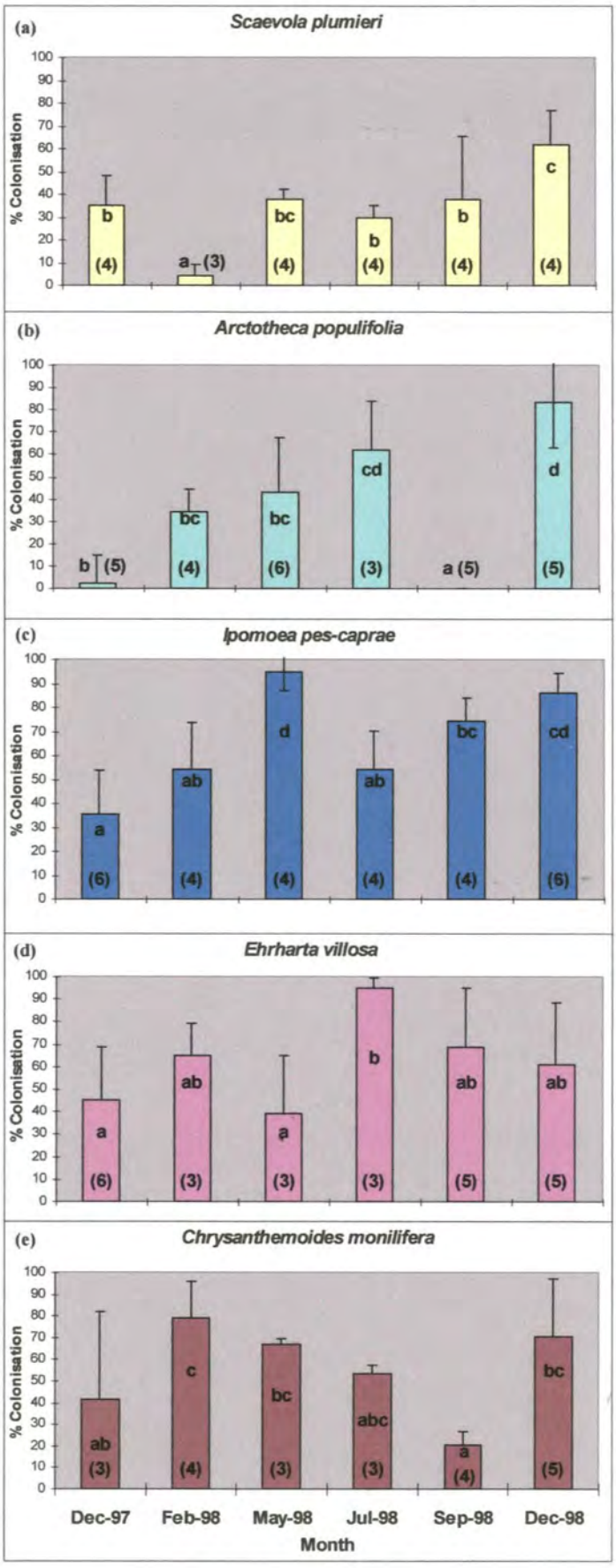


Figure 4.3 Monthly percentage AM colonisation for each plant species. Lines on bars show (+/-) standard deviation. Parentheses contain number of replicates (n). Bars sharing the same letters are not significantly different.

C. monilifera was the only species to reach a peak in mycorrhizal colonisation early in the year (78.7% in February), and then decrease through the autumn, winter and spring months, increasing again in December 98 (Figure 4.3e). Lowest colonisation was 20.6% in September (spring). There were no highly significant differences ($F_{5,16}$=3.10; p≤0.038) between AM colonisation throughout the year, except that in September values were significantly lower than those of February, May and December 98, and February had a significant increase in colonisation from December 97.

The combined mean colonisation of all plant species (Figure 4.4) was lowest in December 97 (31.86%) and highest in December 98 (72.56%). Mean percentage colonisation of all species combined did not differ significantly between months, except that December 98 had a significantly higher AM occurrence than all other months, and December 97 had significantly lower AM than May and July. These variations were significant at 0.01%, where $F_{5,116}$= 5.6; p≤0.0001.

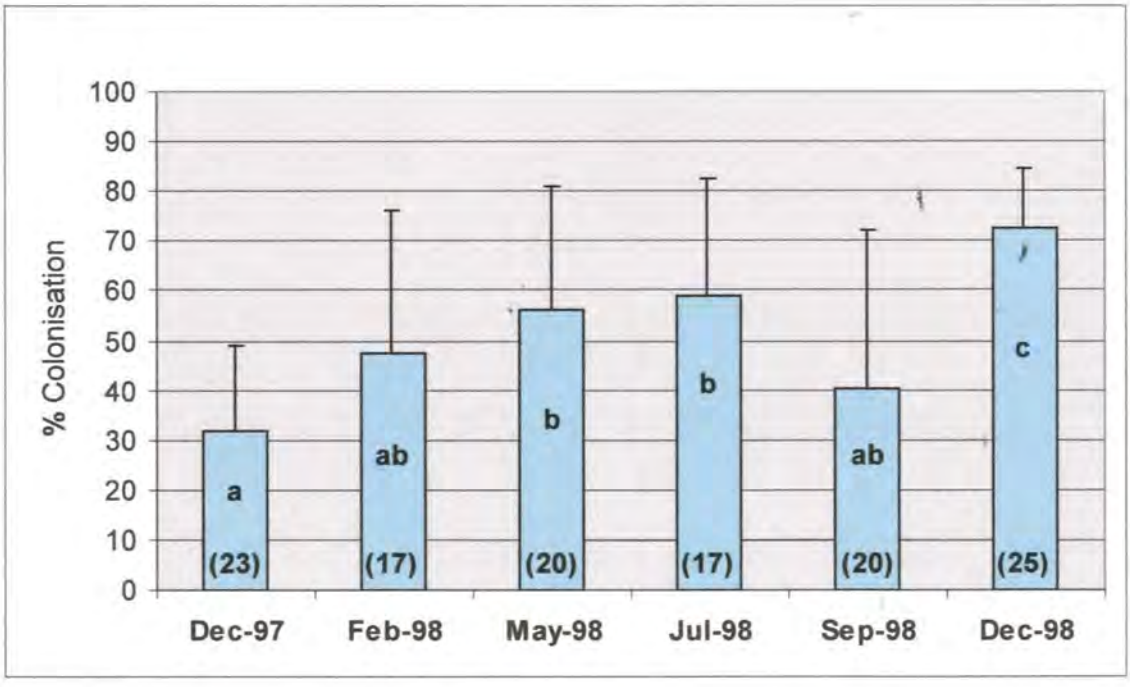


Figure 4.4 Percentage AM colonisation of all plants combined for each month sampled. Lines on bars show (+) standard deviation. Parentheses contain number of replicates (n). Bars sharing the same letters are not significantly different.

4.2.2 POSITION ALONG FOREDUNE

Plant species were plotted on the graphs in the successional sequence in which they occurred on the foredune (Figure 4.5 a-f). *S. plumieri* occurred on the foredune hummock nearest to the shore, I. *pes-caprae* and *E. villosa* on the blow-out behind the foredune hummock, *A. populifolia* in an otherwise unvegetated area of the blow-out, and *C. monilifera* at the start of the dune scrub (see Chapter 2).

ANOVA and multiple range analysis at a 95% LSD were used to determine whether significant differences occurred between the different plant species at each month sampled. There were no significant differences in colonisation between plant species in both December 97 and December 98. February ($F_{4,12}$=13.95; p≤0.0002), May ($F_{4,15}$=9.71; p≤0.0004), July ($F_{4,12}$=13.27; p≤0.0002) and September 1998 ($F_{4,15}$=14.77; p≤0.0001) did show significant variations between plant species, as shown in Figure 4.5 b-e.

In December 97, there were no significant differences between plant species. The lowest colonisation level was in *A. populifolia,* whilst the rest of the species showed very similar AM levels. February showed an increase in AM colonisation levels with increasing distance from the foreshore. *S. plumieri* had a significantly low colonisation (mean = 9.4%). Colonisation levelled out during May, with all species showing similar percentages, except for *I. pes-caprae*, which had a significantly higher AM occurrence. By July, *A. populifolia* and *E. villosa* had the highest mean percentage colonisations (of 52.4 and 79.6% respectively). *E. villosa* had a very small standard deviation, illustrating the fact that all roots observed had colonisation values greater than 90%. In September, the secondary colonisers, (*I. pes-caprae* and *E. villosa*) had the highest levels of AM fungi, while *A. populifolia* had 0% colonisation in the roots sampled. In December 98, colonisation between all plant species did not differ significantly.

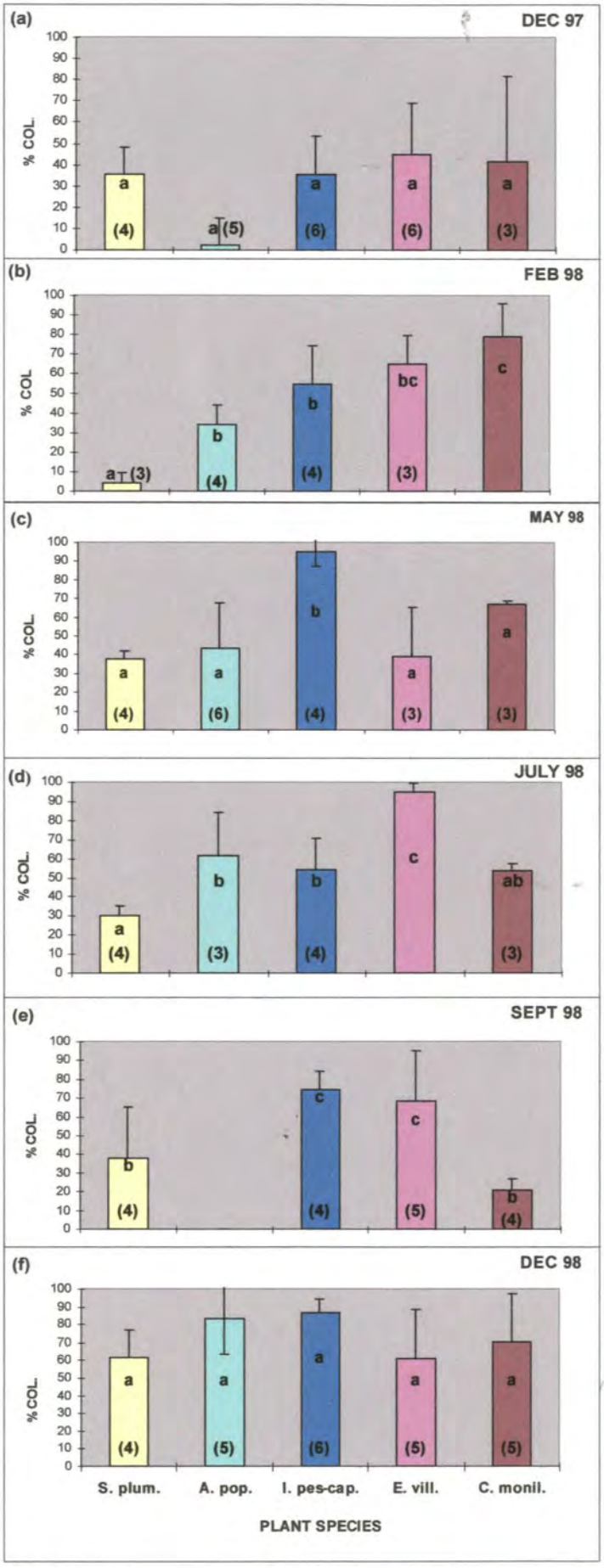


Figure 4.5 Percentage AM colonisation of each plant species at each sampling month. Lines on bars show (+/-) standard deviations. Parentheses contain number of replicates (n). Bars sharing the same letters are not significantly different.

4.2.3 MYCORRHIZAL STRUCTURES

The types of mycorrhizal structures intersecting with the eyepiece cross hair were also recorded (Chapter 3.2.2). These data were recorded as percentages of the total mycorrhizal colonisation shown for each root segment examined. Arbuscules were the mycorrhizal structures that occurred most frequently, and showed the greatest fluctuations, in all plant species. Vesicles and intraradical spores were most common in *I. pes-caprae* and *E. villosa*, occurring rarely in the other plant species. Hyphal coils had a very low occurrence in all species, as did appressoria. Extraradical spores were also observed and recorded, but since it was impossible to tell whether these originated from the roots or from the soil, they were not included in these results. Table 4.1 and 4.2 summarise the occurrence of appressoria and hyphal coils respectively. Figure 4.6 (a-e) shows trends in the occurrence of arbuscules, vesicles and intraradical spores for each plant species.

Table 4.1: Occurrence of appressoria, represented as a percentage of the total AM colonisation.

	PLANT SPECIES				
MONTH	*S. plumieri*	*A. populifolia*	*I. pes-caprae*	*E. villosa*	*C. monilifera*
DEC 97	9.4	0	8.4	15.5	1
FEB 98	0	0	5.4	0	12.5
MAY 98	0	0	2.2	0	0
JUL 98	0	22.9	0	0	0
SEP 98	4.2	0	9.8	13.4	0
DEC 98	0	0	0	0	4.4
Mean	**2.27**	**3.82**	**4.3**	**4.82**	**2.98**
StDev	3.88	9.35	4.23	7.49	4.96

I. pes-caprae had the most frequent occurrence of appressoria, with these being observed in all months except July and December 98. *C. monilifera* had appressoria during both Decembers, and February 98, though the percentages of these were fairly low. *S. plumieri* and *E. villosa* both had

these structures only during December 97 and September 98. The highest percentage of appressoria was observed in *A. populifolia*, which only had these at one sampling time, in July 98.

Table 4.2: Occurrence of hyphal coils, represented as a percentage of the total AM colonisation.

	PLANT SPECIES				
MONTH	*S. plumieri*	*A. populifolia*	*I. pes-caprae*	*E. villosa*	*C. monilifera*
DEC 97	2.1	0	0	13.2	1
FEB 98	0	0	0	0	10.2
MAY 98	0	0	1.2	0	0
JUL 98	3.6	3.9	0	0	0
SEP 98	3.1	0	5	0	0
DEC 98	0	0	0	0	0
Mean	**1.47**	**0.65**	**1.03**	**2.2**	**1.87**
StDev	1.68	1.6	2.0	5.39	4.10

Hyphal coils were recorded rarely, and when they were, their percentages were very low (never higher than 13.2%). None of the plant species recorded the occurrence of coils in December 98. *S. plumieri* had them most often, and *E. villosa* had the highest percentage of coils.

Arbuscules in *S. plumieri* occurred in 0% (Feb 98) to 100% (Dec. 98) of observations (Figure 4.6a). The highest occurrences of arbuscules were in May 98 (winter) and December 98 (summer). Vesicles and intraradical spores were rare in this plant (Figure 6a), both occurring in low percentages (vesicles 3.1%, intraradical spores 4.2%) in September (Plate 2D).

A. populifolia had similarly low percentages and occurrences of vesicles and intraradical spores (Figure 4.6b). Both occurred in July (winter), with percentages of 1.2 and 4.4% respectively. In December 98, 5.7% of mycorrhizal structures were intraradical spores. Arbuscules were present in May (82%), July (4.2%) and December 98 (51.2%).

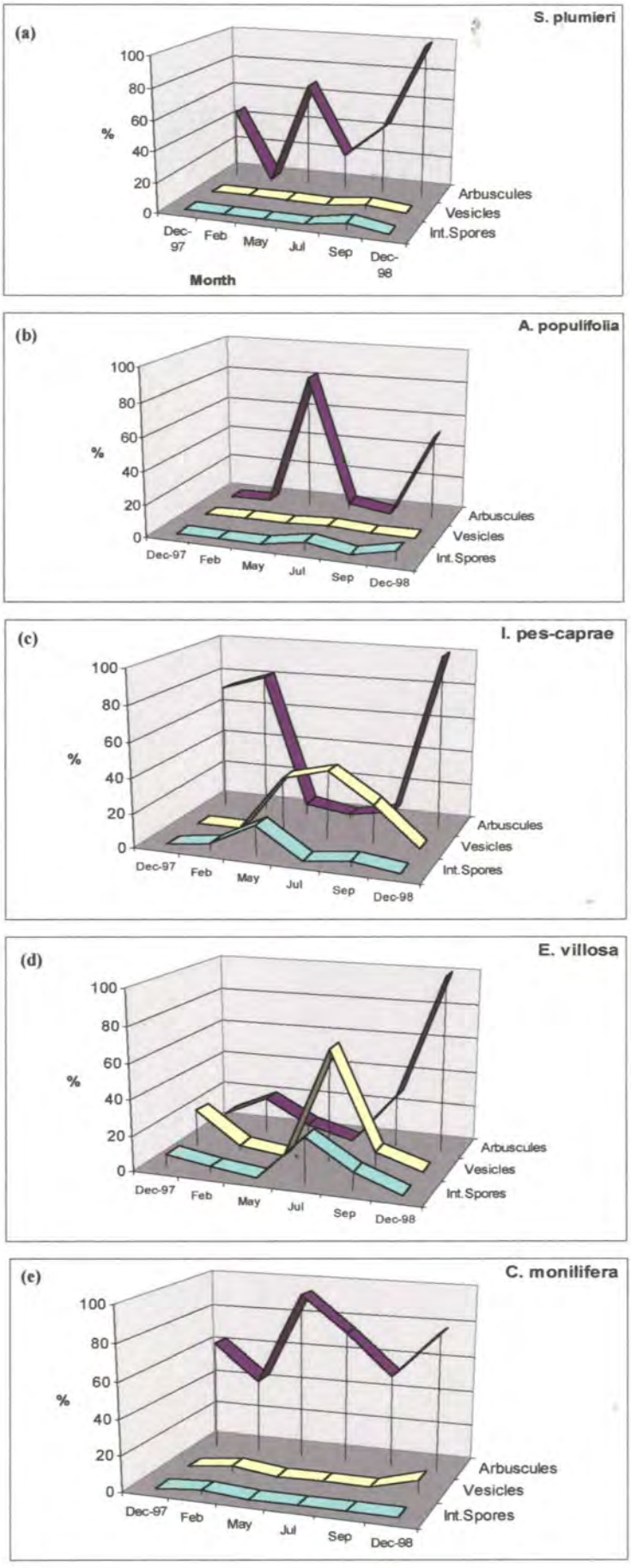


Figure 4.6 Graphs showing occurrence of arbuscules, vesicles and intraradical spores for each plant species. Data are represented as a percentage of total mycorrhizal colonisation.

I. pes-caprae had vesicles during the winter and late winter months (May to September), which accounted for 22-40% of the mycorrhizal structures observed (Plate 2C). Intraradical spores were observed in late summer, early winter (Feb, May) and again in spring (September). Arbuscules were observed throughout the year (Plates 3A and 3B), with the lowest level (2%) in July, and the highest (100%) in December 98 (Figure 4.6c).

E. villosa contained vesicles and intraradical spores during all months sampled, except May and December 98 (Figure 4.6d). In July these reached higher percentages than in any other plant species: 63.8% of mycorrhizal structures were vesicles, and 25.7% were intraradical spores (Plates 2A and 2B). Arbuscules occurred in all months except July and ranged from 7.2% in May to 100% in December 98.

C. monilifera had low percentages of vesicles: 3% and 7.1%, in February and December 98 respectively (Figure 4.6e). A very low number of intraradical spores (1.9%) occurred in February. Arbuscules were prevalent throughout the year, never falling below 42% of mycorrhizal structures. Highest arbuscule counts were in May (94%). In September 98, an echinulate auxiliary cell was found in association with *C. monilifera* roots. Auxiliary cells are only produced by members of the subfamily Gigasporineae (Morton, 1997). Cell surfaces of *Gigaspora* species are echinulate, or narrowly papillate, whilst those of *Scutellospora* are knobby to smooth (Walker and Sanders, 1986). Therefore the auxiliary cell observed was one of a *Gigaspora* species (Plate 3C). This was the only observation of an auxiliary cell.

PLATE 2

PLATE 2: VESICLES

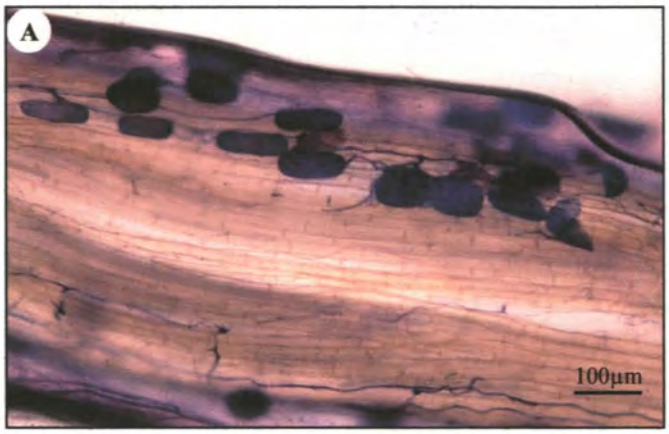
Figure A: Vesicles and AM fungal hyphae in a root of *Ehrharta villosa.*

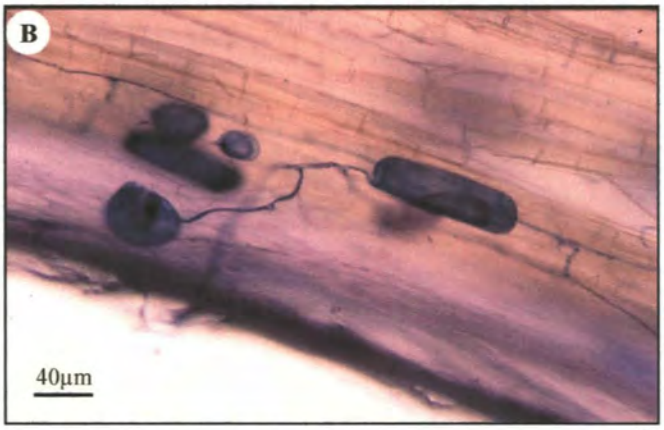
Figure B: Magnified view of *E. villosa* root showing vesicles linked by intraradical hyphae.

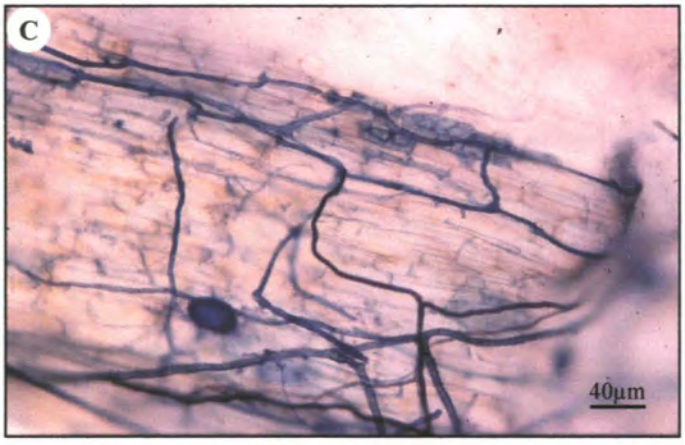
Figure C: AM hyphae and vesicle in an *Ipomoea pes-caprae* root.

Figure D: Vesicles within the root tissue of *Scaevola plumieri.*

PLATE 2







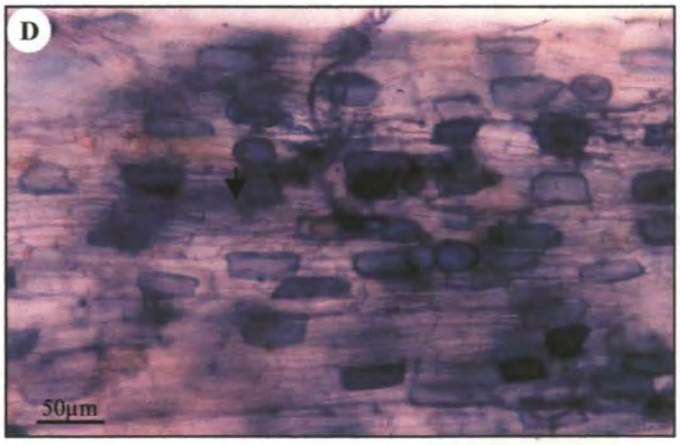


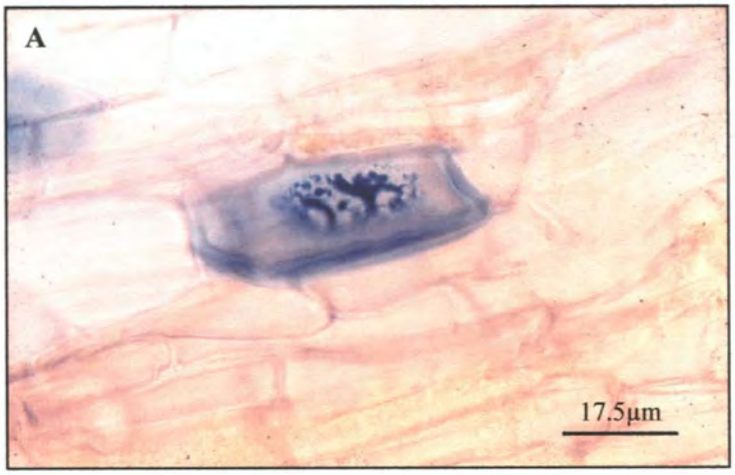
PLATE 3

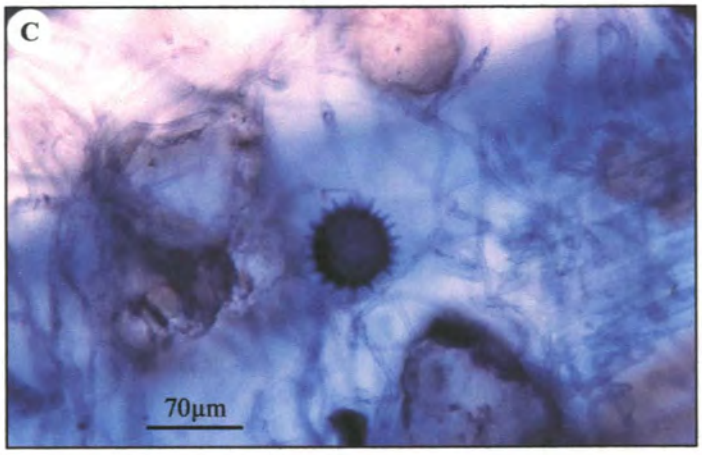
PLATE 3: ARBUSCULES and AUXILIARY CELL

Figures A & B: Arbuscules in the root cells of *I. pes-caprae.*

Figure C: Auxiliary cell extracted from rhizosphere sand of *C. monilifera.* The spiny or echinulate nature of the auxiliary cell indicates that it was produced by a *Gigaspora* species.

PLATE 3





4.2.4 DEPTH

The difference in percentage AM colonisation in *S. plumieri* roots collected at two depths from the same foredune in February 1998 is shown in Table 4.3. There is a 90% difference between the two, with the deeper roots exhibiting almost 100% colonisation levels. A two-sample t-test was conducted at a 95% confidence level with 6 degrees of freedom.

The computed t statistic was -7.24934 which indicated that a significant difference existed between the plant roots from different depths.

Table 4.3: Differences in percentage AM colonisation and types of mycorrhizal structures found in *S. plumieri* roots from different depths in February 1998.

Scaevola plumieri	**30cm Depth**	**100cm Depth**
Mean % AM Colonisation	4.3	91.3
Standard deviation	5.1	14
Arbuscules %	0	4.1
Vesicles %	0	45.7
Intraradical Spores %	0	28
Hyphal Coils %	0	58
Appressoria %	0	9.2

4.3 BIOASSAYS

4.3.1 ASSESSMENT OF AM COLONISATION

Out of 1 500 intersections of sterilised roots observed, 5 contained AM hyphae, giving a negligible mean colonisation value of 0.33% (SD=0.9%). This was a significantly lower AM colonisation (at $F_{2,42}$=121.6; $p \leq 0.0001$) than was found in roots grown in both *Scaevola* and *Ipomoea* sand. However, plants in all three sand treatments had significantly different percentage colonisations, the highest being in the *Scaevola* sand. These results are illustrated in Figure 4.7c.

4.3.2 SHOOT AND ROOT MEASUREMENTS

Figure 4.7a and 4.7b show the results of the *Sorghum* shoot and root lengths, and shoot and root dry weights respectively. Most of the plant biomass was in the roots, which had the greater length and dry weight values (Plate 4C). Mean shoot lengths ($F_{2,71}$=9.38; $p \leq 0.0002$) and dry weights ($F_{2,71}$=6.68; $p \leq 0.002$) in foredune hummock sand were significantly lower than in sand from the *Ipomoea* dune and the sterile treatment (Plate 4A). Absolute lengths were 4-5cm shorter in foredune hummock sand than in the other treatments (Figure 4.7a), though dry weights were only lower by 0.05g (Figure 4.7b). Mean root lengths only differed significantly between foredune hummock and sterile sand ($F_{2,71}$=6.67; $p \leq 0.002$), with roots from hummock sand having a lower mean by 12.8cm. Root dry weights were significantly higher in foredune hummock sand ($F_{2,71}$= 4.68; $p \leq 0.01$). For all treatments, standard deviations were highest in sterile sand measurements.

Plants grown in unsterilised dune sand had a healthy green appearance for the duration of the growth trial. The leaves of plants grown in sterilised sand began to turn purple after two and a half weeks' growth, and remained that way (Plate 4B).

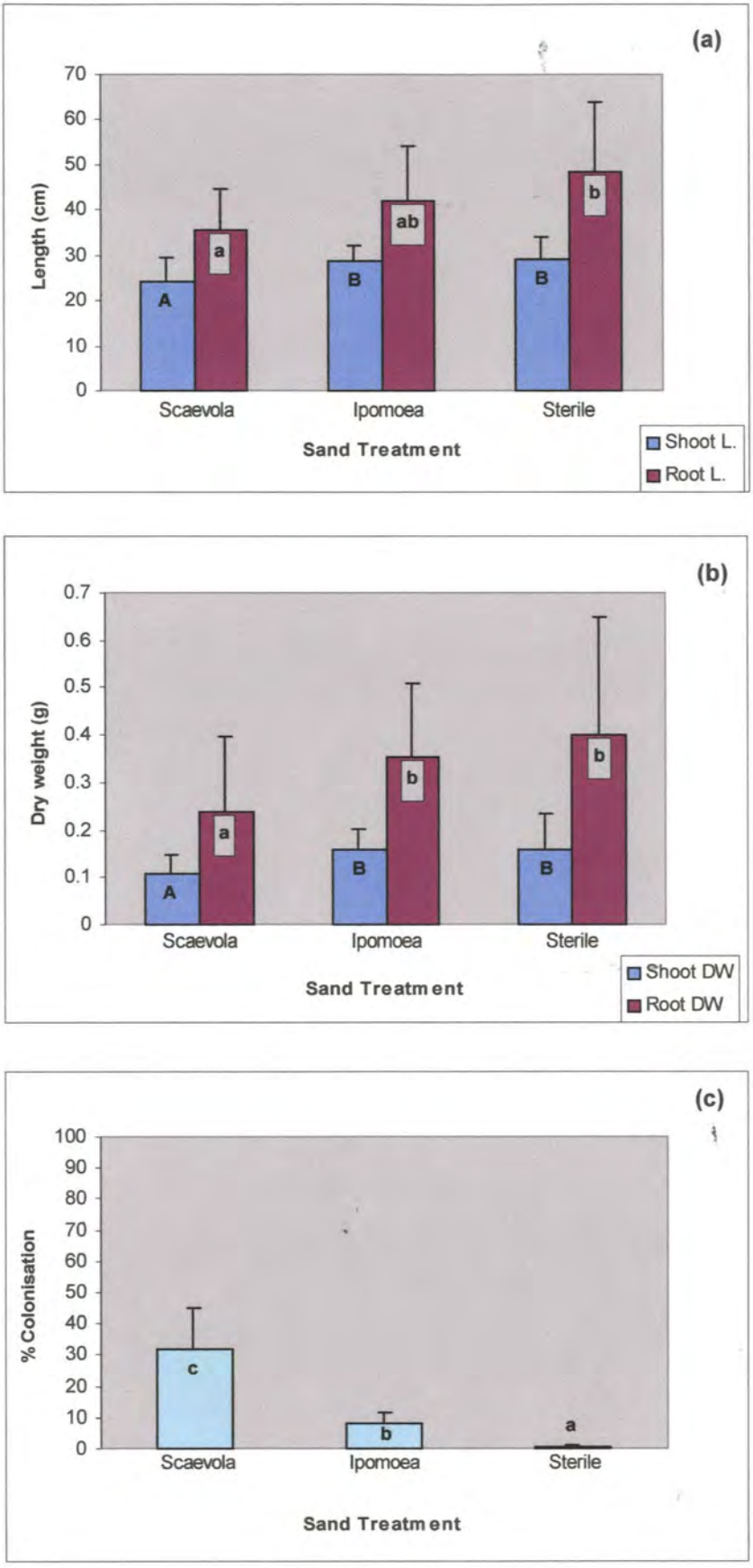


Figure 4.7 (a) Mean shoot and root lengths (n=25); (b) Mean shoot and root dry weights (n=25); (c) Mean percentage root colonisation (n=15) of *Sorghum* plants grown in three sand treatments. Lines show (+) standard deviations. Bars sharing the same letter are not significantly different.

Root to shoot ratios for each treatment were as follows:

Table 4.4 Root to shoot ratios of sorghum plants grown in different treatments.

TREATMENT	Root L : Shoot L (cm)	Root DW : Shoot DW (g)
S. plumieri Hummock	1.5 : 1	2.2 : 1
I. pes-caprae Blow-out	1.5 : 1	2.2 : 1
Sterile	1.7 : 1	2.5 : 1

4.3.3 CORRELATIONS

Simple linear regression analyses were run to determine whether any correlation existed between root and shoot measurements. No correlation existed between root lengths and shoot lengths, or between root dry weights and shoot dry weights. The highest correlation coefficient obtained was 0.68 (R-squared=46.67%) at a 0.1% probability level, between root and shoot dry weights of plants grown in *I. pes-caprae* sand.

PLATE 4

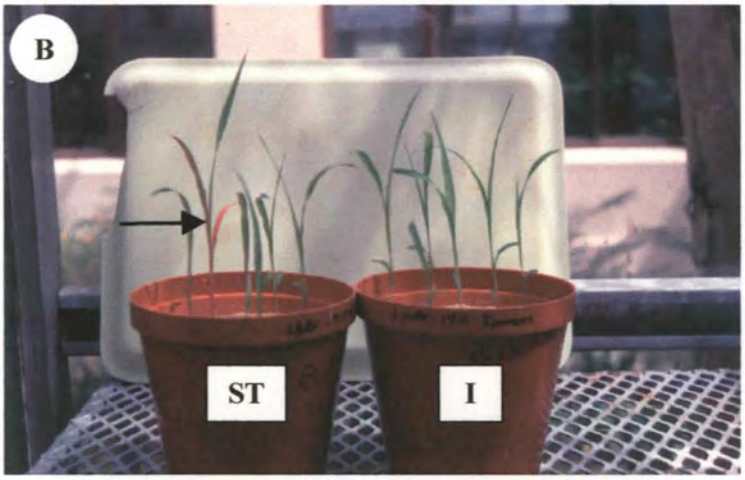
PLATE 4: *SORGHUM* PLANTS

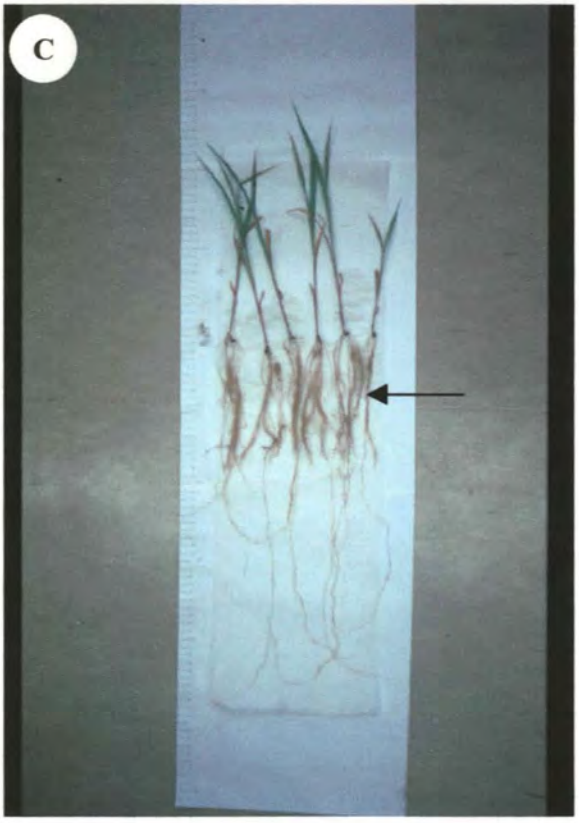
Figure A: *Sorghum* plants growing in the greenhouse. Differences in leaf production between plants grown in sterilised and non-sterilised sand can be clearly seen. Sterilised (ST) treatments had a much higher leaf biomass than plants in *Ipomoea* (I) or *Scaevola* (S) sand.

Figure B: Sterilised (ST) and *Ipomoea* (I) sand treatments after two and a half weeks' growth. Leaves of plants in sterilised sand are turning purple (arrow) indicating phosphorus depletion.

Figure C: Harvested *Sorghum* plants on a measuring sheet. Extensive lateral roots (arrow) permeated the plant pots, and roots were much longer and had a greater biomass than shoots.

PLATE 4





4.4 ENUMERATION OF SPORES

Spore numbers ranged from 0 to 48 spores $100g^{-1}$ sand with mean values of replicates ranging from 0.33 to 33.33 spores $100g^{-1}$ sand. Spore numbers did not differ significantly between the different plant root zones ($F_{4,85}$=4.0; $p \leq 0.005$), except those within the root zone of *I. pes-caprae* which were significantly lower than any of the others. The root zone of *S. plumieri* had the highest overall spore numbers, though not significantly so (Figure 4.8).

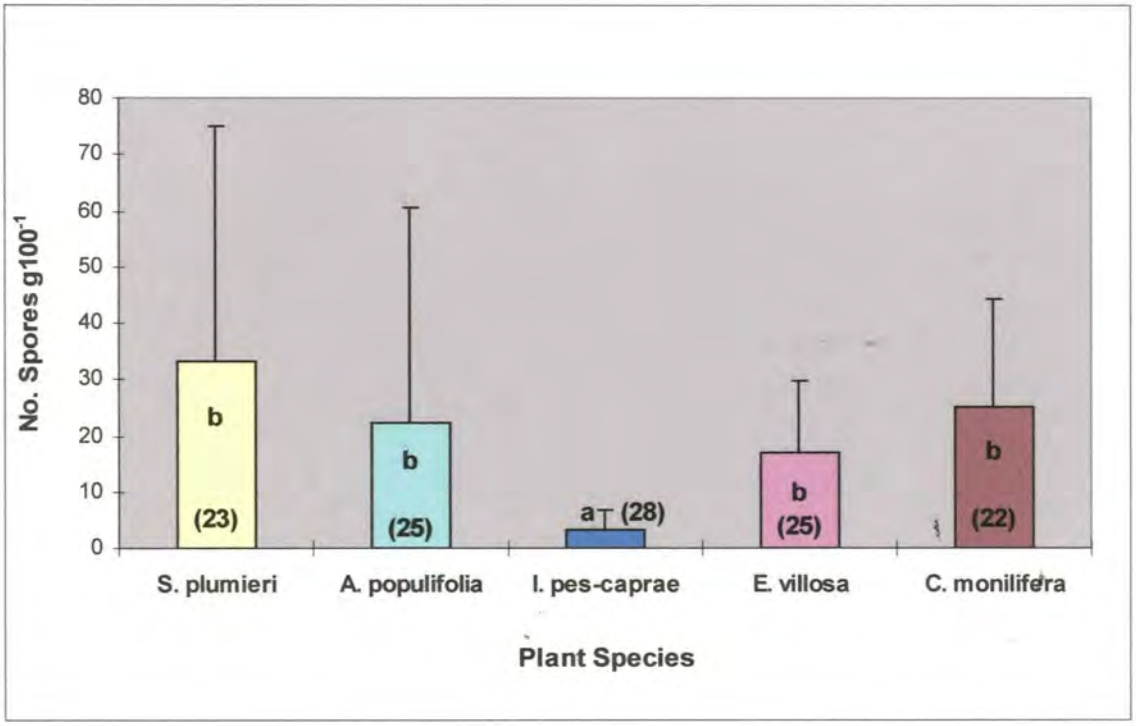


Figure 4.8 Mean annual spore numbers for each plant species. Lines on bars show (+) standard deviations. Parentheses contain number of replicates (n). Bars sharing the same letter are not significantly different.

4.4.1 SEASONALITY

Figure 4.9 shows trends in the spore numbers for each plant species.

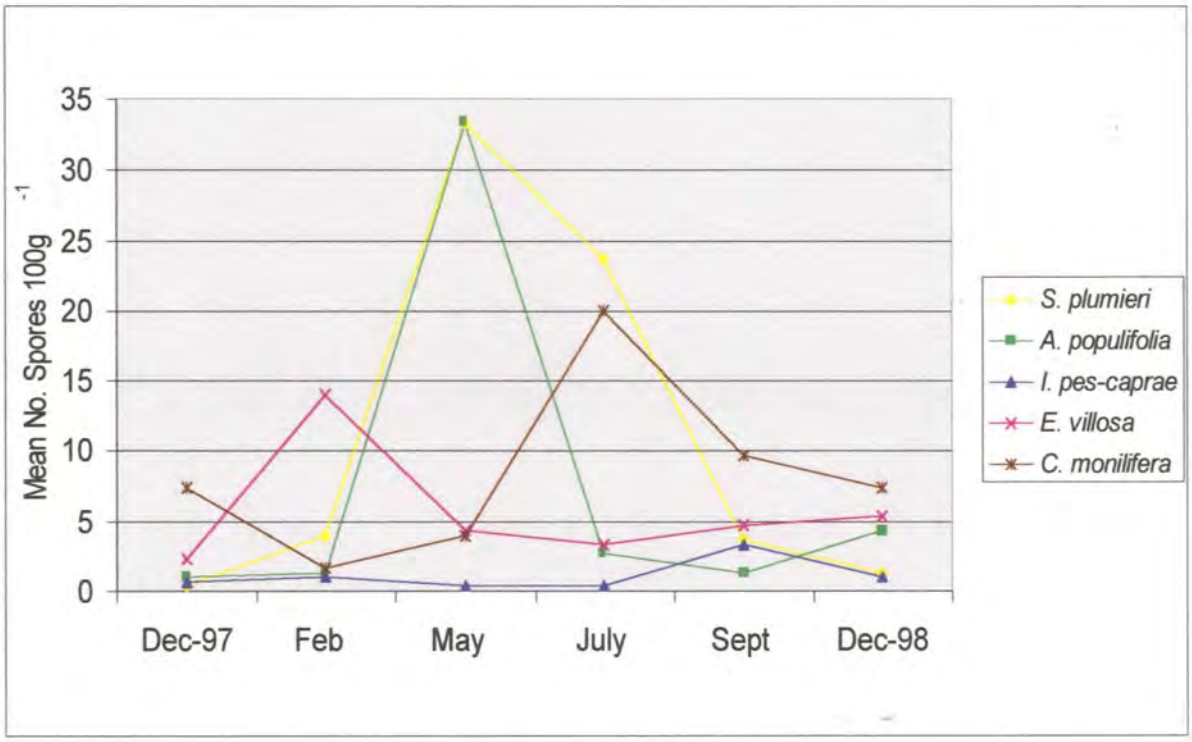


Figure 4.9 Trends in mean monthly spore numbers for each plant species.

The total number of spores extracted from all plant root zones combined was significantly higher in May than in all other months, except July ($F_{5,84}$=3.57; $p \leq 0.0056$). The only other significant difference in total spore numbers was between December 97 (lowest) and July (Figure 4.9). These results are shown in the table below:

Table 4.5 Results of multiple range analysis, showing differences in total spore numbers between months. NS = no significant difference; * = significant difference.

Spore Numbers $F_{5,84}=3.57$; $p\leq0.0056$	Feb. 98	May 98	July 98	Sept. 98	Dec. 98
Dec. 97	**NS**	*	*	**NS**	**NS**
Feb. 98		*	**NS**	**NS**	**NS**
May 98			**NS**	*	*
July 98				**NS**	**NS**
Sept. 98					**NS**

Spore numbers for each plant species reached a peak once in the course of the year, generally close to winter time (May through July). December (summer), both in 1997 and 1998, had the lowest overall spore numbers, followed by September (spring) and then February (late summer). Highest spore numbers were associated with *S. plumieri* and *A. populifolia* root zones in May. One-way ANOVA and MRA showed that *S. plumieri* was associated with significantly higher spore numbers in May and July than in other months. These results are summarised in the table below.

Table 4.6 Results of multiple range analysis, showing differences in spore numbers of *S. plumieri* between months. NS = no significant difference; * = significant difference.

S. plumieri $F_{5,12}=15.51$; $p\leq0.0001$	Feb. 98	May 98	July 98	Sept. 98	Dec. 98
Dec. 97	**NS**	*	*	**NS**	*
Feb. 98		*	*	**NS**	**NS**
May 98			**NS**	*	*
July 98				*	*
Sept. 98					**NS**

A. populifolia root zones had significantly higher spore numbers in May than in other months ($F_{5,12}$=19.68; p≤0.001). No significant differences in spore numbers were exhibited between the other five sampling times.

The root zone of *I. pes-caprae* had significantly higher spore numbers in September than in other months ($F_{5,12}$=4.16; p≤0.02). All other sampling times had similarly low spore numbers. This plant species was associated with the lowest spore numbers for the whole year, as compared to the other plants. Numbers were never higher than 4 spores $100g^{-1}$.

E. villosa was associated with significantly high numbers of spores in February ($F_{5,12}$=4.90; p≤0.01), as compared to other months. This peak in spore numbers occurred just before winter, a trend not shared by any of the other plant species.

C. monilifera had a highest association of spores in July, towards the end of winter. Other differences in spore numbers between months are shown in the table below.

Table 4.7 Results of multiple range analysis, showing differences in spore numbers associated with *C. monilifera* between months. NS = no significant difference; * = significant difference.

C. monilifera $F_{5,12}$=10.98; p≤0.0004	Feb. 98	May 98	July 98	Sept. 98	Dec. 98
Dec. 97	*	**NS**	*	**NS**	**NS**
Feb. 98		**NS**	*	*	*
May 98			*	*	**NS**
July 98				*	*
Sept. 98					**NS**

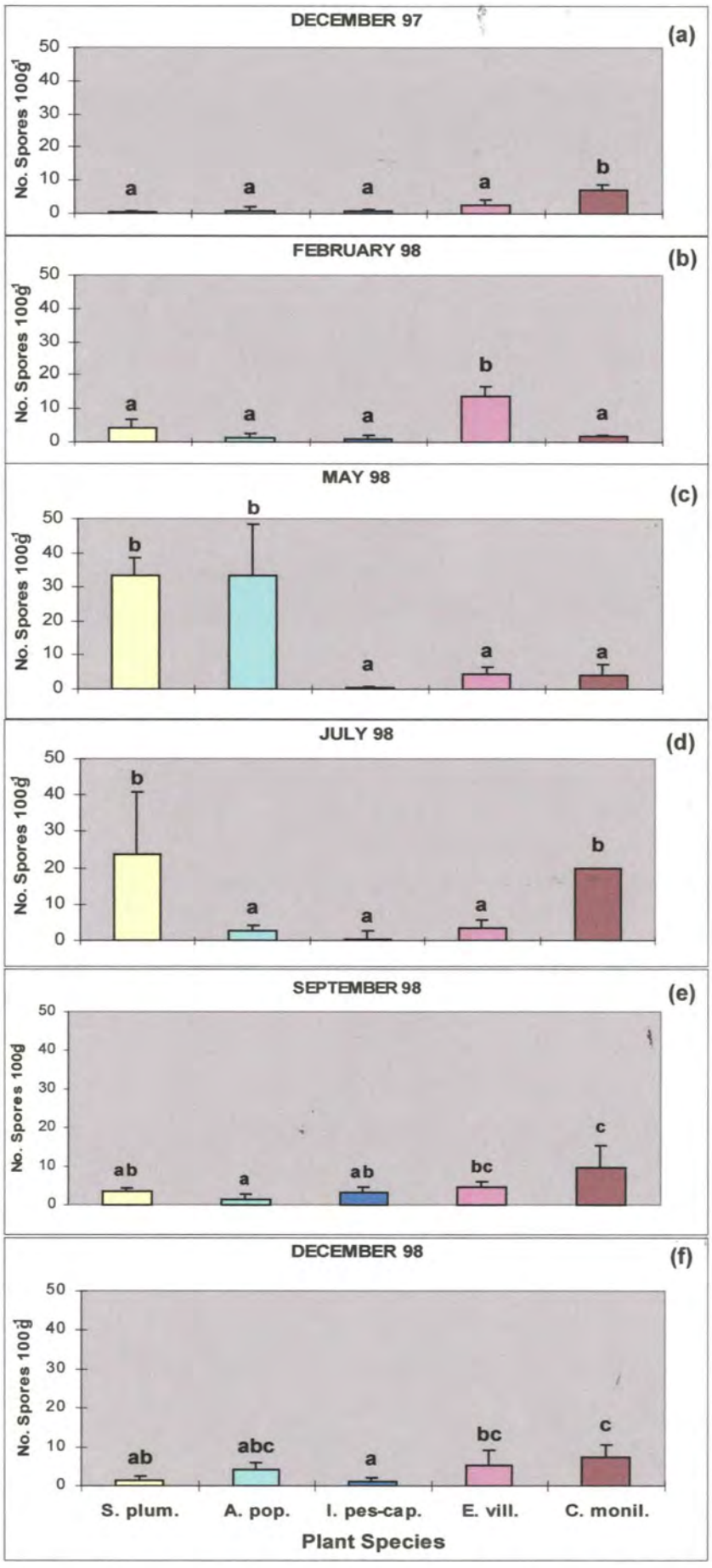


Figure 4.10 Mean monthly spore numbers for each plant species. Lines on bars show (+) standard deviations. Number of replicates = 3 for all samples. Bars sharing the same letters are not significantly different.

4.4.2 POSITION ALONG FOREDUNE

In Figure 4.10 (a-f) plant species are positioned in the same sequence as they were found on the foredune profile. ANOVA and Multiple range analysis at a 95% LSD were used to determine whether significant differences occurred between spore numbers in the root zones of different plant species at each month sampled. Due to the fact that spore numbers were very low in all root zones, trends were not too apparent. However, it did seem that higher spore numbers were associated with the primary foredune colonisers, *S. plumieri* and *A. populifolia,* particularly in May ($F_{4,10}$=26.44; $p \leq 0.0001$) and July ($F_{4,10}$=10.74; $p \leq 0.001$), and in *C. monilifera* the species occurring furthest from the shore, at the start of the more stable scrub area of the dune. In most months, *I. pes-caprae*, the species most prominent in the most mobile zone, had significantly lower spore numbers.

4.4.3 CORRELATIONS OF SPORE NUMBERS WITH COLONISATION LEVELS

Simple linear regressions were performed between spore numbers and percentage colonisation for each month sampled. Results showed no correlation between these measurements.

4.5 IDENTIFICATION OF SPORES

Four genera of AM spores were identified: *Glomus, Acaulospora, Gigaspora* and *Scutellospora.* As a result of the difficulties encountered due to the varying conditions and ages of field spores, it was not attempted to identify these to species level, although in some cases a tentative identification was possible. Morphological differences were used to separate the spores from the 90 soil samples into 15 unidentified or tentatively identified species groups. Each of these groups is described below. All nomenclature for tentative identifications follows the recommendations of Walker and Trappe (1993). In each description, the first number in parentheses (n) refers to the number of spores of that type examined. Colour codes follow those of Brundrett *et al.* (1996). All size measurements are given as minimum to maximum ranges, with the average immediately following in parentheses.

4.5.1 *Glomus* (Tul. & Tul.) Gerde. & Trappe

Five types of *Glomus* spores were isolated and separated according to morphological features. This was an extremely difficult group to try to separate due to the occurrence of very similar morphologies. The groups isolated do overlap in some of their characteristics due to the varying ages and conditions of field spores.

OWR 01

(n=12). Spores were formed on a single undifferentiated hypha. They were brown (0-60-10) to orange-brown (0-80-40) or dark red-brown (20-100-80) in colour. Spore size ranged from 113.7 – 320.1 (208.5)µm. (Plate 5A and C).

Subtending Hypha:

This was generally cylindrical with a width of 7.3 – 14.5 (9.2)µm. Layers 1 and 2 of the spore wall continued into the hyphal attachment.

Wall Structure:

Three spore wall layers were always observed with a fourth sometimes seen. Layers were different colours, and very adherent, even when the spore was broken, making observation of wall layers difficult. Debris was often attached to the outer spore wall, giving it a mucilaginous appearance. The outer wall was hyaline and 0.6–1.3 (0.95)µm thick. A wall layer was occasionally seen beneath this when the spore was sufficiently broken. This may either have been a layer of the outer wall, or of the laminate layer. It appeared as a thin, bright orange layer 0.6–1.2 (0.9)µm wide. L2 was the laminate layer, generally a brown colour and easily discernible on all spores. Measurements of this were 2.7–13.6 (6.7)µm. The third wall layer (L3) had a thickness of 0.8–2 (1.25)µm, and was pale brown to bright orange in colour.

Tentative Identification

These spores fit the description of *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann. The exact resolution of the wall layers, however, was difficult and so does not fit the description exactly.

OWR 02

(n=21). Spores occurred either singly or aggregated into clumps of 5/6. Colour ranged from yellow-brown (0-70-30) to red-brown (0-60-40) in Melzer's reagent. These were some of the smallest spores observed from the study site, with sizes of 40.4 -135 (101)µm. (Plate 5B).

Subtending Hypha:

These were cylindrical in shape, but were often not visible due to sloughing of the outer spore layer. Width of the hyphae was 4.3–9.8 (7.3)µm. Spore L1 and L2 continued into the subtending hypha.

Wall Structure:

Three wall layers were evident, though in some instances it looked as though there may have been two laminate layers. However, this was not observed often, and it was therefore taken that only three layers were present. The spore wall outer layer (L1) measured 0.3 – 1.9 (0.8)µm, and was generally hyaline in appearance. L2 was the laminate layer and measured 2 – 9.7 (3.6)µm. L3 often had the appearance of comprising two layers, but these did not separate easily, and together had a thickness of 0.4 – 2.2 (1.3)µm.

Tentative Identification

Spores were tentatively designated to *Glomus intraradices* Schenck & Smith.

OWR 03

(n=26). Spores appeared to be borne singly on an undifferentiated hypha. Colours ranged from yellow (0-30-10) to yellow-brown (0-70-30) and brown (20-70-60). Sizes varied considerably, 68.6 – 269.5 (156.5)µm, and it is thought this may have been a result of differing ages of spores. Spores tended to become darker and began to slough their outer layers as they aged.

Subtending Hypha:

Spore wall layers 1 and 2 were continuous with the subtending hypha, which was generally cylindrical, sometimes slightly flared, and measured 4.8 – 7.3 (7)µm.

Wall Structure:

Spore wall comprised 3 layers. L1 was the outer layer; this appeared hyaline and was 0.6 - 2 (0.9)µm thick. L2 was the laminate layer and measured 2 – 8.7 (4.4)µm. L3 was fairly thin, 0.3 - 2 (1.1)µm, and sometimes difficult to see.

OWR 04

(n=17). Spore colour varied considerably, from pale brown (0-50-40), through yellow-brown (0-100-20), orange-brown (20-10-60) and brown (20-70-60). Spore size ranged from 95.6 – 261.9 (148.2)µm. (Plate 5D).

Subtending Hypha:

Cylindrical to slightly flared at the site of attachment, 5.6 – 7.4µm wide, with layers 1 and 2 of the spore continuous with the hypha.

Wall Structure:

The spore wall seemed to comprise 3 layers, though it was often difficult to be sure, since these were tightly adherent, and spores did not break easily. L1, the outer spore layer hyaline and smooth, with a width of 0.8 - 2 (1.3)µm. L2 was the laminate layer, measuring 2 – 9.7 (5.2)µm. L3 was 0.8 – 7.3 (1.9)µm, and looked as though it might comprise two layers.

OWR 05

(n=28). These were the most frequently observed *Glomus* spores. When mounted, colours ranged from yellow-gold (0-60-10) to orange-yellow (0-70-40) in PVLG, and orange-brown (0-50-60) to brown (0-50-30) in Melzer's. Spore sizes were 125.8 - 397 (253)µm. (Plate 5E and F).

Subtending Hypha:

The subtending hyphae of this group were distinctive in that they were flared to funnel shaped at the site of the attachment, with L1 and L2 of the spore continuing into this attachment in a funnel shape characteristic of *Glomus* species. The flared attachment then narrowed into the rest of the subtending hypha. This group was at first confused with *Scutellospora*, but the spores did not have as bulbous

an attachment as is found in this group. The flared portion of the attachment measured 24.2 – 43.6 (33.2)µm, narrowing to 9.7 – 14.5 (12.2)µm at normal hyphal width.

Wall Structure:

Three layers of the spore wall were apparent, though occasionally there seemed to be an extra layer above the laminate layer. L1 appeared mucilaginous and was often sloughing from the spore; it measured 0.6 – 1.8 (1)µm. The layer sometimes seen below this measured 1.2 – 1.4 (1.35)µm, and may have been a layer of either L1 or L2. L2 comprised the laminate layer, and had a thickness of 3.8 – 14.7 (9.1)µm. L3 measured 1 - 4 (1.7)µm and was often difficult to see.

Tentative Identification

Spores fit the description of *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. The spores from OWR, however, were much larger than those described by Morton (1997).

4.5.2 *Acaulospora* (Gerde. & Trappe) Berch

Two types of *Acaulospora* spores were identified, though the majority fell into the first group.

OWR 06

(n=30). Spores were formed singly in the soil, and were yellow-straw (0-30-10) in colour. Spores were globose and 115-300 (167.7)µm in size. (Plate 6A and B).

Wall Structure:

The Spore Wall comprised two layers.

L1: Outer wall (W1) was 0.7-1.8 (1.12)µm, hyaline, smooth and rigid.

L2: Laminate Layer: 1.2-7.35 (5.3)µm. This was a darker layer with concave depressions 0.8-1.3 (1.04)µm deep and 2-4.9 (2.5)µm wide.

Flexible Inner Walls: Three were visible. It was not possible to determine the number of layers comprising each wall.

IW1: Inner Wall One measured 0.6-1.8 (1.08)µm. It was very closely adherent to the laminate layer of the spore wall, and difficult to separate from this, even when the spore was broken.

IW2: measured 1-4 (2.02)µm, and was thus somewhat thicker than IW1. On some occasions it looked as though it may have been composed of 2 layers. This wall stained a pale pink in Melzer's Reagent. Very adherent to IW3.

IW3: The third inner wall was 0.8-3.6 (1.38)µm thick, with a granular appearance. Stained a dark rose-pink in Melzer's (0-20-20).

Cicatrix: often clearly visible, 9.7-14.5 (12.9)µm in diameter.

Tentative Identification

These spores fit the description of *Acaulospora scrobiculata* Trappe.

OWR 07

(n=8). Spores were borne singly in the soil. They were yellow (0-20-10 to 0-30-10) in colour and measured 101.6-183 (130.8)µm. Spore shape was globose to sub-globose.

Wall Structure:

The Spore Wall comprised two layers.

L1: Outer Wall. 0.8-1 (0.9)µm. Often mucilaginous with debris attached, and sloughing, making the thickness difficult to measure. The spores were also very difficult to break, making any further observations of the walls very difficult.

L2: Laminate Layer. 4-5 (4.7)µm thick.

Flexible Inner Walls: These were only visible on one spore, and the dimensions were: IW1: 2µm, IW2: 1µm, IW3: 2.5µm.

4.5.3 *Gigaspora* (Gerde. & Trappe) Walker & Sanders

Four groups of *Gigaspora* were isolated.

OWR 08

(n=3). Spores were borne singly in the soil, and occurred terminally on a bulbous sporogenous cell. Colour was hyaline (0-20-10) to pale yellow (0-30-0). Spore dimensions were 373.5-533 (463.8)µm.

Sporogenous Cell: These were 60.5µm long and 46µm wide.

Wall Structure:

Three layers of the spore wall were apparent, the first two being adherent.

L1 was a rigid, permanent outer layer, measuring 1-1.2 (1.1)µm. L2 was a laminate layer consisting of 2-3 sublayers, which together measured 5-7 (5)µm. L3 was a thin (0.8µm) layer, which was often difficult to see.

OWR 09

(n=14). Spores were borne singly in the soil, on the end of a bulbous sporogenous cell. Spore colours ranged from yellow-gold (0-20-10) to yellow brown (0-100-40), with dimensions of 164.2-388 (288)µm. (Plate 7C and D).

Sporogenous Cell: the yellow suspensor cells were 34.3-56.9 (47)µm long and 25.4-48.4 (34.3)µm wide. They were always present.

Wall Structure:

The spore wall comprised three layers. L1 was very adherent to L2, and these did not separate easily, even when the spore was broken. L3 was less adherent, but very thin and hyaline. The outer spore layer (L1) was hyaline to pale yellow, and 0.7-4µm thick. It looked as though it might comprise two sublayers. L1 tended to slough as the spores aged. L2 was the laminate layer, yellow in colour and 5-12.3µm thick. L3 appeared to be a bilayer, 0.3-2.5µm thick.

OWR 10

(n=10). Spores were borne terminally on a bulbous sporogenous cell and occurred singly in the soil. Colours ranged from yellow-gold (0-40-20) to yellow-pink (0-30-10) and yellow-brown in Melzer's reagent. Spores measured 155-329.8 (177.8)µm. (Plate 7A).

Sporogenous Cell: Yellow in colour with lengths of 49-58.8µm (54.7) and widths of 36.8-46.6 (41.8)µm.

Wall Structure:

Spore wall comprised three layers, which separated fairly easily when the spore was broken. L1 measured 0.7-0.8 (0.78)µm and was hyaline in colour. L2 was adherent to this and made up the laminate layer, 3.2-4.2µm wide. L3 had dimensions of 0.7-1.8 (1.2)µm, and was pale yellow in colour.

OWR 11

(n=10). Spores were borne singly in the soil at the end of a bulbous sporogenous cell. Spore colour was a clear yellow-gold (0-20-10) which stained a dark red-brown in Melzer's. Size range was 210.7-305 (257.8)µm. (Plate 7B).

Sporogenous Cell: This was pale yellow in colour, 43.7-60µm long and 29.1-46.5µm wide.

Wall Structure:

The spore wall was made up of three layers. L1 was fairly thick, 0.8-2.5 (1.5)µm, hyaline, smooth, and a clear yellow colour, even in Melzer's. L2 was the laminate layer with dimensions of 2-7.4 (5.1)µm. L3 was a thin and pale layer, 1-1.8 (1.3)µm thick.

Tentative Identification

Spores were thought to fit the description of *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe.

4.5.4 *Scutellospora* Walker & Sanders

Four *Scutellospora* groupings were made. In all groupings, the flexible inner walls were generally clearly discernible from the spore wall layers. However it was often difficult to distinguish between the different flexible inner walls. Germination shields were occasionally observed on the innermost flexible inner walls, but these were difficult to resolve and measure, so are not included in the descriptions.

OWR 12

(n=19). Spores were borne terminally on a sporogenous cell, and occurred singly in the soil. Colour was yellow-gold (0-30-0 / 0-20-10 / 0-50-10). Inner walls stained pink in Melzer's reagent (0-20-10 / 0-30-20 / 0-30-30 / 0-50-30). Spores were mainly globose and measured 90.7-470 (373)µm. (Plate 8A-C).

Sporogenous Cell: Length was 38.8-84 (64)µm, and width 41.7-71.2 (49.6)µm. Colour was pale yellow.

Wall Structure:

Spore wall comprised 2 layers that were very closely adherent and did not separate easily.

L1: Outer wall. Rigid, smooth and hyaline, measuring 0.8-1.2 (0.92)µm. No reaction to Melzer's reagent.

L2: was the laminate layer and had dimensions of 2.5-9.8 (6.5)µm. No reaction to Melzer's. A thin wall seemed to occur beneath the laminate layer, and was closely adherent to it.

Flexible Inner Walls: Two walls were formed, each bilayered.

IW1: Comprised two layers, closely adherent both to the laminate layer of the spore wall, and to each other. L1 was 0.3-1.2 (0.7)µm thick, and L2 approximately 1.2µm thick. Both layers together measured 1.0-3.1 (1.8)µm. This wall stained pale pink in Melzer's reagent (0-20-10).

IW2: Bilayers were fairly adherent, but separated easily from IW1 when the spore was broken. L1 measured 1-2.8 (2.1)µm, and L2 0.3-1 (0.8)µm. Both layers together had dimensions of 2.48-4.2 (2.9)µm. This wall stained a darker pink in Melzer's (0-20-20 to 0-50-30). L2 had a granular appearance.

Tentative Identification

Spores fit the description of *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders.

OWR 13

(n=14). Spores were borne terminally on a sporogenous cell, and occurred singly in the soil. Colours ranged from yellow-brown (0-70-30) to brown (20-70-60). Spores were globose and 210-320.1 (268.3)µm in size. (Plate 8D).

Sporogenous Cell: Most were not present, though sometimes remnants of the suspensor cell were observed. Therefore, these spores were often to distinguish from spores of OWR 05. However, on

the occasions when the sporogenous cell was observed, it was undoubtedly consistent with those of *Scutellospora*. Thus, cell lengths were approximately 61µm, and widths approximately 53µm.

Wall Structure:

Spore Wall: Comprised 2 layers.

L1: The outer spore wall, which was hyaline, and sometimes had a mucilaginous appearance. Dimensions were 0.8-1.8 (1.08)µm.

L2: The laminate layer, which was very thick, 9.7-17.2 (13.6)µm and gave most of the structural integrity to the spore. Sublayers were very apparent.

Flexible Inner Walls: Three walls were observed, although these did not separate easily, and therefore were not always evident. It was not possible to tell whether any of the walls were made up of more than one layer. All walls together measured 3.6-16.4 (6.3)µm.

IW1: Very adherent to the laminate layer, measuring 0.5-1.2 (0.86)µm. No reaction in Melzer's reagent.

IW2: Only clearly seen once, and measured 14.7µm. No reaction in Melzer's.

IW3: Dimensions were 1.2-2.5 (2.05)µm. No reaction to Melzer's.

OWR 14

(n=7). Spores were borne terminally on a sporogenous cell, and occurred singly in the soil. Colours were red-brown (20-60-20 to 0-50-60) and did not change colour in Melzer's reagent. Spore sizes were 215-388 (309.9)µm.

Wall Structure:

Spore Wall: made up of two layers which were fairly closely adherent.

L1: The outer wall, which was generally hyaline, smooth and rigid, approximately 1.2μm thick. In some cases, this layer had begun to slough, and looked much thicker (up to 4.5μm).

L2: The laminate layer. This was a very thick, robust layer 6.1-19.6μm wide. Generally brown.

Flexible Inner Walls: These were evident in all spores examined, but they did not separate easily, and so could only be measured twice. Three FIW's were present, none exhibited any reaction to Melzer's reagent.

IW1: A bilayer which was closely adherent to the spore wall. Measured 2-4.2μm.

IW2: A bilayer, though it was often difficult to see both layers. L1 measured 1-2.5μm. L2 was 0.8-1μm.

OWR 15

(n=2). Spores were borne terminally on a sporogenous cell, and occurred singly in the soil. Spores were a very hyaline colour (10-20-0) and measured 83.3-213.4μm. (Plate 8E).

Sporogenous Cell: Pale hyaline colour, and 4.9μm long.

Wall Structure:

Spore Wall: Comprised two very adherent layers.

L1: The outer spore wall was made up of two sublayers, measuring 0.8μm and 2μm respectively.

L2: The laminate layer did not separate easily from the outer wall. Dimensions were 2.5-4.9μm.

Flexible Inner Walls: Only one FIW was observed. This occurred as a bilayer 1-1.2μm thick. Other FIWs may have been present but not observed, but due to the low replicate number of these spores, this could not be resolved.

PLATE 5

PLATE 5: ***GLOMUS***

Figure A: An aggregation of spores of isolate OWR 01 (tentatively identified as *Glomus caledonium*).

Figure B: Spore of isolate OWR 02 (tentatively identified as *Glomus intraradices*) showing hyphal attachment (H/A). These were some of the smallest spores extracted from the dune sand and wall layers were often difficult to resolve.

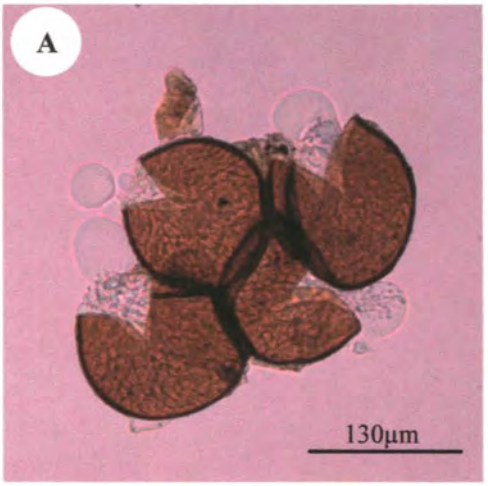
Figure C: Higher magnification of isolate OWR 01. Spore wall layers are visible. Layer one (L1) is the outer wall and layer two (L2) the laminate layer. The third layer has become separated (L3) and has a hyaline appearance.

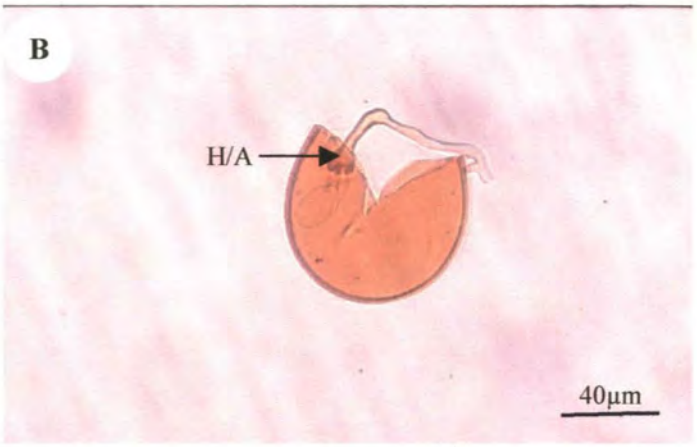
Figure D: Spore of isolate OWR 04 showing continuation of spore walls into hyphal attachment (H/A). Spore wall layer one (L1) was the outer wall, L2 was the laminate layer, and L3 often had the appearance of a bilayer but did not separate easily from the laminate layer.

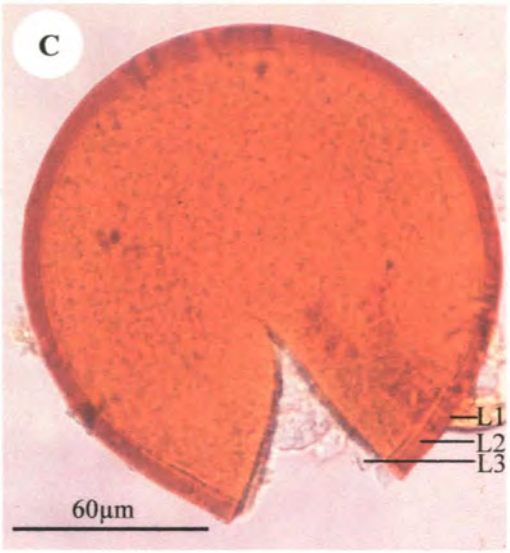
Figure E: Spore isolate OWR 05 (tentatively identified as *Glomus mosseae*). The large size of these spores and the flared hyphal attachment (H/A) made them very distinctive.

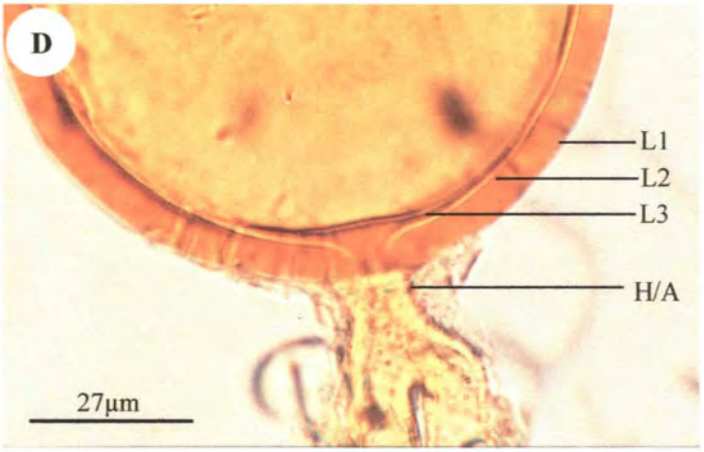
Figure F: Hyphal attachment and pore of OWR 05. Layers one (L1) and two (L2) of the spore wall extend into the subtending hypha.

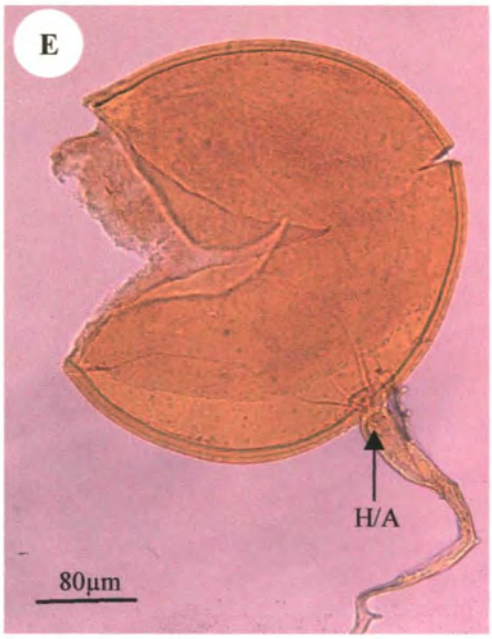
PLATE 5


A
130µm


B
H/A
40µm


C
L1
L2
L3
60µm


D
L1
L2
L3
H/A
27µm


E
H/A
80µm

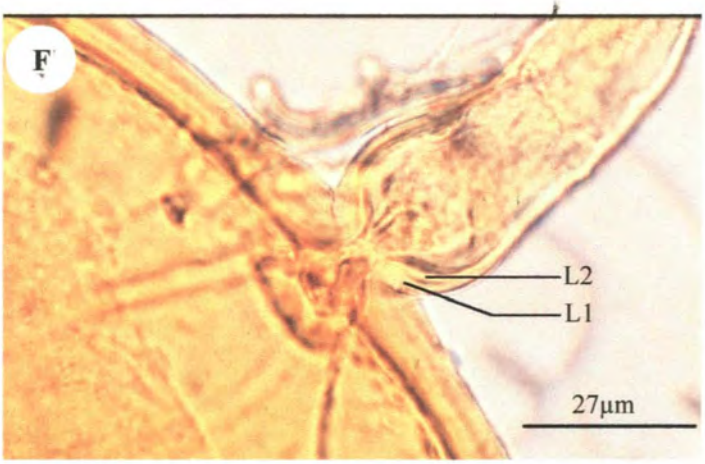

F
L2
L1
27µm

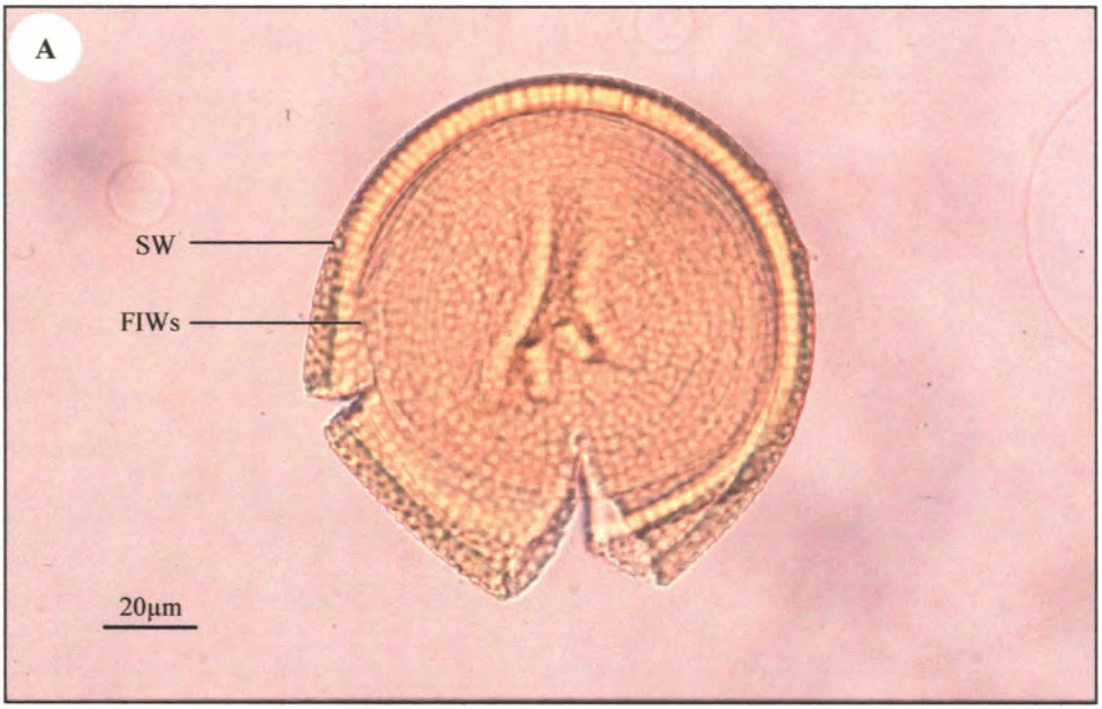
PLATE 6

PLATE 6: *ACAULOSPORA*

Figure A: Isolate OWR 06 (tentatively identified as *Acaulospora scrobiculata*). The two wall groups have separated due to breakage of the spore wall (SW). The flexible inner walls (FIWs) have stained a pink colour in Melzer's Reagent.

Figure B: Magnified view of wall groups of OWR 06. The laminate layer (L2) occurs below the outer wall (L1). Three flexible inner walls are visible: IW1 is tightly adherent to the laminate layer, but IW2 and IW3 separate easily.

PLATE 6



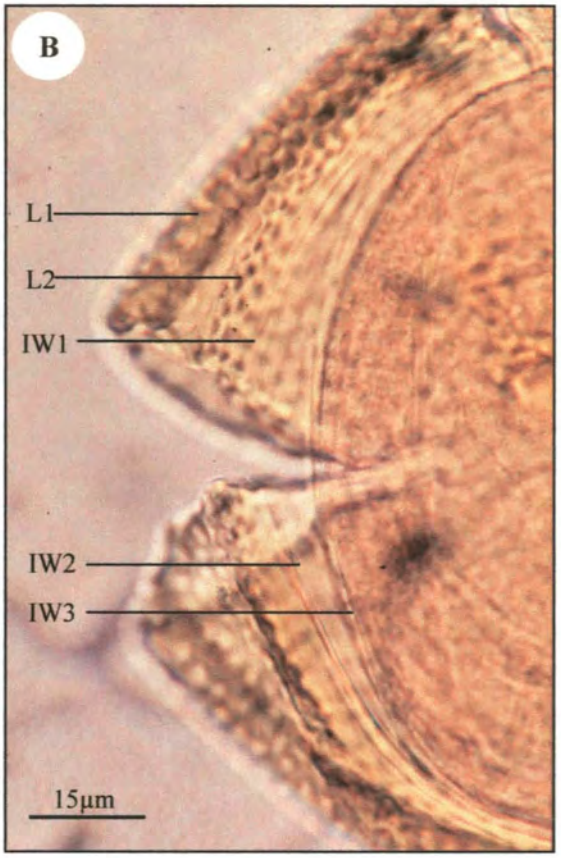


PLATE 7

PLATE 7: ***GIGASPORA***

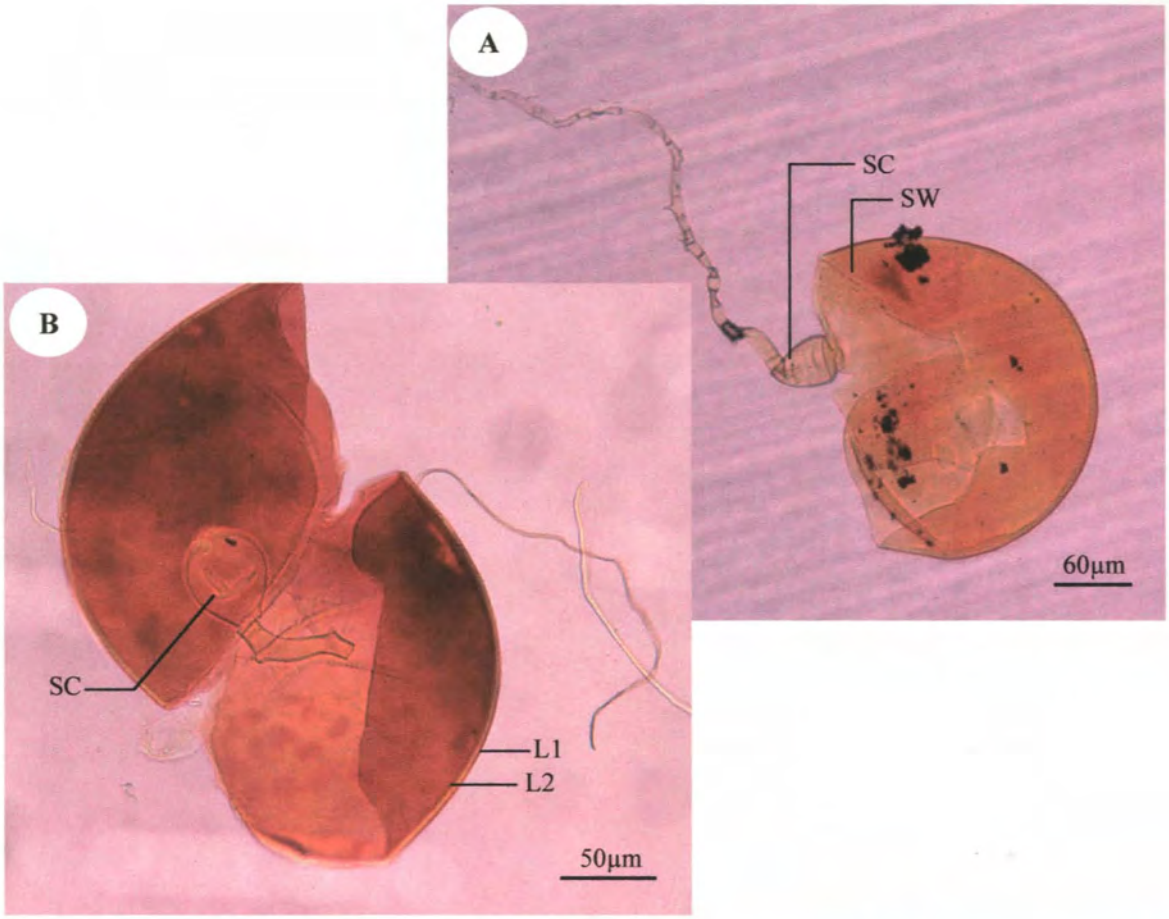
Figure A: Isolate OWR 10. Spore wall layers (SW) stained yellow-brown in Melzer's Reagent. The sporogenous cell (SC) is still attached to the spore. Septations can be seen on the subtending hypha. It is thought that these form as hyphal contents retreat after spore production (Hayman, 1983; Smith and Read, 1997).

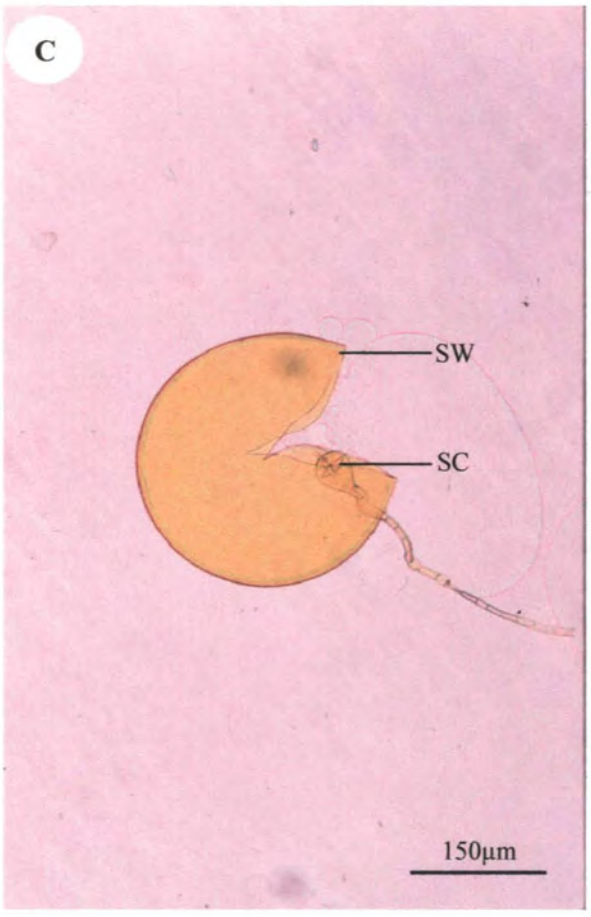
Figure B: Isolate OWR 11 (tentatively identified as *Gigaspora gigantea*). The first spore layer (L1) did not change colour in Melzer's, but L2 stained dark red-brown.

Figure C: Isolate OWR 09. Spores did not react in Melzer's and spore wall (SW) layers were tightly adherent. The sporogenous cell (SC) can be seen attached to the spore.

Figure D: Magnification of the wall layers of OWR 09. L1 is the spore wall. Laminations can be clearly seen in L2. L3 did not separate easily from the laminate layer.

PLATE 7





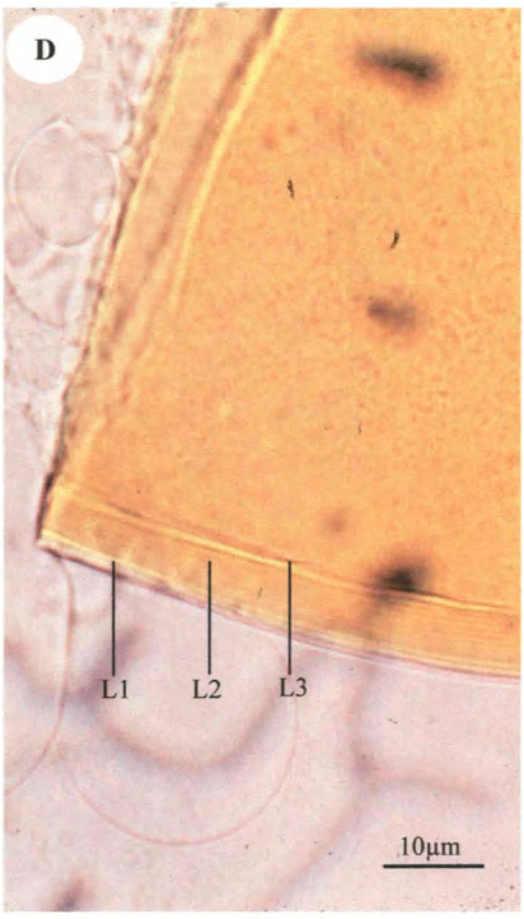


PLATE 8

PLATE 8: ***SCUTELLOSPORA***

Figure A: Isolate OWR 12 (tentatively identified as *Scutellospora calospora*). Spore wall layers (L1 & L2) have separated from the flexible inner walls (FIWs). Spore wall layers had no reaction to Melzer's; IW1 stained pale pink and IW2 and IW3 stained a darker pink. SC = sporogenous cell.

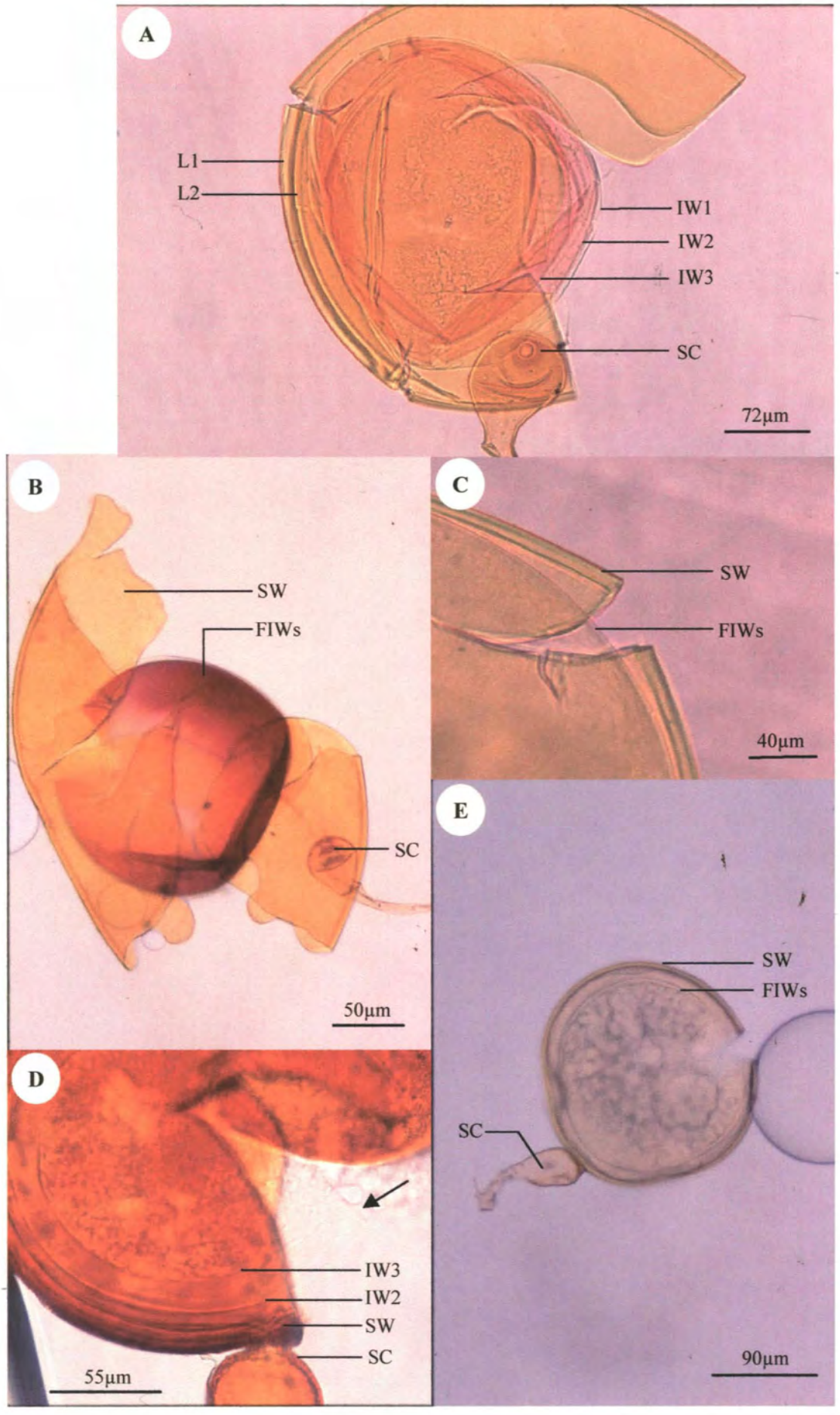
Figure B: Another spore of isolate OWR 12. FIWs had a stronger reaction to Melzer's in this spore.

Figure C: Unstained walls of OWR 12. SW = spore walls; FIWs = flexible inner walls.

Figure D: Isolate OWR 13. The spore wall layers (SW) can be seen continuing into the sporogenous cell (SC). IW1(unlabelled) is difficult to see; IW2 is very clear and extends out to the arrow; IW3 has a granular appearance. These spores showed no reaction to Melzer's.

Figure E: Isolate OWR 15 showing wall groups and sporogenous cell (SC). SW = spore walls; FIWs = flexible inner walls.

PLATE 8


A
L1
L2
IW1
IW2
IW3
SC
72µm
B
SW
FIWs
SC
50µm
C
SW
FIWs
40µm
D
IW3
IW2
SW
SC
55µm
E
SW
FIWs
SC
90µm

4.6 DISTRIBUTION AND OCCURRENCE OF FUNGAL GENERA

The occurrence of each of the four fungal genera differed according to plant species, position along the foredune profile and month. This is illustrated in Figure 4.11 (a-f). The graphs show the number and types of fungal genera associated with the root zones of each plant species, for each sampling time. Numbers of spores are given as $300g^{-1}$, due to the fact that overall spore numbers were low (Section 4.4). It was thus decided to present the numbers for spores extracted from all soil replicates, since representing mean numbers tended to mask the presence of some genera, and produced numbers too low for adequate comparisons to be made.

Although the numbers of different spore types were fairly low (0-93 spores $300g^{-1}$), there were obvious fluctuations in their associations with plant root zones. However, due to the low numbers, these data were not analysed statistically. Table 4.8 shows the total numbers of spores for each genus at each sampling time.

Table 4.8 Number of spores from each fungal genus extracted from the dune soil.

Month	*Glomus*	*Acaulospora*	*Scutellospora*	*Gigaspora*
December 97	20	11	4	0
February	18	4	15	28
May	59	20	105	42
July	45	16	83	6
September	44	13	11	0
December 98	37	15	3	3
TOTAL	**223**	**79**	**221**	**79**
Mean	37.2	13.2	36.8	13.2
StDev	15.8	5.4	45	17.6

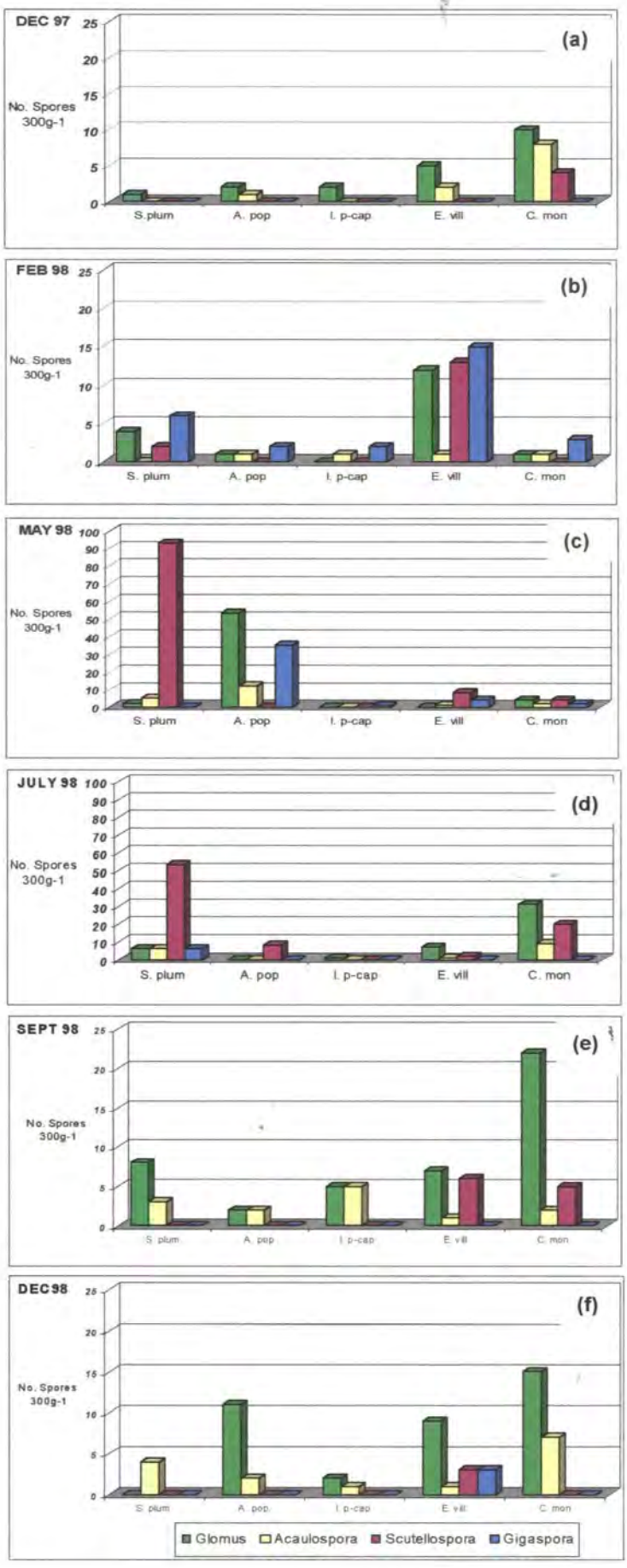


Figure 4.11 The distribution of fungal genera in the root zone of each plant species for each sampling period. (Note that y axis scales differ in graphs c and d).

In December 97 (Figure 4.11a), *C. monilifera* had the highest numbers (also see Figure 4.10a) and types of fungal spores associated with it. *Glomus, Acaulospora* and *Scutellospora* occurred in this zone, and this was the only region in which *Scutellospora* was identified in this month. Spore diversity and numbers increased with distance from the foreshore. In February (Figure 4.11b), *E. villosa* was the plant species associated with greatest spore diversity and numbers, although the occurrence of *Acaulospora* was much lower than other spore types. *S. plumieri* and *A. populifolia* were associated with greatest spore numbers in May (winter) (Figure 4.11c). The very high number of *Scutellospora* propagules with *S. plumieri* at this time was notable as was the lack of substantial numbers of other fungal genera associated with this plant (Figure 4.11c). *A. populifolia* had relatively high numbers of *Glomus, Acaulospora* and *Gigaspora* propagules. The highest numbers of *Scutellospora, Glomus* and *Gigaspora* all occurred during May in these two primary colonising plants. Also during this month, spore numbers decreased with distance from the foreshore. Highest spore numbers and greatest propagule diversity was associated with *S. plumieri* in July (Figure 4.11d), with *Scutellospora* numbers still high. This genus was the only one occurring with *A. populifolia* at this time, and was also associated with *C. monilifera*, which had the highest spore numbers after *S. plumieri.* During September (Figure 4.11e), *Glomus* was the most abundant spore type observed with all plant species, although *A. populifolia* and *I. pes-caprae* had equal numbers of *Acaulospora. C. monilifera* had the highest spore numbers, and *Scutellospora* propagules were confined to the back of the dune, furthest from the shore. *Gigaspora* was not observed during this month. December 98 (Figure 4.11f) again had more *Glomus* than other fungal genera. Overall spore numbers, however, had decreased from the previous month. A greater fungal diversity was associated with plants further back on the dune, as were spore numbers.

CHAPTER 5

DISCUSSION

5.1 ASSESSMENT OF MYCORRHIZAL COLONISATION

The root staining method (Section 3.2.1) proved to be very effective for all roots. However the method did take some time to perfect. Initial trials with dune roots provided varying results. Roots were first collected in October 1997 and stained, but tissues did not clear very well. Further trials with longer bleaching times for some of the roots provided better results, and sampling proper was begun in December 1997. Between December 97 and December 98 the staining technique improved with every sampling month, enhancing the visibility of mycorrhizal structures. This may have influenced the results in that recordings of mycorrhizal structures were possibly higher in later samples. However the percentage colonisation of the different plants relative to each other should have remained accurate.

Obligately mycorrhizal plants are defined as those unable to survive to reproductive maturity without being associated with mycorrhizal fungi in soils of their natural habitat (Janos, 1980), and plants with colonisation levels above 25% have been designated as obligately mycorrhizal, or mycorrhizal dependent (Brundrett and Kendrick, 1988). All plants sampled were mycorrhizal dependent according to this definition, although percentage root colonisation differed on several levels in this site. The plant species examined displayed varying trends in colonisation, which depended both on seasonality (i.e. the month) and the position of the plants along the dune profile. It also depended on depth in the case of *Scaevola.* However, in this study, the position along the profile is synonymous with particular plant species, since each host plant occurred in a fairly discrete zone, separate from the others (Chapter 2.2). The exceptions to this were *I. pes-caprae* and *E. villosa,* which occurred amongst each other, in the same zone. Hence it was difficult to determine whether distance from the shore or plant species was the actual determinant of any

differences in colonisation. The differences in results between *I. pes-caprae* and *E. villosa*, however, indicate that plant species may be more significant in some instances, particularly between plants in the same zone. Bearing this in mind results will be discussed in relation to seasonality and position along the foredune profile.

5.1.1 SEASONALITY

Total colonisation for all species during each month sampled (Figure 4.4) indicates that there was little difference in overall colonisation of AM fungi over the year, with the exception of December 97 (lowest mean colonisation) and December 98 (highest mean colonisation). The high percentage colonisation during December 98 may have been related to climatic conditions (Figure 4.1). Fairly high temperatures (~20°C) and rainfall (35mm) during this month may have increased plant vigour, and the subsequent production of carbohydrate, allowing greater reserves to be apportioned into the fungal symbiont. Gemma *et al.* (1989) suggested that the flowering time of a plant species may be a determinant of seasonal changes in AM fungal dynamics. They propose that in some plants, there may be competition between the AM fungi and the host plant for carbon during flowering. Smith and Read (1997) suggest that mycorrhizal dependency may vary seasonally with respect to periods of high P demand. Concentrations of P in seeds have an influence on plant establishment and response to AM colonisation (Allsopp and Stock, 1992). All plants in the present study flowered during the period December 97 to February 98. It may be that more favourable weather conditions in December 98 reduced the need for competition between the plant and the endophyte for carbon during flowering.

Table 5.1 summarises the findings of various studies investigating AM fungi in sand dunes. The results of this study are presented in the first row. Several of these works indicate that colonisation levels were highest in summer (Ernst *et al.*, 1984) or from summer to autumn (Gemma and Koske, 1988; Nicolson and Johnston, 1979). These studies were conducted mainly on annual grasses, and results resemble those conducted on agricultural crops (Hayman, 1970; Sutton and Barron, 1972).

Table 5.1 Results of sand dune studies, relating to percentage colonisation, spore types and number, plant hosts and region in which the study was conducted. Fungal genera are included. Where species correlated to tentative identifications in the present study these were also included; other fungal species were not listed.

Reference	AM Colonisation	Spores	Spore Types	Plant Types	Region
Present Study:	0-95%, varied with plant species. Increased slightly over the winter months; sharp increase in the second summer.	0-48 spores $100g^{-1}$. Varied between plant zones, and with season. Highest numbers in and around winter.	*Glomus Acaulospora Scutellospora Gigaspora*	*Scaevola plumieri, Arctotheca populifolia, Ipomoea pes-caprae, Ehrharta villosa, Chrysanthemoides monilifera.*	Old Woman's River, East Cape, South Africa.
Bergen & Koske, 1984		Average = 0.2 to 16.2 *Gigaspora* spores $100g^{-1}$ sand	*Gigaspora (gigantea & calospora, Acaulospora.(scrobic ulata),G intraradices)*	*Ammophila breviligulata*	Cape Cod, Massachusetts, USA
Corkidi & Rincón, 1997a and 1997b	0-86% depending on plant species. Seasonal and successional variations also depended on plant species.			All species (=37) distributed along successional gradient of dune.	Gulf of Mexico.
Ernst et al, 1984	Highest in summer.			Perennial grasses	North Holland.
Gemma & Koske, 1988	13-49%. Min. colonisation in spring and summer, max. in late summer – autumn.	10-50 spores $100g^{-1}$. Increase in numbers in early fall.	*Gigaspora (gigantea)*	Ammophila breviligulata.	Rhode Island, USA.
Gemma & Koske, 1989	2-48.8%.			*Ammophila breviligulata.*	Massachusetts, USA
Gemma, Koske & Carreiro, 1989		Seasonal abundance of spores varied between fungal species. Average=+/- 40 spores $100g^{-1}$	*Gigaspora (gigantea) Scutellospora (calospora), Acaulospora (scrobiculata)*	Mainly *Ammophila breviligulata.*	Massachusetts, USA.
Giovannetti, 1985	5-33%. Decreased over summer, constant in autumn, increased over winter.	7-50 spores $100g^{-1}$ sand. Variations showed same trend.	*Glomus Gigaspora*	*Helichrysum stoechas, Ammophila arenaria, Eryngium maritimum*	Italy.
Koske & Gemma, 1997	Increased with greater stabilisation / succession.	Average=0-208.9 spores $100ml^{-1}$ soil. Increased with increasing stabilisation.	*Acaulospora(scrobi-culata),Glomus Entrophosphora Gigaspora (gig-antea), Scutellospora (calospora).*	*Ammophila breviligulata*	Cape Cod, Massachusetts, USA

Koske & Halvorson, 1981	10-80% (average=54%) depending on plant species. Levels varied according to positions of different plant species along the profile.	Average density of all plant species combined = 10-31 spores $10g^{-1}$ soil. Higher numbers in the front half of the dune, decreasing towards back.	*Gigaspora (gigantea & calospora), Glomus(intraradices) Acaulospora (scrobiculata)*	*Ammophila breviligulata, Solidago sempervirens, Lathyrus jamponicus, Myrica pensylvanica*	Rhode Island, USA
Koske, 1975		0-110 spores $10g^{-1}$ sand. Spore density greater in older, more stabilised dunes	*Gigaspora (gigantea & calospora)*	Dune grasses.	New South Wales, Australia.
Koske, 1988	<10-100% (average 61%).		*Sclerocystis, Glomus (intraradices), Scutellospora, Gigaspora, Acaulospora (scrobiculata)*	*Batis maritima, Cocos nucifer, Ipomoea brasiliensis, Pennisetum setaceum, Prosopis pallida, Scaevola toccada, Sporobilis* sp.	Hawaii.
Little & Maun, 1996	10-60%. Hyphae increased from young to old dunes; arbuscules more common on high beach, vesicles varied little			Dune grasses.	Lake Huron, Ontario, Canada. Lacustrine Dunes.
Naidoo, 1987	5-33%. Increased from foredune to scrub.	17-168 spores $30g^{-1}$ sand.	*Gigaspora, (Scutellospora) Glomus, Sclerocystis*	*Scaevola plumieri, Gazania rigens, Eugenia capensis, Chrysanthemoides monilifera.*	Natal, South Africa.
Nicolson and Johnston, 1979	10-90% depending on dune type. Increased in summer and autumn., but considerable infection persisted into winter. Also increased with dune stabilisation.		*Glomus (intraradices)*	*Ammophila arenaria, Agropyron junceiform.*	Scotland
Puppi and Riess, 1987	0-99% depending on plant species. Two of the dunes sampled showed highest levles in spring-summer; the third was even through the year.	0-250 spores $100g^{-1}$ sand. Mean numbers = 0.4-46.7 spores $100g^{-1}$. Highest numbers in spring, late summer and early autumn.	*Glomus (mosseae), Gigaspora,* possibly *Acaulospora*	*Agropyretum mediterraneum, Ammophiletum arundinaceae, Crucianelletum maritimae*	Tirrenian coast of Central Italy.
Rose, 1988		0-46 spores $100g^{-1}$ sand. No significant difference between seasons. Highest numbers from foredunes (most vegetatively diverse region).	*Gigaspora(calo-spora), Acaulospora, Glomus (intraradices)*	All plant species occurring along the entire dune succession.	Northern California, USA.

Other dune studies, however, showed a decrease in colonisation over summer, with an increase in the winter months (Read *et al.*, 1976; Giovannetti, 1985. Table 5.1). Giovannetti (1985) attributed this to high temperatures and dry conditions, which she suggests, cause vegetative stasis in plants. There are also studies which relate seasonal differences in AM colonisation to the plant species involved, and which report that different plant species in the same site are able to show different seasonal variations (Puppi and Reiss, 1987; Corkidi and Rincón, 1997a. Table 5.1).

Environmental effects on plant colonisation are complex and difficult to measure (Smith and Read, 1997). In natural environments, particularly, it is difficult to separate seasonal effects from those related to host development or simple meteorological occurrences (Giovannetti, 1985). The high colonisations exhibited by all plant species in December 98 of the present study is one such case, in which it is difficult to conclude which factors may be responsible, given the available information. Certainly, rainfall and temperature were fairly high at this time, but were not out of the ordinary or unique environmental conditions compared to the figures for the rest of the year (Figure 4.1). They did, however, show a marked difference from the previous December (1997) particularly with respect to rainfall. With the exception of December 98 in Figure 4.4, the general trend is an increase in mycorrhizal colonisation into the winter months, decreasing again in spring (September). The Old Woman's River area did not experience high fluctuations of temperature and precipitation throughout the year, and this may account for the fairly constant monthly colonisation levels (Figure 4.4). In temperate regions, mycorrhizas have to cope with much greater seasonal fluctuations (Baon, 1994; Daft *et al.*, 1980) which would cause more pronounced changes in percentage colonisation. However, Smith and Read (1997) suggest that rapid germination and colonisation would be advantageous in the humid tropics, whereas more seasonal environments may have evolved more subtle interactions between soil moisture and temperature for germination and colonisation. The effects of temperature on colonisation vary between host plants and types of fungus. Percentage colonisation has been shown to increase in temperatures up to 30 or 35°C

(Bowen, 1987). On the other end of the scale, Daft *et al.* (1980) reported increased colonisation in English bluebells in soil temperatures of 5°C.

In the present study, each plant species exhibited a unique seasonal trend in percentage AM colonisation. *S. plumieri* had significantly lower colonisation rates through the year than other plant species (Figure 4.3a), with its lowest levels being in late summer (February). Flowering took place from October 97 through to December 97/January 98, and fruits were observed in February 98. It may be that more carbon was allocated to the plant than the fungus during this time. *S. plumieri* is a primary dune coloniser, with a very interesting belowground architecture, which seems to resemble that of an "underground tree", having just the terminal branches visible above the surface of the dune. The plant causes the development of characteristic hummocks on the dune profile, indicating a large belowground composition (Lubke, 1998c). The parts of the plant occurring below the dune surface seem to comprise thick branching stems, which may have a role in food storage. Smaller, less woody adventitious roots emerge from these stems, and it is these roots that were sampled for AM colonisation. The lifespan of these roots may be of fairly short duration, dying back as accreting sand builds up on the dune, stimulating plant growth and encouraging the formation of new roots closer to the surface which would serve to anchor the aerial parts of the plant. The same may be true of *I. pes-caprae*, which occurred as rapidly-spreading runners, covering a fairly large area, but with a relatively low percentage cover (Avis, 1992). The roots sampled from this plant were shallow (in the top 20cm of sand) laterals, which served to anchor the runners in the soil. *I. pes-caprae* (Figure 4.3c) had the highest annual colonisation of the plants studied, with highest levels in May (winter). Lowest colonisation (December 97) occurred at the same time as flowering commenced, and by February, plants were in full bloom.

A. populifolia had increasing colonisation levels from December 97 through to July 98 (Figure 4.3b). This time span included the latter half of summer and the first half of winter. There was then a sharp decrease in colonisation to 0% in September (spring), before increasing sharply again in

December 98 (summer). There is no obvious reason for this wide fluctuation in colonisation levels, although *A. populifolia* has been recorded as establishing itself fairly quickly, and then disappearing just as fast. Ward (1980) recorded that the species temporarily established after floods at Isipingo in Natal, South Africa. A month later the plants flowered, but no specimen could be found two months after that. Such rapid turnover was also observed within some of the smaller *Arctotheca* communities in the present study site, and it may be that climatic conditions affect cycles of root growth and subsequent mycorrhizal colonisation. *A. populifolia,* as with *S. plumieri*, occurs mainly in discrete communities, not becoming intermingled with other species. It is also a primary dune pioneer species, and was often observed growing in otherwise bare patches on the dunes. This plant species is able to colonise fairly large areas of the dune, with individuals occurring in close proximity with each other. Both *Arctotheca* and *Scaevola*, then, are probably unaffected by competitive factors with other plants, which is perhaps also why their mycorrhizal component was able to remain relatively low, even going down to 0% during part of the year. However, colonisations that were measured as or near to 0% may not be a true reflection of the entire root system of a plant, since only a portion of the root was measured.

E. villosa occurs on seaside dunes in South Africa, up to 1km inland. This grass has a coastal distribution from Langebaan to the region of Old Woman's River with disjunctions in the southern Cape coast (Gibbs Russell *et al.*, 1990). Hence at this study site it is at the edge of its distribution range. Flowering occurs sporadically from September to March (Gibbs Russell *et al.*, 1990). Lowest mean colonisation for the grass occurred in May (early winter), but the standard deviation at this time was fairly high (Figure 4.3d), indicating that some roots were supporting a much higher AM colonisation than others. Highest colonisation occurred at the next sampling time, in July (late winter). It may be that because this plant is a winter rainfall species (Gibbs Russell *et al.*, 1990) it is adapted to lower temperatures. If a greater root growth occurred in May, this would explain the lower colonisation values – i.e. if the growth rate of the root is higher than that of the fungal endophyte, colonisation will never catch up with increases in root length. Colonisation levels may

therefore seem to increase as summer months end and the growing rates of plants slow down (Sutton, 1973). The opposite would apply to plants adapted to warmer growing conditions since the extent of colonisation would depend upon the growth rates of the roots. This is may have been the case with *C. monilifera.* Lowest colonisation for this species occurred in September (spring) when the growth rate of the plants was probably highest, so giving the impression of a reduced mycorrhizal presence. In addition *C. monilifera* was the only species reaching a peak in mycorrhizal colonisation early in the year (Feb), and then constantly decreasing through the autumn, winter and spring months (Figure 4.3e). Without knowing more about the seasonal dynamics of the plant, it is difficult to give an explanation for this.

E. villosa and *I. pes-caprae* had opposite seasonal fluctuations, so as fungal colonisation decreased in *I. pes-caprae* over the winter months, it increased in *E. villosa.* This is interesting, as these two species grow in the same region, with little separation between the individual plants of each. Gemma *et al.* (1989) reported that spore counts for one mycorrhizal species in a sample were often associated with significantly lower levels of sporulation by other species, suggesting antagonistic interactions. This may be the cause of the different levels of percentage colonisation in these dune plants. Alternatively, these plants may be showing varied fluctuations as an adaptation to living in the same niche with limited resources.

5.1.2 POSITION ALONG FOREDUNE

Comparing the mycorrhizal status of each plant species at each sampling time in relation to their position on the foredune gives an indication of whether their situation on the dune is a determinant of AM colonisation. Sand dunes have been used since the work of Nicolson in 1959 as ideal sites in which to study plant succession (see Section 1.3.1). Many mycorrhizal studies have explored the question of whether AM colonisation can be related to the succession of plants along a dune profile (see Table 5.1). In the present study, this was not attempted, since the profile measured does not traverse a complete successional gradient but only constitutes a foredune environment. Foredunes

are characterised by primary colonising plants and a low species diversity (Avis, 1992), although these plants are important for the stabilisation of dune sand, so facilitating the establishment of secondary colonisers (Nicolson, 1960; Allen and Allen, 1990). However, some attempt was made to correlate position of plants on the foredune to colonisation levels, since parameters such as sand stability and protection from salt spray and wind, do decrease with distance from the foreshore (Hesp, 1991), even over the 36 meters sampled.

S. plumieri and *A. populifolia* were found on the front foredunes closest to the surf. It was observed that during very high tides and storms, they were often exposed to wave action and subsequently high levels of salinity. The load and intensity of salt spray is correlated with distance inland from the foreshore (Avis and Lubke, 1985). Juniper and Abbott (1993) reported that high salinity is often linked with a low percentage AM colonisation, although it is not possible to isolate the specific causal factor (i.e. osmotic effects or the effect of specific ions). *S. plumieri* has the lowest annual colonisation levels (Figure 4.2), followed by *A. populifolia*, and this may partly be a result of these factors. Koske (1988) reported colonisations of <10 to 50% for *Scaevola taccada* in Hawaiian dunes. This is similar to the range of colonisations shown in the present study.

Foredune sand is characteristically unstable and mycorrhizal fungi are able to contribute towards the stabilisation of dunes by the formation of sand aggregates held together by the fungal hyphae (Koske *et al.*, 1975). However, Forster and Nicolson (1981a) reported that these fungal aggregates seemed to be more important in lacustrine than in maritime dunes. Nevertheless, numerous studies have documented the importance of AM fungi to sand aggregation in coastal sand dunes, citing this as one of the most important prerequisites for plant succession (e.g. Nicolson, 1960; Koske *et al.*, 1975; Sutton and Sheppard, 1976; Forster, 1979; Forster and Nicolson, 1981a,b; Koske and Polson, 1984; Rose, 1988). Clough and Sutton (1978) used light and scanning electron microscopy to show that AM fungal hyphae associated with bean plants were able to cement to sand grains, suggesting that the hyphae are important in the development of soil structure. This was repeated for sand dune

soils by Rose (1988), who showed that sand aggregation increased along the dune transect, and was correlated to plant community succession.

In the present study, it may be that sand aggregation was less important for the establishment of *S. plumieri*, due to the belowground structure of this species. Forster and Nicolson (1981a), however, report that roots alone had little effect on aggregation, but that there was a noticeable increase in association with micro-organisms such as AM fungi. In light of this, the combined effect of the plant structure and the fungal hyphae may make this a more stable environment than would roots alone. It seems likely that sand aggregation would also be important for the establishment of seedlings of this plant. However, *S. plumieri* seedlings were not encountered along this particular transect and were thus not examined. *A. populifolia* may be more reliant on the sand aggregating properties of AM fungal hyphae than *Scaevola*. These plants were observed establishing themselves on bare regions of the foredune, building up the number of individuals just in those regions. Along the transect studied there was a fairly high turnover of these small, discrete communities. Nicolson (1960) noted that it is often a surprising fact to find high levels of AM colonisation in pioneer dunes. He suggested that propagules are delivered to these regions by wind and by plant fragments deposited on the driftline of the beach. Such may be the case with *A. populifolia*, which occupies otherwise unvegetated zones, and yet had high levels of colonisation in all but two of the months sampled (Figure 4.3 and 4.5).

Highest colonisations were associated with the mid-foredune species (*I. pes-caprae* and *E. villosa*; Figures 4.2 and 4.5). These species occurred adjacent to the blow-out zone and it may be that sand aggregation was more important in this unstable region, and so contributed to the higher colonisation values. *I. pes-caprae* has a distribution generally restricted to the beach, embryo dunes and foredunes (Moreno-Casasola and Espejel, 1986; Corkidi and Rincón, 1997b). Corkidi and Rincón (1997b) stated that various studies have shown *Ipomoea* species to exhibit growth responses of a model pioneer when exposed to AM fungi, in that they were less responsive (i.e. supported a

lower mycorrhizal colonisation at all times) than late seral species. The plants studied included *I. pes-caprae* (Salas, 1994; Pérez-Maqueo, 1995) in the sand dunes of La Mancha, Venezuela, and *I. wolcottiana* (Huante *et al.*, 1993) in a Mexican tropical deciduous forest). In the present study, *I. pes-caprae* was more responsive to AM colonisation than other foredune species (Figure 4.2), but no comparison was made to late seral species. Corkidi and Rincón (1997b) found *I. pes-caprae* to be the least responsive species to AM colonisation in their study on the Gulf of Mexico. However, this plant occurred close to the driftline in that study. It may be that the differing position in relation to the shore in the present study (i.e. behind the first dune hummock on a transverse dune, adjacent to a blow-out zone) affected the mycorrhizal dependency of the species in this site. Further, and perhaps more importantly, the study by Corkidi and Rincón was conducted as a greenhouse experiment, so plants were not exposed to the same conditions as were present on the dunes. In addition to this, all plant species grown in the greenhouse were cultivated in sand from the rhizosphere of only one species (*Palafoxia lindenii*). This excludes the possibility of plants exhibiting different AM colonisations as a result of the fungal genera present in the rhizosphere associated with a particular plant species (i.e. fungal genera may change according to position on the dunes, so that colonisations would vary accordingly). Koske (1988) found colonisation levels of 60 to 100% in *I. brasiliensis* growing in a Hawaiin sand dune. These results are more consistent with those of the present study, and reliable since plants were harvested directly from the dunes.

Naidoo (1987) conducted a preliminary investigation of AM in a natural Natal coastal sand dune ecosystem. She found that AM occurred in all six species of plants that she chose, including *Scaevola plumieri* and *Chrysanthemoides monilifera*. Large variations in colonisation levels within a sample and amongst species were observed. However, it was evident that there was an increase in the mycorrhizal component of mycorrhizas from the foredune to the dune scrub area. In the present study *C. monilifera* did not have a higher mycorrhizal component than all other species, even though it occurred at the very back of the foredune at the start of the more protected dune scrub area. However, it had a large stand of invasive *Acacia* spp. directly behind it which caused a

sudden steep drop in the surface level of the dune, and subsequent instability of the sand. This may well have impacted upon the mycorrhizal component of the roots by causing a less stable environment with greater sand movement than would otherwise be found in this region.

5.1.3 MYCORRHIZAL STRUCTURES

a) Arbuscules

The unifying character of all fungal members of the Glomales is "the ability...to produce arbuscules within the cells of compatible plants" (Smith and Read, 1997). McGonigle *et al.* (1990) state that colonisation cannot be categorised as being produced by AM fungi unless arbuscules are observed. In the present study, arbuscules were observed in all plant species, which therefore confirms that the fungi present within plant roots were of the arbuscular mycorrhizal type. The occurrence of arbuscules showed marked fluctuations between sampling times. Many researchers have documented the difficulties in making observations of arbuscules from field-collected roots, since these are usually relatively short-lived, less than 15 days (Smith and Read, 1997; Brundrett *et al.*, 1996; Isaac, 1992; Sylvia, 1997; Morton, 1997). Morton (*http://invam.caf.edu/myc_info/taxonomy/concepts/lifecyle.htm*) notes that arbuscular differentiation of species in the Gigasporineae persists much longer than that in the Glomineae. Spores of the suborder Gigasoprineae were prevalent in this study, and the correlation between these and mycorrhizal structures will be discussed in Section 5.5.1.

Arbuscules were always present in *C. monilifera*, and in high percentages (42-94%; Figure 4.6e) Highest occurrences of arbuscules in *I. pes-caprae* and *E. villosa* (Figure 4.6c and d) occurred in the winter to spring months (May to September). These species both exhibit very clear seasonal growth dynamics, which may influence this trend. Dexheimer *et al.* (1979) pointed out that while the arbuscule is growing and maturing in the intracellular apoplast, the mycorrhiza can be seen as an association between metabolically active fungal structures and the living cells of roots. It could be that as the older roots senesce through winter the proportion and turnover of arbuscules is reduced,

increasing again as growth commences in the summer months. However, to positively determine whether this is the case it would be necessary to monitor the growth habits of the plants, and the fungi within them, on a more regular basis in order to record the turnover of arbuscules. The rate of growth of the host species seems to affect the arbuscular cycle. Formation of arbuscules in *Triticum aestivum* has been shown to take 2-3 days, with the entire cycle completed in around 7 days (Smith and Read, 1997). However, in slow-growing woodland plants arbuscules appear to last much longer (Brundrett and Kendrick, 1990), although it is not clear why this might be the case. *E. villosa* does not tend to be a slow-growing species, so it could be that arbuscular turnover is fairly rapid. *I. pes-caprae* produces very large stolons beneath the soil surface, which may indicate that the plant is fairly slow-growing with a high annual turnover of runners and lateral roots. These ideas would have to be tested with more rigorous experimentation.

S. plumieri, A. populifolia and *C. monilifera* all had high arbuscule formation in May (winter) and in December 98 (summer). This is difficult to explain without additional information regarding growth of the plants at these times. This is also difficult to compare with other studies, since assessments of mycorrhizal colonisation in field-collected roots are often undertaken without recording the specific mycorrhizal structures observed on a seasonal basis. This is particularly true for the dune studies which have been cited (Table 5.1).

A. populifolia showed very marked fluctuations in the presence of arbuscules. Percentages ranged from 82% in May to 4.2% in July and 51.2% in December 98 (Figure 4.6). Spore numbers associated with the root zone of this plant were also high in May (average of 32 spores $100g^{-1}$ sand; Figure 4.10). Hence it may be that increased numbers of arbuscules were necessary for the acquisition of carbon from the host, which could then be partitioned into spores. In July, when arbuscule levels were low, so were spore numbers. In December 98, when arbuscule levels were intermediate and spore numbers were low (5 spores $100g^{-1}$; Figure 4.10), percentage colonisation of AM fungi in *Arctotheca* roots was very high (80%; Figure 4.3). This may have been due to higher

requirements for nutrients by the host during increased root growth in the summer months. The converse may also be true in that increased production of photosynthate by the host in summer may have allowed greater carbon partitioning into the fungus, and a subsequent increase in colonisation. These ideas would also pertain to *S. plumieri*. However, they would have to be tested by measuring rates of photosynthesis in plants and carbon partitioning between the plant and the fungus.

b) Vesicles

Vesicles are not produced by all genera of AM fungi. *Scutellospora* and *Gigaspora* (Gigasporaceae, Gigasporineae) never develop vesicles; instead auxiliary cells are produced on the extraradical mycelium (Smith and Read, 1997). Vesicles may be present in all other genera (but not verified in *Sclerocystis* (Morton, 1997)), occurring either inter- or intracellularly within the cortex, although the degree to which they occur may vary (Abbott, 1982). Vesicles generally develop after arbuscular colonisation (Morton, 1997). Some species in *Glomus* produce thick-walled vesicles, which eventually cannot be distinguished morphologically from intraradical spores (Morton, 1997). These vesicles are thought to act as infective propagules (Biermann and Linderman, 1983), or spores for some fungal species (Brundrett *et al.*, 1996). Thick-walled vesicles were apparent, and sometimes very abundant in *E. villosa* roots (Plates 2A and 2B). They also occurred, though less abundantly, in *A. populifolia*. Thin-walled vesicles were observed in the remaining plant species (Plate 2C and D), but little is known about the infectivity of this type of vesicle (Morton, 1997). Little is known about the biology of vesicles altogether, particularly with respect to their germination (Smith and Read, 1997).

The proportion of roots occupied by vesicles was highest in *I. pes-caprae* (up to 40%) and *E. villosa* (up to 63%) with the other plants recording very low numbers of observations throughout the year (less than 7.1% - Figure 4.6a-e). The most abundant production of vesicles in both of these plant species occurred in July (mid-winter). This would corroborate the suggestion by some authors that vesicles may form towards the end of the plant's growing season, in order to store reserves, and in

some instances form spores (Smith and Read, 1997). In addition, Morton (*http://invam.caf.wvu.edu/Myc_Info/Taxonomy/Evolution/origin.htm*) states that intraradical vesicles "generally increase in abundance after arbuscular colonisation is well established". In both *I. pes-caprae* and *E. villosa*, this is the case, with highest vesicle recordings occurring after peaks in arbuscule formation (Figure 4.6). The trend is more difficult to follow in the other plant species due to low numbers of vesicles, but appears to be similar. Fungal genotype and environmental conditions affect the occurrence and abundance of vesicles, although they are able to persist in roots for long periods of time (2 years and longer) and remain infective (Morton, (*http://invam.caf.wvu.eduMyc_Info/Taxonomy/Evolution/origin.htm*).

C. monilifera was also associated with an echinulate auxiliary cell produced by a species of *Gigaspora*. There is no evidence to suggest that auxiliary cells are infective, and they have a more transitory storage function than vesicles, though they contain a rich supply of lipid deposits (Morton, (*http://invam.caf.wvu.eduMyc_Info/Taxonomy/Evolution/origin.htm*). They are usually present, and reach a maximum abundance, during early stages of sporulation and then decline (Franke and Morton, 1994). The auxiliary cell was extracted from the root zone of *C. monilifera* in September. This was springtime, and probably when root growth began to increase. Germination of AM spores and an increase in hyphal growth probably also commenced at this time, since there was a significant increase from colonisation in September to that in December 98 (Figure 4.3) in *Chrysanthemoides*. This would correlate with the fact that auxiliary cells form on the hyphae of germinating spores and on the extraradical hyphae of developing mycorrhizas (Morton, (*http://invam.caf.wvu.eduMyc_Info/Taxonomy/Evolution/origin.htm*).

c) Intraradical spores

Intraradical spores occurred in *I. pes-caprae* and *E. villosa*, appearing at the same time as the onset of vesicles, in winter (i.e. May). It is assumed that the intraradical spores developed from vesicles within these roots, and so functioned as a long-term storage structure for the fungal symbiont.

d) Appressoria

Appressoria occur where fungal hyphae first penetrate root epidermal cells (Brundrett *et al.*, 1996), and their measurement can thereby give an indication as to when roots are being actively colonised by new hyphal entry points. All plant species in this study showed evidence of appressoria at some stage during the times sampled though their occurrence was generally low (0-23%; Table 4.1). Isaac (1992) states that there are generally few entry points, which affect a relatively small portion of the root (5-10μm). *I. pes-caprae* had the highest frequency of appressoria, which were recorded in all but two months. In all other plant species except *A. populifolia*, appressoria occurred from spring (September) to late summer (February). The initiation of colonisation is dependent upon the density of propagules in the soil (Smith and Walker, 1981). Yet it seems that at higher densities of propagules there is no increase in entry points, which may indicate a decline in the availability of root length able to be colonised (Smith and Read, 1997). In addition, Tester *et al.* (1986) suggested that photosynthetic rates may influence initial colonisation, and that a lower irradiance decreases the occurrence of appressoria. Hence it seems that in the summer months, as root length increases the opportunity for appressoria formation by the fungus would also increase. The opposite would be true in winter, and this would be compounded by lower irradiance.

The number and structure of appressoria is known to differ significantly between fungal suborders. Bradbury *et al.* (1993) used myc- mutant roots of *Medicago* to show the differential formation of appressoria between two species of *Glomus* and a *Gigaspora.* This may in part help to explain the fact that only *A. populifolia* recorded appressoria in mid-winter (July; Table4.1).

e) Hyphal Coils

Once the fungus has penetrated the host plant cells, hyphal coils may develop in the outer cortex (Isaac, 1992). The importance of hyphal coils relates to the development of different forms of arbuscules. In 1905 Gallaud described *Arum* and *Paris*-type arbuscules. The occurrence of these is still under investigation by various researchers, and the details of them are beyond the scope of this

study. Suffice to say that most typical arbuscular mycorrhizas belong to the *Arum*-type, and are characterised by branching from the terminal branches of intracellular hyphae. Although hyphal coils may be formed in this instance, they do not generally constitute an important part of the intraradical mycelium (Smith and Read, 1997). *Paris*-type arbuscules are characterised by their formation on the branches of extensive intracellular hyphal coils (Smith and Read, 1997). Most plant species in the present study exhibited hyphal coils at the same time as appressoria were observed. However, the occurrence of hyphal coils was relatively low (Table 4.2), their form was not compared to arbuscule type, and hence no attempt will be made to extrapolate the function of the coils from these results.

5.1.4 DEPTH

Scaevola had the lowest mycorrhizal levels (Figure 4.2). However, the single sample taken from a 1m depth produced vastly different results with root colonisation levels of close to 100% (Table 4.3). It is very likely that at greater depths in this dune, AM fungi are able to colonise the *Scaevola* roots more effectively. Virginia *et al.* (1986) found some species to be mycorrhizal only after taking depth into account. The root systems of all these plant species are very different, and it is probable that root architecture has a substantial influence upon AM colonisation rates. Most of the root samples for this study were extracted from the top 75cm of soil. It could be that the more exposed communities of *Scaevola* and *Arctotheca* benefit more by being colonised by the fungus at greater depths to reduce the effects of sand movement. Friese and Koske (1991) attempted to map the root system of *Ammophila breviligulata* to associate the root architecture with the spatial distribution of AM fungal spores. They did this from 5-35cm into the soil profile, and found no association between spore clumping and the occurrence of roots. They did not examine the effect of depth on root colonisation. Nehl *et al.* (1998) studied patterns of mycorrhizas down a heavy textured soil. They found that most fungi associated with cotton occurred near the surface of the soil, and that there was no evidence of propagules at 1m depth. The authors suggested that mycorrhizas are initiated at the soil surface, and then undergo a secondary spread through the

profile in order to colonise the root system (i.e. fungal mycelia do not occur independently deep in the soil). This may well be the case in other systems, and there seems little reason for hyphae to occur in soil devoid of host roots. In the case of a plant such as *S. plumieri*, in which stems and lateral roots of the plant sometimes occur over 2m below the surface of the hummock, it does not seem unreasonable that AM fungi would occur at 1m depth in association with these roots. It also seems plausible that the regions deeper down would be more stable, and therefore able to maintain a high colonisation level in roots. In addition, all of the foredune plants are exposed to high levels of sand deposition, and roots that were once close to the surface of the sand may become buried much deeper, but maintain their mycorrhizal status. This would suggest that higher colonisation values may be associated with older, still active roots, particularly if these remain in contact with an actively growing and undisturbed extraradical mycelium (see Section 5.5.1). These possibilities are merely suggested in the present context, due to the lack of suitable replicates and a more detailed analysis. Further studies would have to be undertaken in order to confirm these observations and ideas.

5.2 BIOASSAYS

The percentage colonisation of roots in the dune plants is affected by various propagules in the soil. However, it is difficult to determine which types of propagules have greater effects on the different plant species and communities. The potential for plants colonising bare or moving sands to become inoculated with AM fungi, particularly in the more unstable regions of the foredune, may be very important for the maintenance and survival of those plants. Hence bioassays were undertaken to test the inoculum potential of the sand (Section 3.4) from the two separate regions on the dune, one of which was more unstable (*Ipomoea* dune) than the other (*Scaevola* hummock) and subject to greater sand movement. The bioassay did not test the progression of colonisation in roots, or the effect of the AM fungi on the actual dune plants. Its role was solely to test for the presence of infective propagules in the soil. The results of the bioassay then, are a function of spore numbers and types

as well as other propagules in the dune soil, and need to be considered in that context, rather than as a separate experiment.

5.2.1 ASSESSMENT OF AM COLONISATION

A very low mean colonisation of 0.33% occurred in the roots of plants grown in sterilised soil (Figure 4.7c). Whilst every precaution was taken to avoid contamination of the treatments, it is thought that an isolated infection (propagule) was the cause. This may have originated from other plants in the greenhouse, in which case it would have been carried by water splash or air movement caused by fans in the greenhouse. Alternatively, spores may have blown into the greenhouse when the door was opened.

Results showed that plants grown in sand from the hummock and the *Ipomoea* dune were significantly different in terms of percentage colonisation, with *Scaevola* hummock sand producing much higher values. The sand samples were taken from the dunes in July 1998. This month had the second highest spore numbers of all the sampling times (Section 4.4.1). Most of the spores, however, occurred in association with *S. plumieri* (with an average of 23.7 spores $100g^{-1}$) and *C. monilifera* (Figure 4.10). *I. pes-caprae* was associated with very low spore numbers (an average of 0.33 spores $100g^{-1}$) which may have been associated with the greater mobility of sand in this area. Therefore, the potential for spores to germinate and colonise roots was much greater in the *S. plumieri* hummock sand. It is thought that this was a major determinant of differences in colonisation levels of *Sorghum* plants. Due to the low numbers of spores in *I. pes-caprae* sand, it is also suspected that propagules other than spores (i.e. infected root fragments and hyphal fragments) had a large role in the colonisation of these plants. The effects of each cannot be separated or verified without further testing, including the extraction of spores from the bioassay soil. Having made this statement, it follows that the same holds true for the hummock sand. Suffice to say then that infective propagules were present in both sets of sand from the foredune, and were for the most

part absent in the sterile control. Furthermore, the inoculum potential of the *Scaevola* sand was greater than that of the *Ipomoea* sand.

5.2.2 SHOOT AND ROOT MEASUREMENTS

These were recorded in order to test whether differences occurred between plants grown in non-sterile and sterile soil. Maximum measurements were from *Sorghum* plants grown in sterile soil, and lowest measurements were from plants grown in sand from the *Scaevola* hummock. Plants from the *Ipomoea* dune showed values intermediate between the two (Figures 4.7a and 4.7b).

Shoot measurements (length and dry weight) of *Sorghum* plants were significantly lower in *Scaevola* hummock sand than in both the *Ipomoea* and sterile sand (Figure 4.7a and b). However, it should be noted that in terms of actual values, shoot measurements were not that much lower in *Scaevola* hummock soil (lengths were 4-5cm shorter, weights were 0.05g lower). It should also be noted that although root dry weights from the hummock sand were significantly lower than those of other treatments, this does not seem to be apparent from the graphs when the standard deviations are taken into account (Figure 4.7b). All values were checked and it was confirmed that the ANOVA was correct. It is thought that the cause may have been outliers in the data (i.e. measurements outside of the normal distribution) which were infrequent enough so as not to affect the overall variance between the samples.

5.2.3 COLONISATION VS ROOT AND SHOOT MEASUREMENTS

A comparison of Figure 4.7c with Figures 4.7a and b shows that as percentage colonisation decreases, root and shoot measurements increase (i.e. highest colonisation values are associated with lowest root and shoot lengths and dry weights). It was hypothesised that some correlation would exist between root measurements and shoot measurements, and that this would differ between mycorrhizal and non-mycorrhizal hosts. However, no correlation within treatments was shown by the regression analysis.

Azcón and Ocampo (1981) found that wheat mycorrhizal dependency decreased as root weight increased. However, Corkidi and Rincón (1997b) found no significant difference between root:shoot dry weight ratio between mycorrhizal and non-mycorrhizal dune plants, although most species allocated a greater biomass to aerial than to below-ground parts of the plant. It is thought that plants maintain a functional equilibrium between shoot and root growth (Brouwer, 1963). This means that shifts can occur in the allocation of growth resources between above- and below-ground parts of the plant, and that these shifts will in essence reflect the abundance of above- and below-ground resources (van Noordwijk *et al.*, 1998). Plants are thought to use a range of warning methods to adjust resource allocations to roots and shoots before resource deficiencies are felt (Brouwer, 1983). Since mycorrhizas improve plant vigour, it was hypothesised that the root:shoot ratios between mycorrhizal and non-mycorrhizal plants would differ. These differences were observed (Table 4.4) but were not very high. A higher root:shoot ratio implies that plants are apportioning a higher component of their resources into root growth. Such an implication cannot be extrapolated from such close ratios. Therefore, there was no real difference between root:shoot ratios of the different treatments.

These results are interpreted with caution. Roots were difficult to extract from the sand, and many of the fine roots may have been lost. They also held the sand grains to them very closely, and many of these could not be removed without breaking the fine roots. Plants were manipulated as carefully as possible, however, and all of the main adventitious roots remained intact. Roots from all treatments were removed with approximately the same measure of success. Therefore, although the length and dry weight values of roots may be biased to some degree, the relationship of these between the different treatments is thought to remain valid.

It may be that the higher shoot and root measurements of plants grown in the sterilised sand is due to the fact that none of the carbon produced by the plant is being apportioned into AM fungal structures. Hence all carbon produced can be used directly for the benefit of the plant. A lack of

association with AM fungi however, means that the plant may have to work harder to acquire all the nutrients it needs. This would mean increased root growth in order to exploit soil reserves. Only plants grown in sterilised sand exhibited a purple colouring in their leaves, which is indicative of a phosphorus deficiency (Marschner, 1995). This implies that although plants were bigger than those grown in unsterilised sand, they were not able to acquire all essential nutrients, particularly phosphorus. Mycorrhizal plants, however, seemed to be able to do this. Another factor which probably affected growth patterns of *Sorghum* in sterilised sand is a lack of all other soil organisms. Sterilisation would also have removed bacteria, other fungi, nematodes etc., be they beneficial or not. This means that *Sorghum* plants in this treatment were protected from the effects of harmful soil organisms. However, some soil organisms enhance the effect of mycorrhizal fungi (see Section 1.3.5) and may have contributed to the health of the mycorrhizal *Sorghum* plants.

The bioassays showed that there were infective propagules in the dune sand, and that those from the more aggregated sand of the foredune hummock had a greater inoculum potential than those from the more mobile *Ipomoea* dune. This is a direct result of the number and type of propagules in the dune sand (Section 5.3). The diversity of AM genera in the soil, the rooting systems of plants and the properties of different AM fungi will also determine the extent to which roots may become colonised. This is discussed further in Section 5.5.

5.3 ENUMERATION OF SPORES

Table 5.1 summarises the results of spore counts in other studies of mycorrhizas in sand dune environments. Sand dunes generally have lower spore numbers than other environments. For example, spore numbers in the savanna regions of Nylsvley nature reserve in South Africa ranged from 22 to 687. $30g^{-1}$ soil (Dames, 1991); 4-200 spores g^{-1} have been found in clay soils used to grow cotton in eastern Australia (McGee *et al.*, 1997), and soils from eleven native sites in Hesse, Germany, contained 31-97 spores g^{-1} dry soil (Loth, 1996). Spore numbers may have been

underestimated in this study, but this may be the case in most studies (Schenck, 1982). Although the sucrose centrifugation method used for spore purifications was the most effective of the methods tried (Section 3.2.4), spores were found in the supernatant and these made a fairly large contribution to the final spore numbers. It is also thought that any very small *Glomus* spores (< 45μm) may have been lost. However spore numbers in this study are comparable with those of other dune systems (Table 5.1).

5.3.1 SEASONALITY

Highest spore numbers in this system occurred in winter, and lowest numbers in summer (Figure 4.9). Spore density and species diversity can be extremely variable, and in some habitats spores are not present during all seasons (Smith and Read, 1997). At Old Woman's River, spores were found throughout the year, although their densities were very variable between plant species. In agricultural soils supporting annual host plants, spore populations have been shown to reach maximum abundance at the end of the host's growing season (Hayman, 1970; Sutton and Barron, 1972). Sylvia (1986), however, suggests that the AM fungal species associated with perennial plants in sand dunes may sporulate at different times throughout the year. Such was the case in this system. It may also be that a more favourable year-round climate for the growth of roots may inhibit sporulation of AM fungi, or favour species which have a reduced ability to sporulate (Baylis, 1969). Although spore numbers associated with different plant species varied considerably, most peaked during or on either side of winter. Root growth cycles may differ between host plant species, and root turnover may actually be greater in summer than at the end of the growing season for some plants (Huisman, 1982). Rather than death of plant roots, some authors (e.g. Gemma *et al.*, 1989; Sylvia, 1986) therefore suggest that various external (environmental) and internal (produced by the plant) stimuli may be the main determinants of sporulation. These would include temperature and light (Furlan and Fortin, 1973), photosynthate production (Wallen, 1980), root exudates (Fluck, 1963) and root hormones during flowering and the end of growth (Torrey, 1976). Research at INVAM and in the lab of Abbott now seems to be indicating that sporulation only

occurs after a critical threshold of biomass has been reached in roots. As mycorrhizal development continues thereafter, sporulation progresses asynchronously along with it (Morton, *http://invam.caf.wvu. edu/myc_info/taxonomy/concepts/lifecyle.htm)*. Hence, Morton states "it is a myth that arbuscular fungi behave similar to many other fungi by initiating sporulation after nutrient deprivation". He suggests that the timing of sporulation varies between species and with growing conditions, but under most conditions occurs 3-4 weeks after commencement of mycorrhizal colonisation.

Sand from the *S. plumieri* zone had high spore numbers only in the winter months. In summer, there was a drastic decrease in numbers. Samples from the *A. populifolia* root zone showed the same trend (Figure 4.9). *C. monilifera* had a peak in spore numbers towards the end of winter, continuing into spring. Summer spore numbers were much higher in this plant than in the other species. *E. villosa* exhibited highest spore numbers from late summer into autumn. Spore numbers decreased through the winter and then began to rise again in spring. This grass is a winter rainfall species, and is therefore suited to cooler conditions. Temperatures in February were the highest for the whole year, and rainfall was low (Figure 4.1). The plants may have been under stress from these conditions, causing a high sporulation rate. Spore germination may have commenced during conditions more favourable to the plant from July onwards, so decreasing the reserves of spores in the soil. Vesicles and intraradical spores peaked in *E. villosa* in July (Figure 4.6d). This may indicate that storage structures occurred in a much closer association with the roots at this time, so that the supply of spores into the surrounding soil was reduced. *I. pes-caprae* showed little seasonal variation in spore numbers (Figure 4.9). This species also had significantly lower annual spore numbers, as compared to the other plant species. Without additional information on the growth parameters of the plant, it is difficult to speculate why seasonal effects are negligible.

Louis and Lim (1987) found that in tropical soils seasonal spore abundance was not related to reproduction or the cessation of growth of the host plants. Spore production and AM colonisation

appear to be influenced by a combination of environmental conditions, host species and soil type. Giovannetti (1985) suggests that "within environmental conditions seasonal variations play the most important role in physiological changes of the host, which directly influence the mycorrhizal symbiosis". Statistical analyses from several studies (Sylvia, 1986; Gemma, 1987; Gemma *et al.*, 1989) indicate that more meaningful studies of AM fungi in natural sites would require intensive seasonal sampling. This would help to develop a more accurate description of seasonal dynamics and spore abundance of different AM species (Gemma *et al.*, 1989).

5.3.2 POSITION ALONG FOREDUNE

Figure 4.10 (a-f) shows that spore numbers in this dune system were generally very low and, although there were significant differences in spore numbers across the dune profile, the numbers themselves were not very different – except in May and July. In these winter months, *S. plumieri* (and *A. populifolia* in May) showed quite dramatic increases in spore numbers.

Rose (1988) found that AM fungi in a Northern California sand dune were associated with succession. Highest spore numbers were recovered from the foredune community. However, the foredune area sampled was associated with a high plant species richness, which indicated competitive interactions between plants. The foredune studied at Old Woman's River had a very low species richness. It would seem that the structure and definitions of these dune types differ somewhat, and care has to be taken in comparing these results. Nicolson (1960) studied dune grasses in a complex dune system in Scotland, and found an increase in mycorrhizal activity from the foredunes to the recently fixed dunes, though levels decreased in older fixed dunes as soil conditions became more suitable for plant growth. In a study of plantings of American beachgrass, Koske and Gemma (1997) found that the youngest sites had fewest spores, as did Koske (1975). Koske and Halvorson (1981) found that areas on the dune which had the greatest cover of *Ammophila* also had greatest fungal densities and diversity in the rhizosphere. They also reported that maximum spore densities tended to occur in the front half of the plant's distribution range,

nearest the dune crest. The consensus in dune studies seems to be that higher mycorrhizal activity is associated with increased vegetation cover and soil stability (Koske and Polson, 1984), and it seems that spore numbers may be more closely related to plant species richness in a site than to the position of that site. Giovanetti and Nicolson (1983) suggested that this was a result of seasonal and edaphic factors.

In the present study, species on the foredune closest to the shore had highest annual spore numbers. Sand on the embryo and mobile dunes is generally very unstable, and as Nicolson (1960) stated: "the main problem regarding infections in embryo dune grasses is the method of spread and dissemination of the endophytes to this isolated community". He did however follow this by saying that small amounts of inoculum can give rise to a much greater fungal population. *A. populifolia* occurred in areas on the foredune often devoid of other plants. It may be that fungal propagules carried into the root zone of this plant by wind or waves were able to colonise the roots fairly quickly, and eventually lead to the production of spores. Puppi and Riess (1987) suggested that one of the major problems limiting optimal mycorrhizal performance in dune habitats is the spread of colonisation and the dispersal of mycorrhizal endophytes. In the mobile zone of a dune, the plants do not form a continuum but constitute 'vegetational islands' as sand is continuously accumulated or removed. The authors state that an effective mycorrhizal endophyte in dune habitats should be able to survive in the soil or move with the sand, and once it meets a root, can spread rapidly into it, producing extensive colonisation, even from individual entry points. *S. plumieri,* on the other hand, is exposed to high levels of wind and sand movement, but its belowground structure may lend some degree of stability, allowing propagules to build up in the root zone.

Puppi and Reiss (1987) reported high numbers of intraradical spores (although they did not give actual numbers) particularly in plant species typical of unstable dunes. They suggest that of the spores from stabilised dunes those enclosed in root fragments may be preferred in dispersal to more mobile dunes, as they are protected from the rigours of the environment. *I. pes-caprae* and *E.*

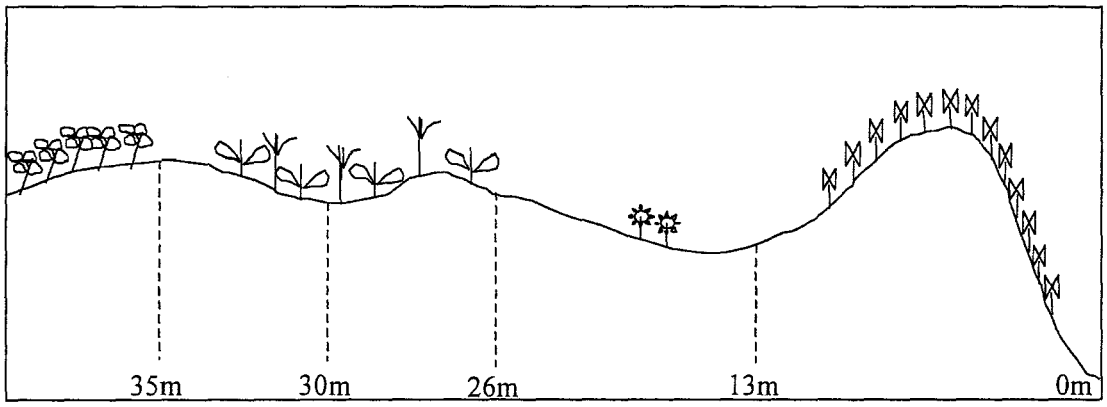
villosa produced the greatest numbers of intraradical spores (Figure 4.6). These plant species occurred in an exposed part of the foredune which is often subjected to a high incidence of sand movement and instability. On the foredune sampled, this zone tended to be much more unstable than the *S. plumieri* hummocks. This may explain why *I. pes-caprae* in particular was associated with significantly low spore numbers, even though it had the highest annual percentage colonisation.

C. monilifera was exposed to lower levels of sand movement. Colonisation was generally high in this plant (Figure 4.3e) and arbuscules were particularly prevalent (over 40%) all year round (Figure 4.6e). In addition, seasonal fluctuations in spore numbers were not as dramatic as in *Scaevola* and *Arctotheca*. It therefore seems as though the fungal symbionts were comfortably established in this host plant and may have supported a strong hyphal network. Hyphal networks are thought to play a more important role than spores in later successional species or more stable regions, as has been shown by studies in pasture soils and mine spoils (e.g. Evans and Miller, 1988; Jasper *et al.*, 1989; Francis and Read, 1994).

It is difficult to draw conclusions regarding spore densities in field soils, or the factors influencing spore production (Smith and Read, 1997). Belowground AM fungal communities are closely associated with aboveground plant community development and changes, and AMF can affect the success of plant species, be they mycorrhizal or not (Miller, 1979; Janos, 1980; Read and Birch, 1988; Koske and Gemma, 1990; Francis and Read, 1995). It should be remembered that sporulation is a survival mechanism for the fungus, allowing it to overcome unfavourable conditions (Smith and Read, 1997) and these conditions may be affected by plant species, environmental conditions, seasonal effects, physical position of the plant and the fungal species involved. It seems that species of host plant, together with physical characters of the site are the most influential factors determining the occurrence and spore density of AM fungal species in sand dunes (Koske, 1981) and could account for the variations observed in this study.

5.3.3 CORRELATION OF SPORES AND MYCORRHIZAL COLONISATION

No correlation was found between spore numbers and mycorrhizal colonisation values of any host plant. Figure 5.1 summarises these results diagrammatically and shows the mean annual spore numbers and percentage colonisation for each dune plant. Results of other studies have been variable. Giovannetti (1985) found a good correlation between percentage root infection and total number of spores every month in Italian sand dunes. Louis and Lim (1987) found an inverse relationship between spore density and root colonisation in a lowland tropical rainforest, in that spore populations declined as root colonisation increased. Some investigations, however, show no correlation between spore populations and infectivity (e.g. Powell, 1977). In a sand dune at Rhode Island, Gemma and Koske (1988) found that spore abundance did not coincide with maximum germination or soil infectivity. Rather, infectivity was found to be affected by dormancy, which was overcome at a certain age and temperature (5 weeks at 5°C). Thus spore abundance and its relationship with root colonisation probably vary according to a range of factors including spore production, dormancy and infectivity potential. Furthermore, populations of spores may not be a true reflection of the contributions of component fungi to root colonisation (Smith and Read, 1997) as discussed in Section 5.5. Another determinant that deserves mention is sampling technique. Giovannetti (1985) reported that significant correlations between mycorrhizal colonisation and spore density have been found where samples were collected from the same plant species in exactly the same site over a year. However, no correlation has been found when samples have been collected from many plant species, in different sites during a single collection. In the present study, samples were taken from the same plant species, in the same area, but not exactly the same place, over a year. This may have reduced the likelihood of finding a correlation, and the movement of sand in the dunes may have exacerbated this effect.



	C	E	I	A	S
% Colonisation:	55.3	62.1	66.7	37.5	34.5
Spores $100g^{-1}$:	25	17	3.3	22.2	33.2

Figure 5.1 Mean percentage AM colonisation and spore numbers in relation to position along the profile. Values are for *S. plmieri* (S), *A. populifolia* (A), *I. pes-aprae* (I), *E. villosa* (E) and *C. monilifera* (C).

5.4 IDENTIFICATION OF SPORES

Spores are the only structures produced by arbuscular mycorrhizas that can be used to identify genera or species with any degree of certainty (Smith and Read, 1997). However, as has been stated before, it is often not possible to identify spores from field soils accurately. Morton (*http://invam.caf.wvu.edu/FAQs/faqID.htm*) states that it is not always necessary to identify species in a study, but that at the very least the number of species present should be estimated. This may be accomplished by separating spores into classes based on major differences in their appearance. This is what was done in the present study. The limitation to inconclusive identifications, however, is that positive comparisons with other studies cannot be drawn. Different species of spores, even within the same genus, can have varying responses to different host plants or ecological conditions (Smith and Read, 1997). In order to make such comparisons it would be necessary to set up trap cultures using sand from field soils. Trap cultures are an isolation procedure often used to propagate spores, and so increase their quality and quantity so that they may be accurately identified to species level (Brundrett *et al.*, 1996). Such a procedure was not attempted in the present study. Nevertheless, comparisons of fungal genera isolated in other dune studies can still be made. These comparisons are important, since it is believed that a good deal of the functional diversity within the Glomalean fungi occurs at the isolate, rather than species, level (Brundrett, 1991; Morton and Bentivenga, 1994). It is thought that over millions of years, populations of AM fungi have adapted to changes in conditions within the same sites (Trappe and Molina, 1986). Therefore habitat information is just as important as the identity of the fungi, particularly when comparing the results of experiments. Table 5.1 shows the spore genera found in other dune studies, many of which are reflected in the present study. Of the species tentatively identified all except *Glomus caledonium* have been reported from past dune studies around the world. The most frequently reported spore types in other dune studies are *S. calospora, Gi. gigantea, G. intraradices* and *A. scrobiculata,* whilst *G. mosseae* was common in Italian dunes. These are all consistent with the tentative identifications presented in this study.

The major difference between the present study and many of the dune systems reported in the literature is the fact that the dominant fungal spores were often those of *Gigaspora*. In the present study the dominant genus, besides *Glomus*, was *Scutellospora*. Two factors may help to explain these differences. The first is that most other dune studies have been undertaken in temperate climates, as well as one or two from the tropics (Table 5.1). The Eastern Cape of South Africa, however, has a transitional climate with variable conditions (Chapter 2.2). The second factor is that *Gigaspora* and *Scutellospora* were not considered to be separate groups until 1986, when Walker and Sanders placed species of *Gigaspora sensu lato* with inner walls and a germination shield into the genus *Scutellospora* (Bentivenga and Morton, 1994). Since then further divisions have been made, with more and more spores formerly classified as *Gigaspora* being placed into *Scutellospora*. Considering that many of the mycorrhizal studies conducted on dunes were undertaken before or around this time, some of the definitions of *Gigaspora* may no longer hold. Such is the case with what used to be *Gi. calospora* (Nicol. &Gerd.) Gerdemann & Trappe, a commonly reported species in past dune studies. This is now *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders, and was tentatively identified as a fairly common species in the present study (Section 4.5.4).

5.5 DISTRIBUTION AND OCCURRENCE OF FUNGAL GENERA

According to Chuang and Ko (1981), the number of spores in a volume of soil has a strong correlation to spore size. This means that although different sized spores should have the same capacity to colonise roots, AM fungi which produce small spores should produce higher spore densities than those with larger spores (Brundrett *et al.*, 1996). This was not the case in the present study. Total figures for the year (Table 4.8) show that roughly the same number of *Glomus* (size range 40.4-397μm) and *Scutellospora* (83.3-470μm) spores occurred, and exactly the same number of *Acaulospora* (101.6-300μm) and *Gigaspora* (155-533μm). However, spore numbers for each fungal genus varied considerably both between months and plant species. The overall trend that emerged seemed to be that spore numbers and diversity were greatest on the back dune during the

warmer summer months, and greatest on the front dune amongst the primary colonisers in the winter months. *Gigaspora* occurred with all plant species only in February, *Glomus* occurred with all plants during both Decembers and September, *Acaulospora* was present with all plants in September and December 98, and *Scutellospora* never occurred with all plant species at the same time (Figure 4.11a-f).

Through the course of the six sampling times, the abundance and type of spores associated with any particular plant species varied considerably. This did not seem to be a result of seasonal effects for all species. Even though spore numbers were high in the winter months and low in summer, not all plant species showed high fluctuations (see Section 5.3.1). Position along the profile does not seem to explain the results either. This is particularly evident if *I. pes-caprae* and *E. villosa* are considered. These plants occurred intermingled in precisely the same zone, but showed very different results in terms of spore associations. Several factors may contribute to these results and help to tie them together with all of the preceding sections. These factors include fungal properties, root systems and sporulation, interactions between fungal genera and plant-fungus interactions.

5.5.1 FUNGAL PROPERTIES

Hayman (1983) stated that several factors need to be considered when trying to elucidate the efficiency of an endophyte: 1) it is difficult to standardise the inoculum of different species, for example in trying to compare species which form spores to non-sporing species. Stutz and Morton (1996) suggest that in arid habitats, a high proportion of AM fungi may be non-sporulating in the field. However, non-sporing species were not accounted for in this study. 2) When counting spores, one needs to work out whether it is practical to compare many small spores with fewer much larger ones. Smaller spores were compared with larger ones here, but as was stated before, the number of spores did not seem to relate in any way to their size. 3) Some spores produce several germ tubes whilst others produce only one, although the spores themselves may be similar sizes. This would have an impact upon the inoculum potential of the propagules (Hayman, 1983).

We know that infective propagules of AM fungi include spores, mycorrhizal root fragments containing hyphae, vesicles (Glomineae only) and auxiliary cells (Gigasporineae only), though there is little evidence as yet that the last are infective. The success of the fungal endophyte in colonising host roots depends on these different types of propagules. There is evidence that there are significant differences in infectivity at the subfamily level (Morton, 1993). The only infective propagules of *Gigaspora* are spores, since hyphae and auxiliary cells consistently fail to produce new mycorrhizas. Furthermore, healthy spores of this genus collected from the field have been found to be as infective as those propagated in trap cultures (Morton, 1993). These facts together imply that counts of *Gigaspora* from field soils can give a real measure of the inoculum potential of the genus in that region (Gemma and Koske, 1988). Walker (1992) suggests that *Gigaspora* has a limited distribution in Europe. If this is the case in other regions as well, it may be that spores of *Gigaspora* are not disseminated easily, and since only the spores act as infective propagules this would limit the range in which establishment of new mycorrhizas could occur (Morton, 1993).

Gemma and Koske (1988) demonstrated that dormancy occurred in newly formed spores of *G. gigantea* under field conditions, and showed seasonal trends in spore abundance and germination of this species. They suggested that dormancy serves to maintain a large amount of inoculum in the soil during the non-growing season of the host plant. Wilson (1984) suggested that spore size reflects the amount of nutrients available to spores, and so may influence the growth rate and number of hyphae produced from spores. This means that if a spore begins germination and does not encounter a root it may be able to re-enter a dormant phase and try again, germinating several times if necessary.

Scutellospora is thought to share many of the properties of *Gigaspora*, but there is as yet not enough evidence for this genus. However, these factors may help to explain the low frequency of

both *Gigaspora* and *Scutellospora* in the present study (where frequency is referred to as the number of samples in which the genus was present, regardless of the number of spores).

Glomus and *Acaulospora* on the other hand, occur with much greater frequency at this site. Morton (1993) documents that "almost all parts of the soma of fungal organisms in *Glomus* appear to function as infective propagules". This includes spores, detached hyphae and mycorrhizal roots, and in some cases it has been found that the latter are more infectious than spores, particularly in the case of field-collected soils. More rapid colonisation has been reported from hyphal fragments and colonised root fragments than from spores (Hall, 1976), probably due to the active status of fragments and the time it takes for spores to germinate. Wilson (1984) showed that *Glomus intraradices*, a species that typically forms intraradical spores, was better able to spread into the root via individual entry points than other species tested. The range of propagules produced by *Glomus* also infers that spore count data has little value, other than as a measure of species diversity (Morton, 1993). *Glomus* propagules thus have a wide distribution, occurring in many habitats. *Acaulospora* is thought to share the same properties, except that it is affected by factors such as dormancy, disturbance and drying, which may cause differences in infectivity (Jasper *et al.*, 1989; Tommerup and Abbott, 1981). *I. pes-caprae* was found to be mainly associated with spores of *Glomus* and *Acaulospora*. This host species generally had low spore numbers, high colonisation values, and a high occurrence of intraradical spores and vesicles during winter. It therefore seems that fungal hyphae, infected root fragments and vesicles perpetuated colonisation in this plant, rather than spores. Addy *et al.* (1997) suggested that spores and persistent hyphae might represent two different survival strategies for the fungus: spores have large reserves and are thereby suited to long-term survival, resulting in staggered germination times. Hyphae on the other hand may not be suited to long-term survival, but might have a more rapid recovery of metabolic activity when favourable conditions return. McGee (1989) also suggested that different propagules might have different roles at different times within a habitat, or between different habitats. This wide range of

infective mechanisms may, at least in part, explain the much higher frequencies of these genera in the present study.

Furthermore, Merryweather and Fitter (1998a; 1998b) developed an objective assessment which allowed them to identify different fungal taxa in the roots of bluebell. Using this method, they compared the results with spores extracted from surrounding soil, and found that the types of spores in the soil were not a true reflection of which taxa were colonising the host roots. In particular, they found that *Glomus* spores were rare in field collections, but *Glomus* morphotypes were an important mycorrhizal component of the roots. A previous study on this host plant, using PCR-based methods gave similar results (Clapp *et al.*, 1995).

As a final thought regarding the properties of various fungi, there is another adaptation which may account for high mycorrhizal colonisations in the absence of spores. Many researchers have claimed that carbon is able to be transported between plants by a common mycorrhizal network (CMN) (Read *et al.*, 1985; Grime *et al.*, 1987; Newman, 1988; Simard *et al.*, 1997). Such a mechanism involves the sharing of carbon via hyphal links (of AM or ectomycorrhizal fungi) between plants of the same or different species. This would serve to reduce competition between neighbouring plants. It would also be beneficial to the growth of new seedlings, which could immediately become attached to the existing hyphal network, rather than relying upon the propagules in the soil (Smith and Read, 1997). However, a recent study by Robinson and Fitter (1999) refutes these claims. They report that although large amounts of carbon are transported between linked plants via the CMN, this carbon never reaches the shoots of "receiver" plants, but remains in the roots and most likely within the fungal structures. This would mean that carbon is moved according to the demands of the fungi, not the host. This is an important concept in terms of resource allocation to, and perpetuation of, the fungal symbiont. Whether the host would benefit in that less C would have to be allocated to the endophyte is not known.

5.5.2 ROOT SYSTEMS AND SPORULATION

No detailed examinations were performed on the host roots in this study, but some general observations were made. During the collection of sand samples from the dunes care was taken to remove only sand from the root zones of individual plants of each species. This is difficult in field sampling since roots are often intermingled (Brundrett *et al.*, 1996). Yet in the foredune sampled this was a less challenging task as plant communities were very discrete. The exception to this was the *Ipomoea/Ehrharta* dune, but it is felt that results do reflect the individual components of the root zones of *I. pes-caprae* and *E. villosa*, since the abundance and diversity of spores varies so markedly between the two host plants. This may be a result of very different rooting systems in these plants. *Ipomoea* produces runners and fairly shallow lateral roots which anchor these in the sand. The roots sampled were generally no more than 15cm long. *Ehrharta* had much deeper and more fibrous roots, and lengths of over 1m were often extracted from the sand. Hence it may be that AM fungal communities were quite localised around these different roots, causing the differences observed. Koske and Halvorson (1981) reported that sand samples taken from the same site often had such enormous variations in spore densities that it was not possible to demonstrate significant differences. Part of the explanation may be that some plants were growing in sand that had been colonised for years, while other plants were growing in newly colonised sand. It was mentioned previously that Giovannetti (1985) documented the poor mobility of spores in the dune sand. In such cases one might assume that any spores recovered from a particular root zone were produced there (Koske and Polson, 1984). A very detailed study by Friese and Koske (1991) (mentioned in Section 5.1.4) revealed that high degrees of spore clumping were not significantly correlated with the position of roots, or with the abundance of spore populations only 10cm away. They suggest that the poor dispersal of spores causes their clumping at the site of formation, and that in some cases the roots associated with these aggregations die back and are undetectable. This then leads to seemingly random clumps of spores in the soil. It also means, however, that during any subsequent cycle of root growth any new roots coming into contact with these spores will be infected. This may be particularly important for plants such as *S. plumieri* and *A. populifolia* which

are concentrated into hummocks, and may account for the high spore numbers associated with them (Figure 4.8). Sporulation patterns then, are closely related to the rooting patterns of the host plant. The result of uneven spore patterns is that field sampling will probably never give a true reflection of spore abundance and diversity in a particular site. Sampling techniques will also affect this (Section 5.3.3).

5.5.3 INTERACTIONS BETWEEN FUNGAL GENERA

Arbuscular mycorrhizal fungi are not known to be host specific, though they may show preferences for some host plants over others (Smith and Read, 1997). What is not well understood is how different fungal genera or species interact within the same host, and to what degree competition between them regulates host colonisation. It is unlikely that all fungal species contribute equally to the functioning of the symbiosis (Merryweather and Fitter, 1998a) yet it is difficult to separate colonisation by different fungi within host roots, and spores do not give a realistic interpretation of which fungi may be present inside roots. Different isolates of AM fungi have been shown to result in different effects on plant growth (Jakobsen *et al.*, 1992), but few studies have shown the effects of different fungal isolates on plant growth in an ecological context (Sanders *et al.*, 1996).

Research by van der Heijden *et al.* (1998a) indicates that naturally co-occurring AM fungal species have the potential to determine plant community structure. The authors showed firstly that plant species differ in their mycorrhizal dependency, secondly that a mixture of AM fungal species caused different growth responses in different host plants, and thirdly that plant species respond differently to single AM fungal species, or to a community of AM fungal species. These results highlight the need for ecological studies to consider the composition of the fungal communities, and the effect these have on plant community structure. In the present study, detailed experiments regarding the effect of the fungal community on the host plants was not conducted. However, the fungal genera underwent distinct seasonal variations (Figure 4.11) with respect to their association with specific host plants.

Spore numbers, regardless of plant species, were highest for each fungal genus around the winter months (Table 4.8). Highest spore numbers from all genera were extracted from sand in May, and the next highest numbers for all except *Gigaspora* were in July. Yet these high spore numbers were associated with only some of the plant species (Figure 4.11), mainly *S. plumieri* and *A. populifolia* in May, and *S. plumieri* and *C. monilifera* in July. Further, the main contributor to these spore numbers was *Scutellospora* which had much lower numbers at any other time of the year. *I. pes-caprae* exhibited consistently low associations with fungal spores, and most of the spores found in this root zone belonged to *Glomus* and *Acaulospora*. It is likely that the few spores found in September and December were those produced within the roots as intraradical spores during the winter months.

Gemma *et al.* (1989) reported that interspecific competition between fungal species in a Massachusetts sand dune determined the spore abundance of the five fungal species studied. A high spore density of a particular species in a sample was associated with low numbers of other species in the sample. This seems to be somewhat true regarding the fungal genera in the present study, although some exceptions to this exist. During all months, *Scutellospora* propagules were associated with regions on the foredune and plant species that exhibited high spore numbers and a reasonably high fungal diversity. This was unlike *Glomus*, which occurred in all areas of the foredune during most sampling times (Figure 4.11). *Acaulospora* propagules showed the same tendency, though in lower numbers. Interestingly, *Acaulospora* generally occurred in the same sites as *Glomus* spores – i.e. it very seldom occurred on its own or in association with only spores other than *Glomus*. Propagules of *Gigaspora* were only really prevalent in February and May, occurring in the root zones of all plant species concurrently with all other fungal genera, particularly during the former month. In May, the highest *Gigaspora* numbers for the year were found in association with *A. populifolia*, whilst numbers were very much lower in the other plant species during this month.

At least once during the year, each plant species (with the exception of *I. pes-caprae*) appears to have a "turn" at being associated with the greatest spore numbers, coupled with the greatest diversity of spores as compared to the other plant species. This occurs in *C. monilifera* in both December 97 and September 98; *E. villosa* in February and December 98; *A. populifolia* in May (spore numbers are the same as for *S. plumieri* during this period, but the diversity is greater in *A. populifolia*), and *S. plumieri* in July. Koske and Halvorson (1981) also reported that individual samples showed a positive correlation between spore density and fungal diversity. Gemma *et al.* (1989) interpreted seasonal differences between fungal species as being either a strategy that would minimise direct competition for the host substrate, particularly during sporulation, or being the result of one species out-competing the others for host photosynthate or cortical cells. Thus it seems that the effect of different fungal genera on the same host is an important factor to consider in exploring the results of this and other studies.

5.5.4 PLANT-FUNGUS INTERACTIONS

Different plant-fungus combinations have been shown to produce fairly large variations in percentage colonisation of host roots. This may be linked to the growth rate of roots, the susceptibility of different roots to AM fungi and differences in root colonising strategies of fungi (Smith and Read, 1997). Although AM fungi are not host specific (Chapter 1) there are degrees to which different plants, even within the same species, may become mycorrhizal. To quote Smith and Read (1997): "The converse is also true: different species or isolates of fungi colonise the roots of the same species of plant to different extents and in a few cases the range of potential partners appears so restricted as to constitute specificity".

Sanders *et al.* (1977) examined the spread of infection and effects of four species of AM fungi (three *Glomus* and one *Gigaspora*) on the growth of *Allium cepa*. They found that *G. mosseae, G. 'macrocarpus* var. *geosporus'* and *Gi. calospora* (now *S. calospora*) colonised roots quickly, and increased P uptake and growth of the plant, whereas *G. 'microcarpus'* was slow in colonising roots

and had a negligible effect on P uptake and plant growth. Numerous other authors have performed similar studies which identify the various types of fungi found within specific plant roots, or investigate the effects of inoculating plants with several fungal taxa (Giovannetti and Hepper, 1985; Douds *et al.,* 1998; van der Heijden *et al.*, 1998a, 1998b; Boddington and Dodd, 1998; Merryweather and Fitter, 1998a, 1998b).

Although spore types do not give an accurate or complete reflection of the AM fungi present within host roots, they do give an indication of the types of associations that are present, particularly with respect to the Gigasporineae. The results from this study indicate that all plant species were associated with each of the four fungal genera, with the exception of *I. pes-caprae*, which was never associated with *Scutellospora.* The extent to which any particular fungus colonised each plant host was beyond the scope of this study. However, this concept requires mention here since it may be a causal factor of the percentage colonisation and spore numbers associated with the different host plants.

CHAPTER 6

CONCLUSIONS

This study found that all plant species sampled on the dune were mycorrhizal and that typical AM structures such as arbuscules and vesicles occurred in many of the roots examined. Arbuscules in particular were very prevalent, confirming the identity of the fungal endophyte within host roots. Spores were effectively extracted and identified to genus level, although this was often difficult given their varying conditions. However, the results of spore numbers, genera and tentative species identifications are comparable with those of other dune mycorrhiza studies. Spore numbers differed, sometimes considerably, between the root zones of different plants. Both colonisation and spore data seemed to be affected by the seasons to some degree, but this seemed to depend more upon the seasonal cycles of the individual plant species than on seasonality *per se*. It is thought that this may be due to the transitional nature of the climate in this region, as well as to the fact that seasonal changes in climate may not be as marked as in temperate regions. Hence fungal isolates may be more sensitive to daily or weekly climatic changes than to seasonal changes over months. This would have to be tested with more frequent sampling and perhaps a greater number of replicates. The position of plants along the profile did not seem to influence AM colonisation to a great degree, and it is thought that this is more likely due to the different rooting strategies of the dune plants. Position along the foredune did seem to have more of an influence on spore numbers. However, the dependence of mycorrhizas on plant position in the dune would probably be more evident if a full dune succession was sampled. The AM component of the rhizosphere could then be related to changes in species richness and diversity, as well as percentage cover, edaphic factors etc. Such sampling might also offer the opportunity of examining the same plant species from different successional stages on the dune. Depth appeared to play a significant role in the percentage colonisation of *Scaevola* plants, with deeper roots supporting a much higher AM fungal component. This needs to be examined further in all dune plant species, with roots sampled at set

depths. The lack of correlation between spore numbers and percentage colonisation is a reflection of the properties of different types of propagules in the soil. Studies which identify and quantify the mycorrhizal component of roots, as well as the extraradical mycelium would bridge the gap between colonisation and soil-borne spores. In some cases plant species had greater affinities with the spores of particular fungal genera, but this seemed to depend very much on season. Most AM fungi were associated with most plants at some stage, although the timing of this differed between hosts. This may have been influenced by competitive interactions between fungal groups. The bioassays confirmed that viable propagules were present in the dune soil and that the *Scaevola* hummock supported a greater inoculum potential than the *Ipomoea* dune. However it may be more correct to say that the rate of infection was faster in plants grown in *Scaevola* sand. Bioassays could be run for a longer period of time, with more replicates, to allow the harvesting of plants at set intervals in order to show the progression of root infection. Trap cultures could also be propagated from dune sand in order to bulk and purify spores and make more conclusive identifications.

Several other areas of research could build upon the results of this study. The extent and role of the external fungal mycelium in the soil needs to be elucidated. This could include an assessment of the sand aggregating properties of hyphae using techniques such as the assessment of glomalin content in the soil. Molecular studies could be used to identify the different AM fungi present within roots or soil. The presence of other soil microbes which occur in soil and roots alongside AM fungi also needs to be examined in this ecosystem, and their role and effects on both plants and AM fungi needs to be examined. Physiological studies should be undertaken to measure the functional role of mycorrhizas in nutrient uptake and exchange, as well as carbon partitioning by the plant. Furthermore, seedlings need to be studied with respect to the rate at which colonisation is initiated in their roots.

In any site, the unique combination of climatic, edaphic, host plant and other factors will influence the final result of mycorrhizal assessments, as will sampling techniques and different methods of

extraction and assessment. Therefore it is difficult to address all the questions of how a particular system functions. Yet an appreciation of all the components of an ecosystem is a start in helping us to build up a more integrative approach to an understanding of ecosystem functioning. This study met its stated objectives and provided an assessment and quantification of mycorrhizal distribution in this system. This knowledge is important in paving the way for more in-depth studies of specific aspects on the role of this mutualistic relationship in South African dunes. The Eastern Cape has a particularly striking coastline, which is often exposed to heavy usage. It is also vulnerable to the effects of building and construction. Dune maintenance and often stabilisation and rehabilitation are becoming increasingly important in some regions along the coast. Any coastal vegetation survey or revegetation project should also account for the arbuscular mycorrhizal component of the rhizosphere. As Koske and Polson (1984) wrote: any "mechanical, environmental or chemical factors that reduce or eliminate AM fungi from dune soils could have serious consequences to dune stabilisation programs". Moreover the diversity and functioning of the fungal component of this ancient symbiosis makes a challenging and fascinating study.

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