ARBUSCULAR MYCORRHIZAL ROOT COLONISATION AND THE SUBSEQUENT HOST PLANT RESPONSE IN YOUNG GRAPEVINES IN A SOUTH AFRICAN COMMERCIAL VINEYARD

By

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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SUMMARY

Arbuscular mycorrhizal (AM) fungi facilitate the uptake of nutrients, improve growth and alleviate drought stress in grapevines. Consequently, AM fungal root colonisation contributes to the optimum performance of grapevines. It is for this reason that young grapevines are sometimes inoculated with commercial AM fungal strains to reduce environmental stresses during transplant. In the past, soil fumigation has often been considered as a prerequisite for soil conditioning with commercial AM fungal strains. However, grape growers opting to inoculate with these fungal strains will have to do so in unfumigated soils, since the use of fumigants in South African agricultural soils is currently being phased out. Since little is known about the nature and scope of indigenous AM fungi that may be present in SA vineyard soils, it is difficult to predict the grapevine's response to artificial inoculation in soils already containing adequate concentrations of these fungi.

In the first part of the study, commercially available AM inocula were tested under field conditions that would prevail on a typical farm. This entailed measuring vine growth, nutrition, drought stress resistance and percentage root colonisation, over two consecutive seasons, from the onset of planting new commercial grapevines. The field trial carried out at Groenland, a commercial farm in the Stellenbosch Region. Merlot grafted onto 101-14 Mgt, 110 Richter (110 R) and 99 Richter (99 R), was planted in December 1998. These rootstocks were selected to accommodate different soil forms: 101-14 Mgt and 110 R on a Westleigh soil form, which was ridged and 99 R on an unridged Fernwood soil form. Vine roots were inoculated during planting with different AM inocula, i.e. Biocult®, Vaminoc® and *Glomus* sp. 1054. One treatment was left uninoculated and treated with a combination of the fungicides Benlate® (active ingredient: benomyl) and Rovral Flo® (active ingredient: iprodione). The control received neither fungicides nor inocula.

Microscopic examination of the vine roots revealed that, apart from a significantly higher level of root colonisation observed in Biocult-treated 99 R vines during the first season, the level of AM root colonisation was similar in both the uninoculated (control) and inoculated vines. Infected control vines indicated that indigenous AM fungi were present in the vineyard soil. This level ranged between 40% and 85%, and 70% and 90% in the first and second season, respectively. Apart from the significant growth improvement observed in 110 R vines inoculated with *Glomus* sp. 1054 during the first season, no growth improvement was observed for the other rootstocks or treatments. Furthermore, generally no alleviation of water stress and nutritional benefits could be detected for both the seasons. Despite this, less than 1% dieback was recorded for the vines.

In the second part of the study, additional information on the diversity of indigenous AM fungal species was obtained, which included the quantification and identification of these fungi present in the soil. The AM fungal spore numbers in the vineyard soil ranged from 1000 to 3779 spores/100 g dry soil. The results confirmed that the majority of AM fungal species found in the soil was not part of the commercial inocula, but originated either from the vineyard and/or the nursery where the vines were obtained. The uncovered AM fungal species belong to the genera *Acaulospora*,

Gigaspora, *Glomus*, *Sclerocystis* and *Scutellospora*. This is similar to the AM fungal genera recorded in vineyards by other workers.

To the best of our knowledge, this study provided the first documented evidence on the diversity of indigenous AM fungi present in SA vineyard soils. Although it may be preliminary in nature, the results clearly showed that a wide diversity and abundance of indigenous AM fungal populations may occur in a typical SA vineyard. Depending on the superiority and possible masking effects on the part of the indigenous AM fungal populations, positive responses to inoculation with commercial AM fungal strains in grapevines grown in such vineyard soils may consequently be unlikely. Thus, before reconditioning of vineyard soils with these fungi can commence, it is essential for farmers to first assess the mycorrhizal status of their soils and nursery vines. Since the majority of SA farmers are not yet familiar with inoculation practices and are still unacquainted with the mycorrhizal status of their soils, the findings from this study could be of great benefit to particularly wine grape growers opting to inoculate with commercial AM fungal strains on a large-scale.

OPSOMMING

Blaasagtige Struikvormige Mikorrisa (BSM) swamme bevorder die opname van voedingstowwe, verbeter groei en verlig droogtestres in wingerd. Gevolglik is die kolonisasie van wingerdwortels deur hierdie swamme voordelig om die prestasie van wingerd te optimiseer. Om hierdie rede word jong wingerd somtyds op kommersiële skaal met mikorrisa swamme geïnokuleer om omgewingstres tydens oorplanting te oorkom of te verminder. In die verlede is grondberoking dikwels as 'n voorvereiste beskou vir grondkondisionering met kommersiële BSM swamme. Boere wat van voorneme is om met BSM te inokuleer, sal dit egter in onberookte grond moet doen, aangesien beroking tans in SA uitfasseer word. Omdat daar baie min bekend is oor die voorkoms en aard van inheemse mikorrisa spesies, is dit moeilik om te voorspel wat die wingerdreaksie na kunsmatige inokulasie sal wees in grond wat reeds genoegsame konsentrasies mikorrisa swamme bevat.

In die eerste deel van die studie is kommersieel beskikbare BSM inokulums getoets onder veld kondisies wat op 'n tipiese plaas sou heers. Dit het die meting van groei, voeding, droogtestres en persentasie wortel kolonisasie van jong wingerd oor twee opeenvolgende seisoene ingesluit en het onmiddelik 'n aanvang geneem na die jong wingerdstokkies geplant is. Die proef is op die plaas Groenland, net buite Stellenbosch uitgevoer. Merlot, geënt op 101-14 Mgt, 110 Richter (110 R) and 99 Richter (99 R), is geplant in Desember 1998. Die onderstokke 101-14 Mgt en 110 R was geplant op operdwalle op 'n Westleigh grondvorm en 99 R op 'n Fernwood grondvorm (geen operdwalle nie). Wingerdwortels is tydens plant geïnokuleer met verskillende BSM inokulums. Die inokulums was Biocult, Vaminoc and *Glomus* sp. 1054. Een behandeling was ongeïnokuleerd gelaat en behandel met 'n kombinasie van die swamdoders Benlate® (aktiewe bestandeel: benomiel) en Rovral Flo® (aktiewe bestandeel: iprodioon). Die kontrole het nóg swamdoder nóg inokulum ontvang.

Mikroskopiese ondersoeke het getoon dat behalwe vir 'n betekenisvolle hoër vlak van wortelkolonisasie by die Biocult-behandelde 99 R stokke, die vlak van wortelkolonisasie in ongeïnokuleerde (kontrole) en geïnokuleerde wingerdstokke soortgelyk was. Gekoloniseerde kontrole stokke het gedui op die teenwoordigheid van inheemse BSM fungi in die wingerdgrond. Die vlak van gekoloniseerde stokke het tussen 40% en 85% tydens die eerste seisoen en tussen 70% en 90% tydens die tweede seisoen varieer. Behalwe vir 'n betekenisvolle toename in groei wat tydens die eerste seisoen by 110 R stokke, geïnokuleer met *Glomus* sp. 1054 waargeneem is, is geen ander groeiverbeterings by ander stokke of behandelings waargeneem nie. Verder kon geen algemene verligting van waterstres en voedingsvoordele tydens beide seisoene waargeneem word nie. Ten spyte hiervan, is minder as 1% terugsterwing aangeteken vir die wingerdplante.

In die tweede deel van die studie is addisionele inligting bekom rakende die diversiteit van inheemse BSM spesies. Dit het die kwantifisering en identifisering van BSM swamme ingesluit. Die BSM spoorgetalle in die wingerdgrond het tussen 1000 en 3779/100 g droë grond varieer. Die resultate het bevestig dat die meerderheid BSM spesies wat in die wingerdgrond teenwoordig was, nie deel van die oorspronklike kommersiële inokulums was nie en was óf inheems tot die wingerd óf het sy oorsprong in die kwekery vanwaar die wingerdplante afkomstig is. Die spesies wat identifiseer is, is díe van die genera *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. Dit stem ooreen met BSM genera wat voorheen al in wingerd geïdentifiserd is.

Tot die beste van ons wete, is hierdie studie die eerste van sy soort in 'n SA wingerd. Hoewel die studie voorlopig van aard is, wys die studie duidelik dat 'n wye diversiteit en genoegsame hoeveelhede natuurlike populasie BSM swamme in 'n Suid-Afrikaanse wingerd mag voorkom en dat kunsmatige toediening van kommersiële BSM swamme onder hierdie kondisies, nie noemenswaardige voordele vir die wingerd inhou nie. Daarom is dit van kardinale belang vir boere om eers vertroud te raak met die BSM status van hulle wingerdgronde alvorens hulle met BSM swamme op groot skaal inokuleer. Aangesien die meerderheid SA boere nog nie vertroud is met die inokulasie prosedure nie, kan die inligting wat uit hierdie studie gegenereer is, baie voordelig wees vir boere wat oorweeg om op groot skaal met BSM te inokuleer.

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CHAPTER 1

LITERATURE REVIEW

1.1. INTRODUCTION

In South Africa, a low survival rate of young vines often occurs as a result of a number of adverse conditions, such as drought, heat, saline soils and diseases (van der Westhuizen, 1981). To minimise stress related to diseases in newly planted vines, some soils are fumigated before planting (de Klerk, 1981). However, vineyard establishment on fumigated soils often results in production losses due in part to side effects of fumigants that lead to the destruction of beneficial soil organisms including fungi (Menge *et al.*, 1978a; Menge *et al.*, 1983; Skinner *et al.,* 1988). Furthermore, the use of fumigants in countries such as South Africa is currently being phased out, primarily for health reasons (Koch *et al.*, 2002). It is for the above reasons that inoculation of vine roots with arbuscular mycorrhizal (AM) fungi has been considered as an environmentally friendly alternative to fumigation.

These fungi were shown to be beneficial in the production of both pot-grown and field-grown grapevines. Using sterilised soil as growth medium, it was found that various AM fungal species positively affected the growth and development of young pot-grown vines under greenhouse conditions (Menge *et al.*, 1983). Other pot trial studies have shown that AM fungi allow for greater uptake of nutrients, thus stimulating vine growth (Deal *et al.*, 1972; Karagiannidis *et al.*, 1995; Biricolti *et al.*, 1997). Furthermore, it is known that AM fungi play an important role in plant water relations (Safir *et al.,* 1971; Hardie & Leyton, 1981; Sieverding, 1981) and may alleviate drought stress in vines.

Under field conditions, AM fungi are known to naturally colonise grapevine roots (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Gebbing *et al.*, 1977; Menge *et al.*, 1983; Nappi *et al.,* 1985; Schubert & Cravero, 1985). When subjected to the influence of the indigenous AM fungi in the field, the performance of inoculant AM fungi may differ from that in pot trials under sterile conditions. However, on commercial farms where crops were inoculated with AM fungi in unfumigated soils it has been demonstrated that growth responses as a result of AM inoculation may occur under these conditions (M. Venter, personal communication, 2000). To promote positive responses in young vines it is important that the artificially inoculated AM fungal species be more efficient than the indigenous AM fungal populations. For practical purposes, successful selection of superior AM fungi can only be achieved if their performances are evaluated in the field in unfumigated soil (Abbott & Robson, 1982; Menge, 1983; Schubert & Cravero, 1985).

The aim of this study was therefore to evaluate commercially available AM inocula under field conditions that would prevail on a typical farm. This entailed measuring growth responses, nutrition, drought stress resistance and percentage root colonisation in young commercial grapevines. In addition, to obtain supportive evidence for the presence of indigenous AM fungi in the vineyard, AM fungal spores were isolated from the soil and identified using spore morphology as criterion. However, to fully understand the results of such an investigation, it first was

necessary to highlight the importance of the South African viticulture and wine industries and to review the literature on the biology of AM fungi.

1.2. VITICULTURE IN THE WESTERN CAPE – A PREMIUM WINE PRODUCING REGION

The South African viticulture industry is based mainly in the Western Cape (SAWIS, 2001). More than 90% of all vines planted in this region are used for the production of wine. Only a minor proportion (6-7%) of cultivated vines, are used to produce table grapes. The rest are rootstock mother block plantations. Consequently, it was decided to limit the following discussions only to relevant matters related to wine production in South Africa, particularly in the Western Cape.

1.2.1. Historical overview: Vineyards were first established in South Africa in 1657, two years after Jan van Riebeeck imported the first vines from western France (du Plessis, 1947; SA Wine Industry Directory, 2001). A mere 1200 vines were planted then. But viticulture was soon becoming an established agricultural practice and vines seemed to be adapting particularly well to local conditions. Two years later, on 2 February 1659, the first wine was pressed at the Cape. Governer Simon van der Stel subsequently established a wine estate at Constantia on the lower slopes of Table Mountain. With the arrival of the French Huguenots in 1688, invaluable knowledge of winemaking was brought to the Cape that gave the young wine industry a welcome boost (SA Wine Industry Directory, 2001). During the following two centuries, Cape viticulture underwent a relatively trouble free period in terms of diseases and was soon becoming a competitive industry which grew beyond the boundaries of the Cape Peninsula. In those days the Western Cape vineyards constituted more than 120 million vines, which covered an area of an estimated 44 000 to 45 000 acres, of which 18 million and 13 million vines were planted in Paarl and Stellenbosch, respectively (du Plessis, 1947).

However, over time, the Cape vineyards became infected with several grapevine diseases and in the mid 19th century powdery mildew made its first appearance in South Africa (du Plessis, 1947). Consequently, the Vine Disease Commission was established in 1880, with the main task to investigate diseases in South African vineyards and to assess subsequent damage to vines. Not long after the establishment of this commission, the South African vine industry suffered a major setback due to a severe phylloxera outbreak, which resulted in the destruction of millions of vines. It took the industry several years to recover from this epidemic. However, global politics and market forces send the industry through a troublesome period, culminating in a state of near chaos in 1918 (SA Wine Industry Directory, 2001). It took several developments and initiatives by key role players to reverse this state of affairs, such as legislation that was introduced in 1973, which enabled the South African wine industry to certify wine for the first time under the Wine of Origin System.

1.2.2. The current state of affairs: There are about 4501 primary wine producers in South Africa (SAWIS, 2001). Of the 355 wine cellars, 277 belong to private owners, 69 are co-operative

wine cellars and 9 are producing wholesalers. According to the latest surveys, the total area under cultivation for wine grape production is 105 566 hectares, while 10 985 hectares are used for table grape production. The total quantity of grapes crushed in South Africa amounts to more than a million tons each year of which 65% is utilised in the production of wine; 3% in brandy distillation; 17% for conversion into grape concentrate and 15% in the production of grape spirit. South Africa currently ranks 16th in the world in terms of area used for vineyards. Annually, it produces close to one billion liters of wine, which makes it the seventh-largest wine producer in the world, producing 3.2% of the world's wine. The total wine export has more than doubled over the last 6-7 years.

In addition to the abovementioned wine production, the founding of the Wine Routes, in 1973 has become a major tourist attraction. South Africa is currently divided into 12 wine producing regions (SA Wine Industry Directory, 2001). These include: Constantia, Durbanville, Franschhoek, Klein Karoo, Olifants River, Orange River, Paarl, Robertson, Stellenbosch, Swartland, Tulbagh and Worcester. Each of these regions makes substantial contributions to the South African wine industry each year

1.2.3. Future prospects: The future of the South African wine industry will greatly depend on its ability to sustain itself. Initiatives such as Vision 2020 is just one of many ways to achieve this goal (SA Wine Industry Directory, 2001). Vision 2020 is managed by the Wine Industry Network of Expertise and Technology (Winetech) in collaboration with key role players in the industry and government. The goal of this initiative is to develop a highly successful and international competitive South African wine, brandy and grape juice industry. To achieve this goal, a number of challenges, have yet to be overcome which include amongst others, maintaining good quality plant material and effective control over grapevine diseases. Young grapevines at planting are particularly vulnerable to environmental setbacks and diseases, which is evident from the great number of losses each year, which amounts to thousands of rands. These replant problems needs to be seriously dealt with.

1.2.4. Replant problems: It was stated that if not addressed properly, replant problems may have high cost implications for the South African viticulture industry (SA Wine Industry Directory, 2001). Replanting grapevines on land on which vines were cultivated previously may result in severe growth impairment, such as stunted growth and root system damage (Waschies *et al.,* 1993). A number of factors may contribute to replant problems associated with grapevines.

In South Africa, poor vineyard establishment often occurs as a direct result of environmental and physiological stress factors (van der Westhuizen, 1981). Severe weather conditions have been shown to be particularly costly to the viticulture industry. However, it was also found that replant problems may occur as a result of soil-borne pathogens commonly associated with roots of diseased vines (Deal *et al.*, 1972; Waschies *et al.*, 1993; Waschies *et al.*, 1994). Increases in

populations of fluorescent *Pseudomonas* spp. prior to the reduction in root and shoot weights, indicated that these species were at least partly responsible for replant disease, possibly by increasing the susceptibility of grapevine roots to soil-borne pathogens (Waschies *et al.*, 1994). Pests such as phylloxera (Deal *et al.*, 1972; Granett *et al.*, 1998) and nematodes (Atilano *et al.*, 1981) have also been associated with low survival rates after replanting of vines. Attempts to control these deleterious rhizosphere microflora with fumigants often resulted in the destruction of beneficial soil organisms, which in turn resulted in stunting of vines and loss of production (Menge *et al.*, 1978a; Menge *et al.*, 1983; Skinner *et al.*, 1988). One such group of beneficial organisms is mycorrhizal fungi. It was stated that if left untreated, replant decline might persist in vineyard soils for several years (Waschies *et al.*, 1994). Consequently, to limit replant decline, young vines are occasionally inoculated with commercial cultures of mycorrhizal fungi before planting (M. Venter, personal communication, 2000). However, limited data is available on the response of young vines in vineyards to these inoculations.

1.3. ORIGIN AND TYPES OF MYCORRHIZAL ASSOCIATIONS

The term, "mycorrhiza", was suggested by A. B. Frank in 1885 to describe fungus-tree associations (Harley, 1969). This term is derived from a combination of two words namely, "mykes" (Greek) meaning fungus, and "rhiza" (Latin) meaning root. Currently, "mycorrhizae" refers to highly evolved, mutualistic associations between soil-borne fungi and the roots of about 80% of the known vascular plant species (Harley & Smith, 1983; Brundrett, 1991). In this association, the fungal partner essentially acts as an extension of the root system, by absorbing nutrients for the plant host via an extensive hyphal network, and in return, the plant supplies a constant flow of catabolised photosynthetically derived carbon compounds to the fungus. These fungi are therefore obligate biotrophs that are unable to grow and reproduce separately from a plant host. In the past, mycorrhizae were grouped in only three classes, based on the relationship of the fungal hyphae with the root cells namely, ectomycorrhizae (EM), ectendomycorrhizae and endomycorrhizae (Alexopoulos & Mims, 1979). However, Harley & Smith (1983) proposed a new classification system based on the type of host plant as well as the type of fungus involved. Currently, mycorrhizae are grouped into at least seven types including vesicular-arbuscular, ecto, arbutoid, ericoid, monotropoid, orchid and ectendo (Brundrett, 1991; Smith & Read, 1997). Of these, the ectomycorrhizae and vesicular-arbuscular mycorrhizae are the most widespread and therefore the best described.

Ectomycorrhizae (EM), of which the origin roughly dates back to about 200 million years ago, form hyphae between the root cortical cells resulting in a netlike structure called the Hartig net (Cairney, 2000). Apart from this diagnostic feature, EM fungi also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root. Ectomycorrhizae are mainly associated with the roots of woody plants such as trees.

The term endomycorrhizae, which refers to all mycorrhizal types where the fungus grows into the cortical cells and where no Hartig net is formed (Peyronel *et al.,* 1969), also includes the vesicular-arbuscular mycorrhizae (VAM). The term VAM originally applied to symbiotic associations formed by all fungi in the order Glomales (Morton & Benny, 1990). However, because a major suborder in this order lacks the ability to form vesicles in roots, the term arbuscular mycorrhiza (AM) is now commonly accepted. Arbuscular mycorrhizae form associations mainly with herbaceous plants such as legumes and grasses, but also with certain types of trees.

The ectendo mycorrhizae are intermediate mycorrhizal types that exhibit characteristics of both the ecto- and endomycorrhizae. This type of mycorrhiza is usually associated with coniferous and deciduous hosts in nurseries and burned forest sites. Ectendomycorrhizae form a typical ectomycorrhizal structure and the hyphae in the Hartig net may penetrate the root cortical cells.

The investigation into the origin and history of mycorrhizae is undertaken in a variety of ways, but is basically dependant upon the discovery and study of fossil remains. Several scientists gave accounts of such fossils (Harley, 1969; Taylor *et al.,* 1995) and may have added appreciably to our knowledge of the origin and history of mycorrhizae. One of the most significant discoveries in the search for paleontical evidence regarding the origin of mycorrhizae, was made recently when a 400 million year old mycorrhizal fossil from the Devonian period was found in Rhyne chart (Taylor *et al.,* 1995). In their paper, the authors reported on the first unequivocal evidence of arbuscules in an endomycorrhizal symbiosis. The ancient chart provided a wealth of information not only of early terrestrial plants, but also of the fungi that inhabited this paleoecosystem. One most significant fact that was deduced from these fossil remains is that the origin of mycorrhizae coincided with the appearance of the first terrestrial plants about 400 million years ago. Most recent publications have reconfirmed the origin of AM fungi in which their age is estimated between 450 to 500 million years (Cairney, 2000). Available evidence points largely to an ongoing parallel evolution of the partners in response to environmental changes (Cairney, 2000). Thus, given the fact that this is one of the oldest fungal groups and that these fungi are alive and well to this day, mycorrhizae must surely be of a selective advantage for both the fungus and the plant (Harley, 1969).

1.4. OVERVIEW OF MYCORRHIZAL TAXONOMY

Several landmark publications have led to the current classification system for mycorrhizal fungi. In the 1800's, three genera were erected to accommodate these fungi, namely *Endogone* (Link, 1809); *Glomus* (Tulasne & Tulasne, 1845) and *Sclerocystis* (Berkeley & Broome, 1873). Five decades later, several species were transferred from *Endogone* to *Glomus* (Gerdemann & Trappe, 1974). These genera were included in a single family, the Endogonaceae, which was accommodated in a single order, Glomales (Benjamin, 1979). Two other genera were added,

namely *Acaulospora* and *Gigaspora*, bringing the number of genera in this family to five. Another two genera were later added to the Endogonaceae, *Entrophospora* (Ames & Schneider, 1979) and *Scutellospora* (Walker & Sanders, 1986). The present-day family Glomaceae was erected to accommodate *Glomus* and *Sclerocystis* (Pirozynski & Dalpé, 1989).

About a decade ago, another two families were erected, Gigasporaceae (suborder Gigasporineae) to accommodate *Gigaspora* and *Scutellospora*, and Acaulosporaceae (suborder Glominea) to accommodate *Acaulospora* and *Entherophospora* (Morton & Benny, 1990). They placed these suborders in the order Glomales (phylum Zygomycota, class Zygomycetes). The genus, *Glomites* was erected by Taylor *et al.* (1995) to accommodate fossil fungi that closely resemble modern-day *Glomus* species. Two new families of Glomales were erected recently, Archaeosporaceae and Paraglomaceae, consisting of two new genera, *Archaeospora* and *Paraglomus*, respectively, based on concordant molecular and morphological characters (Morton & Redecker, 2001). Most recently, a new monophyletic fungal phylum, the Glomeromycota, was proposed, based on natural relationships for the AM fungi and related fungi (Schübler *et al.*, 2001). Consequently, AM fungi together with the endocytobiotic fungus, *Geosiphon pyriformis*, were removed from the polyphyletic Zygomycota. Apart from the order Glomales, or orthographically more correctly, the Glomerales (as suggested by the latter researchers), three new orders were placed under the Glomeromycota, namely, Archaeosporales, Paraglomerales and Diversisporales. The Glomerales still represents many of the "classical glomeralean" species as understood by Morton & Benny (1990). Today AM fungi consist of approximately 160 species. The greater part of these species has been described over the last two decades, which is an indication of the increased interest in these organisms.

1.5. GENERAL CHARACTERISTICS OF THE ROOT COLONISATION STRUCTURES AND DEVELOPMENTAL STAGES

To optimally utilise morphology in the taxonomy of AM fungi, it is essential to understand the role of the different fungal structures in the ontogenic stages of an AM fungus. The components of root colonisation and the process of AM fungal ontogeny in angiosperms were reviewed by Bonfanto-Fasolo (1984). Root colonisation by AM fungi hardly ever induces root alterations recognisable with the naked eye, in which case a dissecting and compound microscope are needed to grasp the complexity of the process (Brundrett *et al.,* 1996).

According to Brundrett *et al.* (1996), AM root colonisation occurs in two main phases (Fig. 1), an external phase (in the soil with external hyphae and spores scattered around the roots) and an internal phase (inside root tissue with intraradical, unbranched hyphae, intercellular hyphae, arbuscules and vesicles).

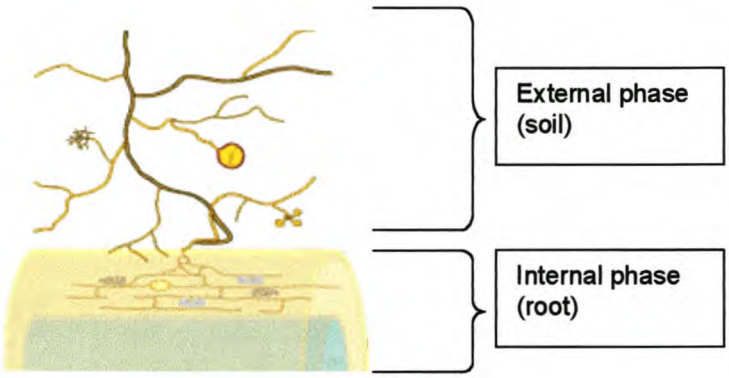

FIGURE 1
A schematic representation of the external and internal phases of the root colonisation process. (Adapted from Brundrett, 2001).

In the following paragraphs the different components of AM association mentioned above, are discussed in more detail:

1.5.1. Soil hyphae: Soil hyphae, also known as extraradical hyphae or external hyphae, are filamentous fungal structures that ramify through the soil (Brundrett *et al.,* 1996). These hyphae are believed to be responsible for nutrient acquisition, propagation of the association and spore formation. Soil hyphae may either originate from germinating spores or from fragments of roots, but in many cases a pre-existing network of hyphae is already present in the soil resulting from previous root activity. Soil hyphae resulting from spore germination have a limited capacity to grow and may die if they do not encounter a susceptible root (Kabir *et al.*, 1997). Interestingly, in *Scutellospora* species, hyphae emerge from a germination shield within the spore (Brundrett *et al.*, 1996). Generally, AM fungi produce soil hyphae of various morphologies and functions including thick "distributed" or "infective" hyphae to thin "absorptive" hyphae as well as "fertile" (spore-bearing) hyphae. The finer hyphae may produce "branched absorptive structures" (BAS), also known as arbuscular-like structures (ALS), in which the hyphae proliferate to form small groups of dichotomously branched hyphae in the soil (Bago *et al.,* 1998). It was found that BAS immediately form after fungal penetration of the host and establishment of the symbiosis. The average period for BAS to develop was found to be seven days, after which the hyphae degenerated, becoming empty septate structures. Certain BAS were closely associated with spore formation, appearing at the spore's subtending hypha. Apparently, these spore-associated BAS initiate spore formation. It was also suggested that BAS may play a role in nutrient uptake by the fungus. Typical BAS are illustrated in Figure 2 A and B.

FIGURE 2
(A) Branched absorptive structures formed by *Glomus proliferum*. (Brundrett, 2001).
(B) Branched absorptive structures formed by *Glomus intraradaces*. (Morton, 2001).

Currently, duration of growth and other properties of each hyphal type are poorly understood. However, infective hyphae are believed to be responsible for the initiation of new points of infection or colonisation on the same root, other roots of the same plant or on the roots of adjacent plants (St John *et al.*, 1983). AM fungal species of the suborder Gigasporineae, have extraradical hyphae that branch into clusters of thin walled, swollen cells containing spines or knobs (Brundrett *et al.*, 1996). These cells are known as accessory bodies, auxiliary cells or external vesicles. The morphology and function of these cells will be discussed later.

1.5.2. Root contact and penetration: Mycorrhizal associations are initiated when soil hyphae respond to the presence of a root growing towards it, establishing contact and growing along the surface of the root (Brundrett *et al.*, 1996). Next, one or more hyphae swell apically and increase in size to form appressoria between adjacent epidermal cells of the root. Figure 3 is a schematic representation of the root colonisation process.

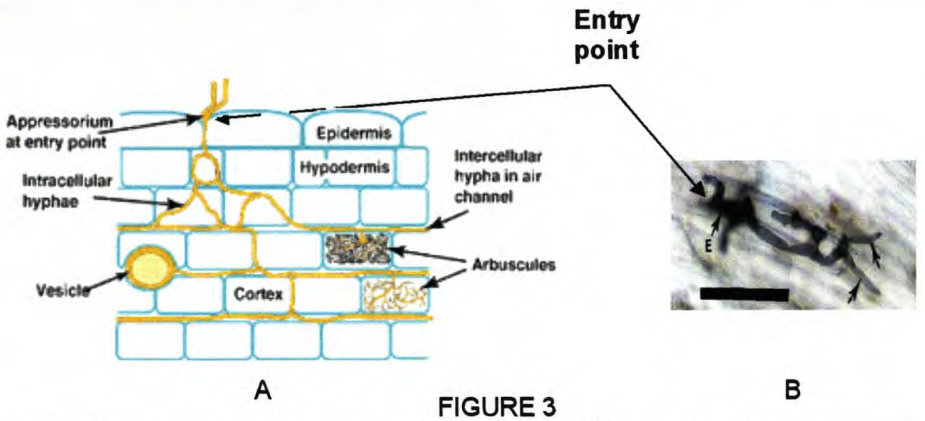

FIGURE 3
(A) A schematic representation of root colonisation resulting from a single entry point. (Adapted from Brundrett, 2001) (B) Hyphae at an entry point (E) penetrating cortex cells (arrows) approximately 1 day after contact with the root. (Bar = 100 µm) (Adapted from Brundrett, 2001).

Hyphae from appressoria, then penetrate and occupy the first epidermal cells to enter the root, after which the aseptate hyphae cross the hypodermis, through passage cells, if these are present in the exodermis (Brundrett *et al.*, 1996). These hyphae start to branch in the outer

cortex forming a colony (infection unit). The extrametrical mycelium may give rise to a number of entry points in the root, depending on the season and age of the root (Mosse, 1959; Nicolson, 1959). As root colonisation progresses, various fungal structures are formed.

1.5.3. Hyphal proliferation in the cortex: The different ways in which hyphae may penetrate roots seem to be linked to the anatomy of the plant root (Brundrett *et al.*, 1996). The way spreading occurs most probably depends on the wall thickening pattern of the outer exodermis cells, since suberin in the walls of these cells, was implicated as a factor influencing the pathway of hyphal root penetration. Therefore, the way the fungus spreads varies and depends on the host and fungus involved. In 1905, Gallaud observed that AM associations between different species formed two distinctive morphology types, the *Arum*- and *Paris*-series, which he named after the host plants. The difference between these two modes of spreading within the root cortex are explained below:

The *Paris*-Type: Associations in which hyphae spread primarily by intracellular growth forming intracellular coils in cortical tissue, because there are no continuous longitudinal air spaces between root cells (Brundrett *et al.*, 1996). Resulting colonies generally have a coiled appearance (Fig. 4).

The *Arum*-Type: Associations in which hyphae proliferate in the cortex by growing along longitudinal, intercellular air channels or spaces between the walls of root cells (Brundrett *et al.*, 1985). A relatively rapid parallel spread of intercellular hyphae may occur along these channels. The resulting colonies of AM fungi have a linear appearance (Fig. 5).

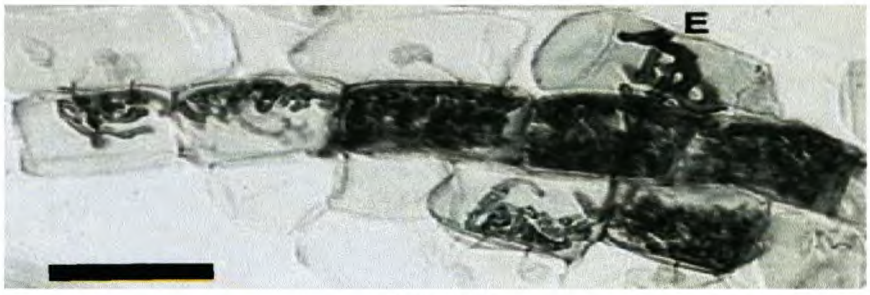

FIGURE 4
The *Paris*-Type: A colony of an AM fungus spreading from the entry point (E) by convoluted hyphae in the inner cortex of an *Erythronium americanum* root. This hyphal growth pattern is typical of roots without cortical air channels. (Bar = 100 µm) (Brundrett, 2001).

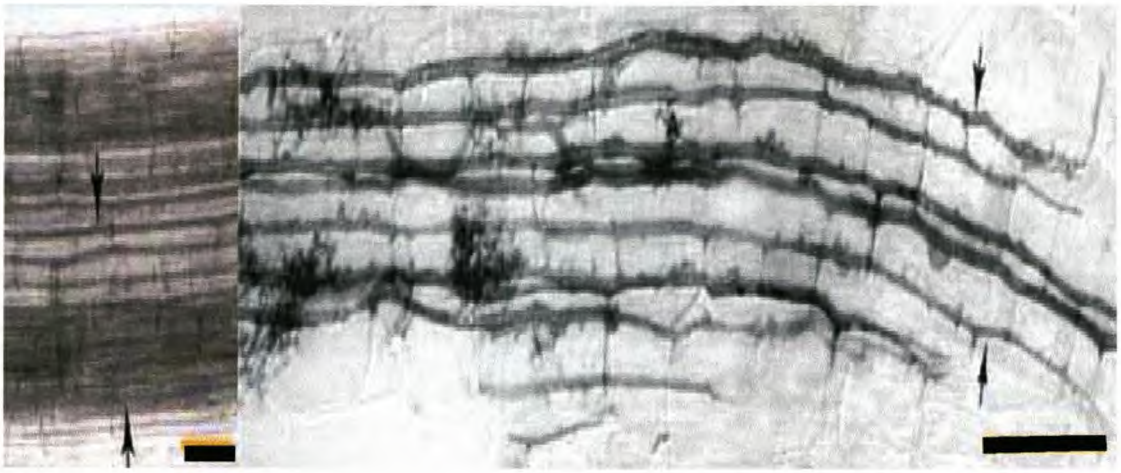

FIGURE 5
The *Arum*-Type: Intercellular air channels (Left) (black arrows) in a whole mount of a living leek root, shown for comparison with mycorrhizal development. These channels run continuously from the apex to the base of roots. (Bar = 100 µm). Longitudinal growth of hyphae of AM fungus *Glomus versiforme* along cortex air channels (Right). Note progressive development of arbuscules (black arrows) with increasing distance from the growing tips of hyphae. (Bar = 100 µm) (Brundrett, 2001).

Thus, this distinction arises as a result of the presence of intercellular spaces between root cells, which are rapidly occupied by hyphae (Brundrett *et al.*, 1985; Brundrett & Kendrick, 1988). When these spaces are not available, the slower *Paris*-type AM association may develop (Cavagnaro *et al.,* 2001). It was found that both time and space influence the formation of hyphal coils during the latter type of association.

1.5.4. Intracellular hyphae in the outer cortical layers of the root: The *Paris*-type AM association that was mentioned above, starts in the outer cortical root layers of the host in which the characteristic looped arrangements of intracellular hyphae are formed (Brundrett *et al.*, 1996). The fungus progresses from the cortical outer layers to the inner ones through cell-to-cell passage. The penetrating or infective hypha of the AM fungus may form intracellular loops in the first cell to be occupied, with similar loops being subsequently formed in adjacent cells. The penetrating hypha may also occupy the first cell without looping and form loops only in adjacent cells. The number and behaviour of the hyphae are probably host dependant, whilst the size of the intracellular unbranched hyphae is fungal dependent (Abbott, 1982). Coils can fill a large part of the cell lumen, but during deterioration, fungal cytoplasm degenerates with subsequent hyphal wall collapse (Kinden & Brown, 1975).

1.5.5. Intercellular hyphae: Intercellular hyphae are usually found in the intermediate layers of the cortical parenchyma where they dilate the intercellular spaces and sometimes occur in bundles of three or four (Brundrett *et al.*, 1996). These bundles sometimes give them a wavy appearance as they follow the outline of the host plant cells, covering considerable distances in

the parenchyma, up to several millimeters (Fontana *et al.,* 1978). They often form intermittent projections and are at times swollen (Abbott & Robson, 1979).

1.5.6. Arbuscules: The arbuscule is considered to be the preferential site for plant-fungus bi-directional nutrient exchange, making it the most significant structure in the AM complex, in particular from a functional viewpoint (Cox *et al.*, 1975; Scannerini & Bonfante-Fasolo, 1983). Gallaud (1905) introduced the name "arbuscule" because of the striking resemblance these structures have with little trees or shrubs (Fig. 6A and B) (Brundrett *et al.,* 1996). These intricately branched haustoria originate from branches of the intraradical hyphae, after these hyphae had penetrated the cortical cell wall (Harley, 1969). Formation of arbuscules is initiated shortly after root penetration (Brundrett *et al.,* 1996), as a result of repeated dichotomous branching and reductions in hyphal width, that starts off with an initial trunk hypha (5-10 μm in diameter) and ending in numerous slender branch hyphae (< 1 μm in diameter).

FIGURE 6
(A) Developing arbuscule of *Glomus mosseae*. (Bar = 10 μm) (Brundrett, 2001). (B) Mature arbuscule of *Glomus mosseae*. (Bar = 10 μm) (Brundrett, 2001).

Actively growing arbuscules enter individual cells of the root cortex by penetrating the host cell wall (Brundrett *et al.*, 1996). However, they remain outside the cytoplasm, but in close contact with the plasma membrane, since this structure forms invaginations or infoldings enclosing the arbuscular hyphae. Arbuscules contain numerous nuclei, mitochondria, glycogen particles and lipid globules. Also, arbuscules contain small vacuoles with electron-dense granules, inside which are high levels of Phosphorous and calcium, which in turn point to the possible presence of polyphosphates (White & Brown, 1979). This is why arbuscules are considered to be the site of intense alkaline phosphatase and possibly ATPase activity (Marx *et al.*, 1982). The fungus may

take up the phosphate from the soil, accumulate it as granules of polyphosphate, and transfer it in this form along the intercellular hyphae by cytoplasmic streaming to the active arbuscule. Here the granules are degraded and Phosphorous is released to the host (Callow *et al.,* 1978).

Although hyphae and vesicles can remain in roots for months or even years, arbuscules are short-lived and may start to collapse only four days after formation. This deterioration is characterised by the collapse of the arbuscular branches until only the trunk remains (Cox & Tinker, 1976; Brundrett *et al.,* 1996). Arbuscules may appear and disappear numerous times in roots over long periods of time, as long as the fungus is growing, which in turn depends mostly on the formation of new roots by the host (Brundrett *et al.*, 1996).

The processes of arbuscular deterioration and collapse were described at the ultrastructural level by numerous researchers and may be summarised as follows: the smaller arbuscular branches show disorganised cytoplasmic contents, loss of membrane integrity, no discernable organelles, and finally appear as an amorphous mass (Holley & Peterson, 1979). Sometimes the arbuscular branches become empty and differentiate a subapical septum that separates empty portions from functional ones (Bonfante-Fasolo, 1978). The walls of the empty zones collapse and subsequently aggregate into clumps. Roots collected from the field often show large arbuscular clumps, originating from smaller arbuscules, filling the host cell (Bonfante-Fasolo, 1978). In nature and particularly in older plant roots, senescent arbuscules are more frequently encountered than active arbuscules (Bonfante-Fasolo, 1984). Active arbuscules are usually associated with young mycorrhizal roots obtained under controlled conditions.

1.5.7. Vesicles: Vesicles are thin-walled intercalary or terminal hyphal swellings formed on internal hyphae, which may be found both in the inner and outer root cortex, within or between cells, and develop to accumulate storage products (e.g. lipids) (Brundrett *et al.,* 1996). The cytological organisation of the vesicles and the fact that vesicle numbers increase in old or dead roots, in which they develop thick walls, suggest that these structures are mainly resting organs (Bonfante-Fasolo, 1984). Vesicles start to form soon after the first arbuscules, but continue to develop when the arbuscules senesce (Brundrett *et al.,* 1996). With age, vesicles become vacuolated (Mosse, 1959; Nicolson, 1959). Fig. 7A and B give examples of typical vesicles formed by AM fungi.

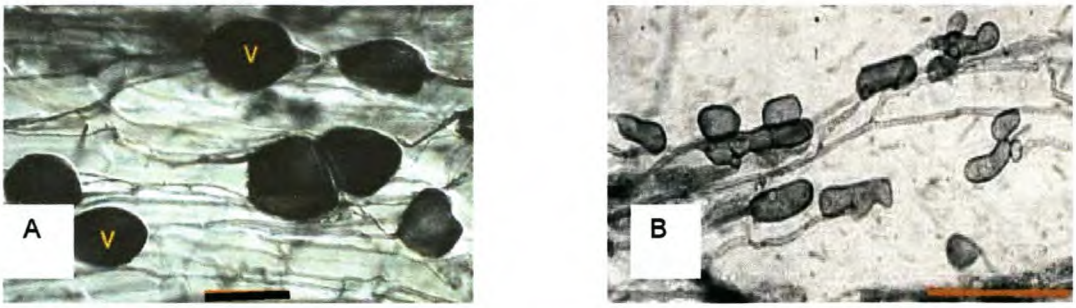

FIGURE 7
(A) Vesicles produced by *Glomus* species in a leek root. (Bar = 100 µm) (Brundrett, 2001).
(B) Lobed vesicles of an *Acaulospora* species in a clover root. (Bar = 100 µm) (Brundrett, 2001).

In the family Glomaceae, vesicles are generally ovoid to ellipsoid (Abbott, 1982, Brundrett *et al.,* 1996). Vesicles in the family Acaulosporaceae often are ellipsoid to irregular and/or knobby or some may be lobed and may even develop inside cells. The outer vesicle surface appears smooth without any ornamentation. The walls are trilaminate, consisting of layers differing in electron density. Intercellular vesicles and host walls are in direct contact whilst a layer of condensed host cytoplasm encloses intracellular vesicles (Holley & Peterson, 1979; Scannerini & Bonfante-Fasolo, 1983).

1.5.8. External vesicles: External vesicles (Fig. 8A and B.), also known as accessory bodies or auxiliary cells, are clustered swellings on external soil hyphae (Brundrett *et al.,* 1996). These structures are often ornamented with spines or knobs (Fig. 8A and B.), which may be used in the identification of genera and species. Only *Scutellospora* and *Gigaspora* species form external vesicles. Apparently these structures do not function as propagules and are believed to have a similar function as normal vesicles.

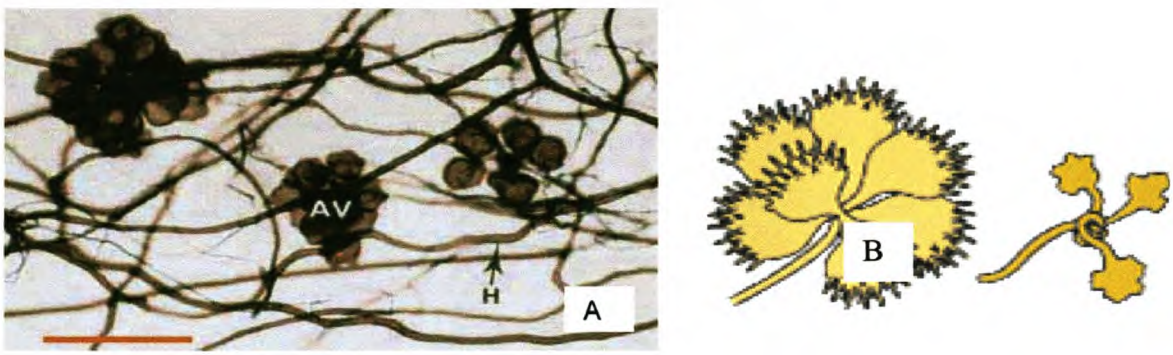

FIGURE 8
(A) Darkly pigmented soil hyphae (H) of a *Scutellospora* species with auxiliary cells (AV). (Bar = 100 µm) (Brundrett, 2001). (B) Auxiliary cells with spines (Left) and smooth surface (Right). (Brundrett, 2001).

1.5.9. Spores: All members of Glomales are classified in the Zygomycota, but none are known to produce zygospores, only asexual or so-called azygospores or chlamydospores are produced (Brundrett *et al.,* 1996). These structures form as swellings on one or more subtending hyphae in the soil or in roots (Fig. 9). Spores usually develop thick walls, which often have more than one layer and may function as propagules.

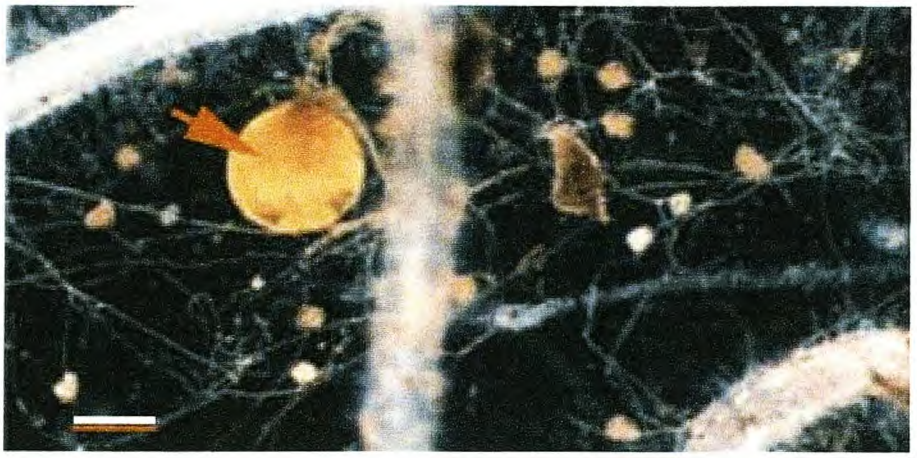

FIGURE 9
A single germinated spore of *Gigaspora* (arrow) in the rhizosphere of a clover plant. (Bar = 100 µm) (Brundrett, 2001).

1.6. CRITERIA USED FOR THE IDENTIFICATION OF AM FUNGI

The identification of AM fungi, e.g., members of the genus *Glomus*, is largely based on morphology (Brundrett *et al.*, 1996). There are, amongst others, four morphological character data sets that are complimentary in identifying an AM fungus (or fungi) to genus level. This includes: (1) mycorrhizal structures when roots are available for study, (2) mode of spore formation, (3) properties of spore subcellular structures, including spore wall layers, flexible inner layers, and (4) mode of spore germination. The abovementioned are usually used in combination with each other, but it is not essential to use all four sets during the identification of a fungus.

According to Brundrett *et al.* (1996), the main spore features used in AM fungal identification, are:

1.6.1. Spore development: Spores of *Scutellospora* and *Gigaspora* species develop from bulbous subtending hyphae, compared to the narrow or flaring hyphae of *Glomus* (Fig. 10A and B). Spores of *Acaulospora* and *Entrophospora* become sessile after detachment from a sporiferous saccule. Many *Glomus* species may form spores within roots, as well as in soil, but other genera do not generally sporulate in living roots.

1.6.2. Spore arrangement: Spores can be produced singularly or may be arranged into groups within sporocarps (Fig. 10C). These spore aggregations may have a peridium (outer covering of hyphae).

1.6.3. Spore shape: Most Glomalean fungi form spores that are spherical, but some species produce spores that are oval, oblong, or sometimes other shapes (Fig. 10 D1 and 10 D2). Subtending hyphae that stay attached to spores can be cylindrical, flared into a conical shape, or swollen. Some spores have multiple or branched subtending hyphae, while other mature spores have spore attachments that may be occluded by wall layers or other material.

1.6.4. Spore size: Substantial differences in spore sizes may help to identify species. Glomalean spore sizes range from small (20-50 µm in diameter) to large (200-1000 µm in diameter) spores. Delicate endophyte spores, such as those produced by *Glomus tenue*, may be as small as 5 µm, and are easily overlooked.

1.6.5. Spore colour: Spore colour varies between and within isolates of Glomalean fungi and may be used to identify them. Colour charts are available for this purpose.

1.6.6. Spore ornamentation: Surface features include pits, reticulations, spines and papillae, which are found most often on *Scutellospora* and *Acaulospora* spores, and are used in the identification of these fungi (Fig. 10E).

1.6.7. Spore wall layers and staining reactions: Walls of Glomalean spores have one or more layers that vary in thickness, structure, appearance, and staining reaction (Fig. 10F). *Acaulospora*, *Entrosphospora* and *Scutellospora* species typically have a complex wall structure consisting of a thicker outer wall and one or more thin inner wall layers. These wall layers can only be visualised when spores are crushed and observed under a compound microscope. Melzer's staining reactions may occur in inner or outer wall layers of spores in all genera, but typical staining reactions may not occur in spores that are old, damaged or have been stored in preservatives. *Glomus* and *Gigaspora* spores are generally simpler in structure than those of other genera, but *Glomus* spores often have several wall layers. Immature *Glomus* spores may have a weak Melzer's staining reaction, which is absent in older spores. Young *Glomus* spores often have a fragile outer wall layer, which is lost as the spores become older.

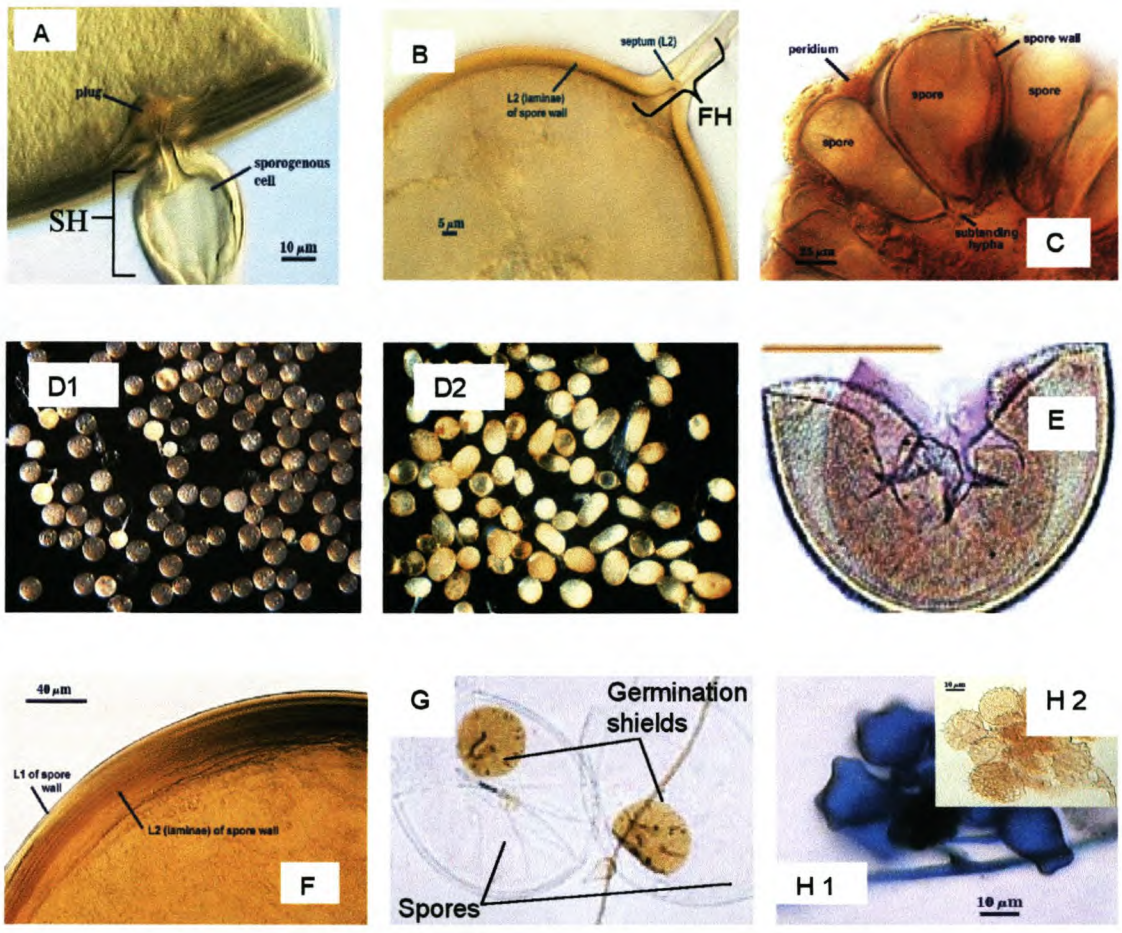

FIGURE 10

(A) Bulbous subtending hyphae (SH) of *Gigaspora gigantea*. (Adapted from Morton, 2001). (B) Flaring hypha (FH) of *Glomus versiforme*. (Adapted from Morton, 2001). (C) Sporocarp of *Glomus sinuosum*. (Morton, 2001). (D1) Spherical spores of *Acaulospora scrobiculata.* (Morton, 2001). (D2) Spores of *Scutellospora calospora* are subglobose to oblong, sometimes irregular. (Morton, 2001). (E) *Acaulospora* spore with deep pits in the outer wall and inner wall layers stained by Melzer's reagent. (Brundrett, 2001). (F) Layers (L1 and L2) in the spore wall of *Gigaspora decipiens*. (Brundrett, 2001). (G) *Scutellospora* spores with prominent germination shields. (Adapted from Morton, 2001). (H1) Auxiliary cells of *Scutellospora fulgida*. (Morton, 2001). (H2) Auxiliary cells of *Gigaspora gigantea.* (Morton, 2001).

1.6.8. Spore contents: Lipids within spores may vary in colour and may be arranged in large or small droplets. The size and arrangement of lipid droplets may help to identify AM fungi, but it must be borne in mind that lipids change when the spores mature. Glomalean spores also often contain parasitic organisms, especially those spores collected from field samples. These parasites subsequently cause spore wall degradation (Lee & Koske, 1994).

1.6.9. Spore germination: The mode of spore germination may also serve to differentiate between Glomalean fungi, especially *Scutellospora* species, which have germination shields containing complex infoldings on their inner walls (Fig. 10G). During germination, hyphae develop from compartments within these shields and subsequently grow through the outer wall. There are also characteristic features of spore germination in other genera such as *Acaulospora* spores, in which germination shields may form and *Gigaspora* spores, in which warts are usually formed inside the spore wall.

1.6.10. Structures associated with soil hyphae: The morphology of auxiliary vesicles or cells, which are clustered structures produced by soil hyphae of *Scutellospora* and *Gigaspora*, may also be of use in the identification of species (Fig. 10 H1 and H2). *Glomus* and *Acaulospora* species may also produce small round "vesicles" in the soil.

By using root colonisation patterns as criterion (Fig. 11A – D), AM fungal genera and sometimes species, may be identified (Abbott, 1982). Morphological features that are important, include variations in vesicles (size, shape, wall thickness, wall layers, position and abundance), hyphal branching patterns, the diamater and structure of hyphae (especially near entry points), and the staining intensity of hyphae (dark or faint) (Brundrett *et al.,* 1996). However, mycorrhizal morphology may be influenced by host root anatomy.

A shortcoming of the abovementioned traditional identification methods is that classification is largely based on spore morphology as a taxon-discriminating tool. Consequently, spore chemical composition such as sterols and fatty acid composition (Morton & Redecker, 2001), as well as spore protein profiles (Xavier *et al.,* 2000), have been used as additional taxon-discriminating characters in the taxonomy of AM fungi.

The taxonomic criteria mentioned above are difficult to employ when measurements of AM fungal diversity in fungal communities in their natural environment are required (Brundrett et al., 1996). It is for example difficult to identify field-collected spores, because of the lack of correlation between functional diversity and morphological diversity of spores used to delineate species (Douds et al., 1999).

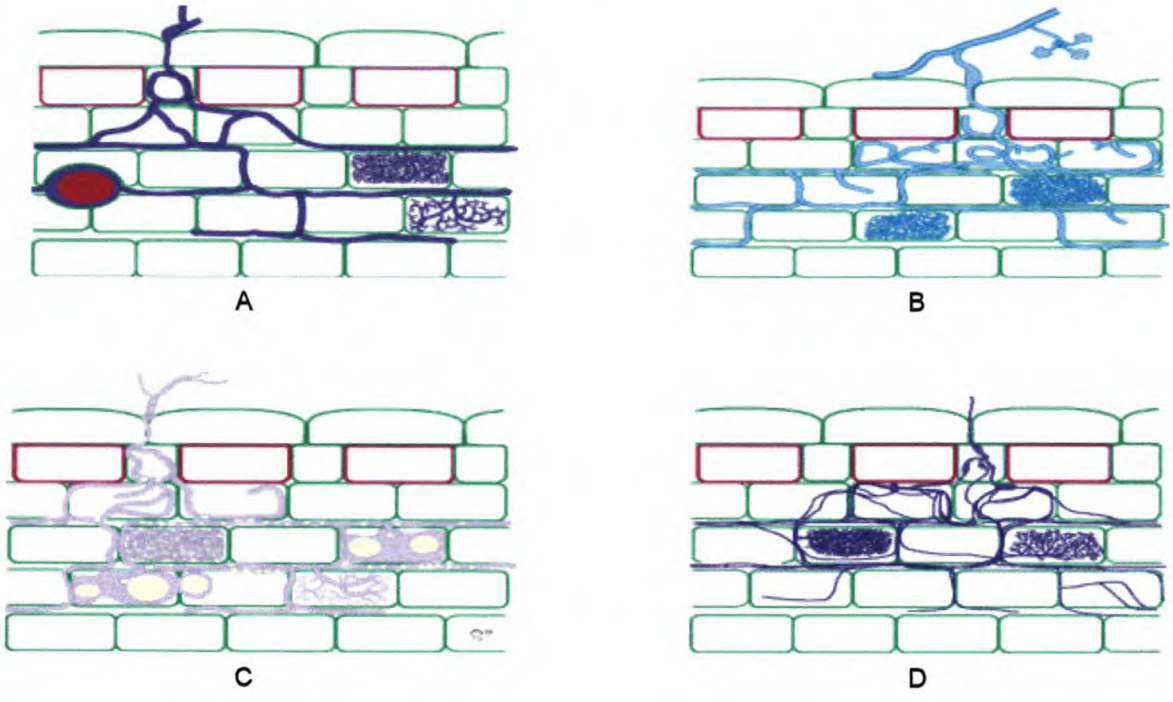

FIGURE 11

(A) Root colonisation by *Glomus* species with relatively straight hyphae, often producing "H" branches which result in simultaneous growth in 2 directions. Staining of these hyphae is usually relatively dark. Arbuscules can be dense and compact. Vesicles are oval, which usually form between root cortex cells. (Brundrett, 2001).

(B) Root colonisation by *Scutellospora* with looping hyphae often present near entry points. This genus has similar root colonisation patterns to *Acaulospora*, but hyphae in the cortex generally are thick-walled and stain darkly. Internal vesicles are not present, instead auxiliary cells are formed outside the roots. Arbuscular trunk hyphae normally are much longer and thicker than those of *Glomus*. The root colonisation pattern for *Gigaspora* is very similar to that of *Scutellospora*, but hyphae are thicker than those of most other AM fungi. (Brundrett, 2001).

(C) Root colonisation by *Acaulospora* species with entry point hyphae that have characteristic branching patterns. Hyphae in the outer cortex generally are more irregularly branched, looped or coiled than for *Glomus*. Colonies in roots are often thin walled, often stain weakly and thus may be very hard to see. External hyphae are usually also very hard to see. Intracellular oil-filled vesicles that are initially rectangular, but often become irregular lobed due to expansion into adjacent cells, which is a characteristic feature of most isolates. These vesicles have thin walls and do not persist in roots. (Brundrett, 2001).

(D) Root colonisation by fine endophytes, previously referred to as *Glomus tenue*. Fine endophyte forms very narrow hyphae (< 1 µm in diameter) and follow a netlike growth pattern in roots. Small hyphal swelling (< 5 µm in diameter)) can occur near entry points and may be analogous to vesicles. (Brundrett, 2001).

In addition, variation was detected in spore size and colour within single isolates of some glomalean fungi (Bentivenga *et al.*, 1997). Variation was also found in progeny spore shape and size amongst single spore cultures (Bever *et al.*, 1999). This so-called heritable variation is ascribed to the segregation of genetically diverse nuclei through dividing hyphae. It appears that the segregation starts off in parental spores containing these genetically variable nuclei, which than segregate into progeny spores to generate the observed differences.

This variation in spore morphology has lead to the search for additional characters as potential taxonomic tools for the identification of AM fungi, one of which is the utilisation of molecular data sets (Redecker, 2000). This new technology is still in its first phase of development, but is expected to make substantial contributions to the current classification system of the Glomalean fungi. However, these aspects are not considered here.

1.7. AM-ALTERED ANATOMICAL AND CYTOLOGICAL CHANGES IN THE HOST

Organs such as stems and leaves undergo noticeable anatomical modifications following AM root colonisation (Krishna *et al.,* 1981). Daft & Okusanya (1973), demonstrated that root colonisation increase the amount of vascular tissue, lignification of the xylem, and the number of vascular bundles. Krishna *et al.* (1981) observed an increase in leaf thickness, size of the midrib vein, as well as numbers of mesophyll cells and plastids. However, changes induced in roots are different (Bonfante-Fasolo, 1984). For example, it was observed that AM root colonisation blocks meristem activity in the apical root meristem of mycorrhizal plants (Fusconi *et al.*, 2000).

Cortical cells in roots also undergo drastic morphological changes upon AM root colonisation, especially in those cells in which arbuscules are formed. In response to AM root colonisation, the host cell increases its volume of protoplasma (Cox & Tinker, 1976). In addition, the host plasmalemma proliferates and surrounds all the fungal branches. The host cell nucleus is also irregularly lobed and Golgi bodies are sometimes hyperactive. All these are signs of a metabolic active host cell (Scannerini & Bonfante-Fasolo, 1983).

During the process of hyphal cell invasion, the large central host vacuole (characteristic of differentiated cortical cells) disappears, as it is split up by the developing hyphae. At the end of the invasion process, the host cell reverts to its normal state and appearance. In fact, the amount of cytoplasm and number of organelles decrease and the large vacuole reappears (Kariya & Toth, 1981). By the time root colonisation has reached a matured stage, starch granules are found near arbuscular clumps (Bonfante-Fasolo, 1984; Brundrett *et al.*, 1996).

The dimorphic nature of the exodermis of *Asparagus officinalis* L. has particularly been informative in studies dealing with cytological variation in AM fungal invaded or occupied root cortical cells (Matsubara, 1999; Matsubara *et al.,* 1999). The dimorphic exodermis in the feeder

roots of asparagus consists of both short and long cells, but only short cells are occupied by AM fungal hyphae (Matsubara, 1999). This phenomenon was also observed in the dimorphic exodermis of *Smilacina racemosa* (Fig. 12). The frequency of hyphal invasion however, differs among fungal species. Appressorium formation starts off in epidermal cells, located above the short cells of the exodermis, after which infective hyphae penetrate the outer tangial walls of the short cells (Matsubara *et al.,* 1999). A hyphal coil forms in short cells and microtubules become closely associated with the coil; some cortical microtubules are retained in occupied short cells. A typical *Arum*-type AM association forms in the cortex, and concomitant to this, a rearrangement of microtubules occurs. During arbuscular formation, microtubules are associated with the large hyphae and with the arbuscule branches. Cortical microtubules, although reduced in numbers, are also present. As the arbuscule degenerates, a few microtubules are still associated with the hyphal clump and many cortical microtubules, oriented primarily in a transverse plane, are present.

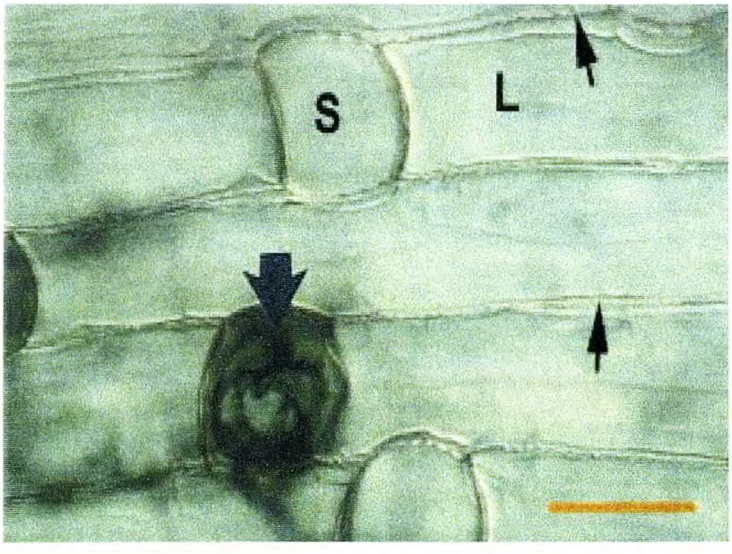

FIGURE 12
Alternating long (L) and short (S) cells in the dimorphic exodermis of a *Smilacina racemosa* root. Hyphae of AM fungi have penetrated unsuberised short cells (arrows). (Bar = 100 µm) (Brundrett, 2001)

1.8. PLANT NUTRITION, GROWTH AND RELATED DISEASES – THE ROLE OF AM FUNGI

1.8.1. AM-facilitated nutrient uptake: In the AM association, the fungus obtains carbon compounds while the host plant receives mineral nutrients (Harley & Smith, 1983). This is commonly referred to as a bi-directional transfer of nutrients (Smith *et al.*, 1994). Thus, the AM roots have a greater nutrient-uptake ability compared to AM-free roots. The external mycelium may acquire up to 80% of plant Phosphorous (P), 25% of plant nitrogen (N), 10% of plant potassium (K), 25% of plant zink (Zn) and 60% of plant copper (Cu) (Marschner & Dell, 1994).

Compared to most horticultural crops, vines have a relatively low nutrient requirement. However, the same nutrients required for normal growth of most plants, are also essential for vine growth (Saayman, 1981). Increased nutrient uptake as a result of AM fungi is, however, restricted to nutrients with low diffusion rates as well as those nutrients that are present in low concentrations. This is particularly applicable to P absorption (Bolan *et al.*, 1987). Considering the importance of P fertilisation as a common practice in agriculture, this immobile nutrient has been studied in more detail compared to the other nutrients.

1.8.2. Phosphorous (P) and AM fungi: Known global phosphate deposits are a finite resource that will run out in about four centuries at the present rate at which it is mined (Mengel, 1997). Consequently, various agronomic measures to save phosphate and avoid losses have been suggested, one of which is the utilisation of AM fungi. The enhancement of Phosphorous uptake by AM fungi is now well recognised. This immobile nutrient plays an essential role in plant growth. Since transpiration rates of AM plants are higher than normal, water uptake per unit root length is also expected to be higher. Subsequently, the mass flow of soil solution to the root surface is higher than normal, and the rate of P absorption is likely to be influenced accordingly. This explains why AM vines exhibit higher P concentrations compared to AM-free vines (Possingham & Obbink, 1971; Karagiannidis *et al.,* 1995).

Phosphorous absorption may take place directly through the roots, but is also acquired via the AM fungi. The mechanisms involved in the uptake of P will be discussed in more detail later on. Generally, AM plants with a coarse-rooted system benefit more from fungal P absorption compared to finer-rooted plant species (Baylis, 1970; Crush, 1973; Linderman, 1988). Indeed, apart from a reduction in root hairs, AM root colonisation has also been shown to reduce root weight in sunflower (Koide, 1985), root branching in tallgrass prairie plants (Hetric *et al.,* 1988), branch root length in *Allium porrum* L. (Berta *et al.*, 1990), root fineness in cotton (Price *et al.*, 1989), and total root length in two grasses (Fitter, 1977). This change in root morphology is the result of increased uptake of P facilitated by AM fungi. P additions independently reduced root fineness in soybeans (Hallmark & Barber, 1984), root system weight in barley, buckwheat and rape seedlings (Schjorring & Jensen, 1984) and root hair formation in rape, spinach and tomato (Fohse & Jungk, 1983). High P supplies via AM fungi may also lengthen the mitotic cycle without blocking the apices, resulting in a steady, slow root growth, which may explain the low root:shoot ratio (mass), so often observed in AM plants (Fusconi *et al.*, 2000).

1.8.3. General concepts regarding P nutrition in vines

P availability: Phosphate is absorbed as $PO4^-$ ions (H_2PO4^-), which are present at low concentrations in the soil solution (Mengel, 1997). This weakly bound phosphate is highly exchangeable and is in isotopic equilibrium with the soil solution, but represents only a small portion of the total soil P content. The greater proportion of P is unavailable to plants due mainly

to phosphate fixation (Mengel, 1997). During this fixation, P becomes adsorbed to the soil surface or is precipitated as Fe and Al phosphate in acid soils or as Ca and Mg phosphates in limy and basic soils (Bolan, 1991). However, based on a 11-year trial, it appears that little P fixation occurs in vineyards soils of the Western Cape in South Africa (Conradie & Saayman, 1989).

Biological assimilation of phosphate may prevent inorganic phosphate from fixation. Organic anions produced during the decomposition of organic matter in soils, as well as the excretion of anions by plant roots, depress phosphate adsorption by competing with phosphate for binding sites at the adsorbing surface. Hence farming systems, which bring much organic matter into soils, contribute to a better use of soil and phosphate fertiliser (Mengel, 1997). The potential role of AM fungi in exploiting such soil phosphates needs to be further investigated.

During P absorption by roots, the zone surrounding the roots has to be filled simultaneously by mass flow of the soil solution, but research showed that diffusion of P to the roots is slower than the rate of P-uptake [Nye & Tinker, 1977, quoted by Koide (1991)]. This zone is referred to as the P-depleted zone and can be up to 2 mm from the root surface [Jungk, 1987, quoted by Koide (1991)]. Subsequently, an insufficient P supply is available to the roots and explain why vines often suffer from P deficiencies even under conditions of adequate P concentrations in the soil. AM roots, however, can overcome this shortage by external hyphae extending beyond the 2 mm zone, reaching soil volumes unavailable to plant roots.

P demand, supply and shortages: Healthy vine growth in terms of phosphorous nutrition basically depends on two factors, namely, P demand and P supply. P demand is the lowest rate of P absorption that enables the plant to reach optimum growth rates (Koide, 1991), whereas P supply refers to the real rate of P absorption under conditions of adequate P. P deficit is a function of the latter two factors and occurs when the P demand exceeds the supply. AM associations promote vine growth by reducing the P deficit. Reduction in P deficits is resultant of an increase in P supply via AM fungi. AM fungi are equipped with several mechanisms to increase the uptake of soil P.

The influx rate of P to AM roots can be up to six times higher than for AM-free roots, (Sanders & Tinker, 1973; Jakobsen *et al.,* 1992). This is mainly attributed to hyphae exploring larger soil volumes compared to root hairs (Li *et al.*, 1991). In essence it shortens the diffusion distances of P ions to the roots (Sanders & Tinker, 1973). Hyphae reach distances beyond the P-deleted zone to reach sites of higher P concentrations [Nye & Tinker, 1977, quoted by Gianninazzi-Pearson & Gianninazzi (1983)].

Another contributory factor to the increased uptake of soil P is the increased surface area by the fine network of hyphae (Sanders & Tinker, 1973). These extremely fine hyphae can also penetrate soil pores that are inaccessible to root hairs [Bjorkmann, 1949, quoted by Bolan (1991)]. This is obvious from the 2-4 µm diameter hyphae compared to the generally larger than 10 µm diameter of root hairs (Barley, 1970). However, there is no substantial evidence for the uptake of P by AM roots from sources that would usually not be accessible to AM-free roots (Bolan, 1991).

There are also other less popular viewpoints to explain the higher P uptake rates by AM roots, amongst which, that AM fungi, like ectomycorrhizae, could change the pH in the rhyzosphere that would create conditions favourable for dissolving P in the soil solution (Raven *et al.,* 1978). One such way that was suggested previously might be that the release of P from organic complexes in the soil is catalysed by extracellular acid phophatase. However, insufficient evidence exists for the presence of such phosphatase or extracellular phytase activity (Marschner & Dell, 1994). It is also possible that hyphae could produce organic acids with chelating properties, such as citrate, which could chemically modified bound P (Bolan *et al.*, 1987). However, chemical modification of the rhizosphere is according to Bolan (1991), not at the forefront of viewpoints to explain the higher P uptake by AM plants.

The process of P uptake, transport and exchange: Inorganic P is actively taken up from the soil solution across a concentration gradient (Gianninazzi-Pearson & Gianninazzi, 1986). Transport of P inside the hyphae is passive and the exchange from the fungus to the roots is active. The absorbed P accumulates as granules inside vacuoles of the external hyphae. Vacuoles are structures that usually have a storage function in both plants and fungi. From here these P granules are translocated to the inter- and intracellular hyphae, which are in contact with the host tissue. Degradation of the polyphosphates by polyphosphatase follows. The activity of these enzymes is located in the vacuoles of the intercellular hyphae and mature arbuscules (Ezawa *et al.*, 1995). Polyphosphate degradation releases the inorganic P in the cytoplasm of the fungus before it is actively transported across the membrane (Smith & Smith, 1990) of the fungus (intracellular arbuscule) to the host cortical cells.

P reserves: At times AM root colonisation may increase the rate of P accumulation that results in excessive P uptake that exceeds the needs of the host plant (Koide, 1991). Such luxury consumption of P subsequently necessitates a mechanism to store P, allowing AM plants to ultimately outperform AM-free plants. Hyphae are also able to store more P than roots (Harley & Loughman, 1963). Absorbed P in AM hyphae is partially stored in soluble forms, namely soluble orthophosphate (Harley & Loughman, 1963) and soluble polyphosphate (Loughman & Ratcliffe, 1984). The bulk of the phosphates, however, is in a solid form of polyphosphate granules and is located inside vacuoles in the external hyphae (Cox *et al.*, 1980). The concentration of inorganic

P inside hyphae may be up to a 1000 times higher than that in soil solution (Gianninazzi-Pearson & Gianninazzi, 1986). Subsequently, when the conditions are favourable, hydrolises of polyphosphates occurs, ensuring the maintenance of a high P concentration in the fungus. Stored P can therefore be supplied to the host if P uptake from the soil is not possible.

Effect of P application on AM-facilitated P uptake: When fertilised vineyard soil was compared with non-fertilised soil, a lower percentage AM root colonisation was recorded for the fertilised soil (Deal *et al.*, 1972). Also, the leaf P concentration was considerably lower in plants grown in fertilised soil compared to plants in non-fertilised soil. In addition, as the soil P concentration increased, no positive growth was obtained (Marschner & Dell, 1994). A seemingly inhibitory effect of high soil P concentration has been observed previously (Mosse & Phillips, 1971; Marschner & Dell, 1994). This is because under conditions of high soil P concentrations, plants absorb soil P directly and not through the mycorrhizae, resulting in increasing P concentrations in the root tissue that inhibits AM root colonisation (Menge *et al.*, 1978b; Jasper *et al.*, 1979). Thus, it seems that it is rather the root P concentration than the soil P concentration that is the determining factor in AM root colonisation (Graham *et al.,* 1981).

It was suggested that membrane permeability of the roots increases under conditions of low P concentrations. The resultant effect is an increased outflow of nutrients from the root tissue, which stimulate fungal growth. As the fungus grows and the subsequent P concentration in the plant increases, membrane permeability of the host root decreases. Consequently, a slower outflow of nutrients from the root occurs. This lack of nutrients from the host then inhibits fungal growth. Therefore, P fertilisation seems to be unnecessary in cases of adequate concentrations of AM fungi in vineyard soil. However, some species of *Glomus* are apparently resistant to high soil P levels (Ravolanirina *et al.*, 1989). For example, Menge *et al.* (1978b) illustrated the ability of *G. fasciculatus* to colonise roots despite conditions of very high levels (600 mg/kg) of soil P. But excessively high levels of available soil phosphate, much higher than required for optimum crop production may eventually lead to bonding of P in forms that are unavailable to plants and may increase the hazard of phosphate loss by wind and water erosion and even leaching (Mengel, 1997). However, P application seems to be a feasible option under conditions of fumigated soils because fumigation would eventually lead to the destruction of the AM fungi. However, the latter must still be weighed up against the option of introducing new AM fungal species to boost the damaged AM populations and thus, restore the mycorrhizal status of the soil.

Apart from the role of P fertilisation and AM root colonisation in vine nutrition, another factor should also be considered, namely, rootstock cultivars (Karagiannidis *et al.*, 1997). It was found that the leaf P concentrations of various varieties differ due to a variety-specific demand for P. The results obtained by Skinner *et al.* (1988), also support this finding. They found that Cabernet Sauvignon leaf nutrient concentrations were significantly affected by rootstock cultivars.

Interactive effects of rootstock and mycorrhizal treatments on leaf nutrient concentrations, however, were not significant.

1.8.4. Nitrogen and Potassium uptake: In addition to the role of improving the uptake of P, AM fungi may also play a role in the acquisition of other slowly diffusing nutrients by plants (Abott & Robson, 1982).

***Nitrogen (N)*:** AM-facilitated uptake of N in vines has previously been demonstrated (Bavaresco & Fogher, 1992), notably with regard to the uptake of nitrate (NO_3^-) and ammonium (NH_4^+). The pH influences the form in which N is available. In alkaline soils NO_3^- is the dominating form of N (Barea *et al.,* 1987). Nitrate is therefore the main nitrogen source and is more available to plants in dry soil conditions (Tobar *et al.,* 1994). However, because NO_3^- is readily mobile under these conditions, AM-facilitated uptake thereof in plants is not likely unless the mass flow and diffusion to the root surface is hampered by the unfavourable drought conditions (Smith *et al.*, 1985). Under these conditions it is expected that the external mycelium of AM fungi will be active in NO_3^--uptake and transport (Tobar *et al.,* 1994). This phenomenon can be regarded as critical for N nutrition of plants in arid and semi-arid agricultural soils.

Although plant roots are able to take up NH_4^+ independently of AM fungi, this ion is relatively immobile in soil and thus, AM-facilitated uptake of NH_4^+ is more likely compared to the freely moving NO_3-ions (Marshner & Dell, 1994). Indeed, the external AM fungal hyphae have been shown to be active in NH_4^+-uptake (Ames *et al.*, 1983). Given the importance in N fertilisation as a common practice in viticulture and considering the costs involved, it is encouraging to learn that N fertilisation seems to be unnecessary in cases of adequate concentrations of AM fungi in the vineyard soils. Nitrogen fertilisation may also eventually result in a decrease in AM fungal concentration and according to some sources may even be more inhibiting to AM colonisation than P fertilisation (Hayman, 1970), even though supporting evidence has not been forthcoming.

Potassium (K): Some studies have shown a relatively low hyphal capacity for K uptake (Karagiannidis *et al.*, 1995). But in other studies, the uptake of this readily mobile ion has been undoubtedly demonstrated (Marschner & Dell, 1994). Different AM fungal species may also differ in K uptake (Sieverding & Toro, 1988). A decrease in K concentration in leaves, as observed by Buwalda *et al.* (1983), might be as a result of a diluting effect caused by an AM-facilitated increase in vigour.

1.8.5. Uptake of other macronutrients: Not much information is available on AM-facilitated uptake of Ca^{+2}, Mg^{+2}, and S^{-2} in plants (Marschner & Dell, 1994). These ions, according to Smith *et al.* (1994), are exchanged (via a bi-directional transfer system) as free ions between symbionts. Evidence of an increase in AM-facilitated Ca^{+2} uptake in vines was obtained previously

(Bavaresco & Fogher, 1996b). Apparently the uptake of Mg^{+2} does not seem to be significantly improved by AM root colonisation (Marschner & Dell, 1994; Karagiannidis *et al.*, 1995). Although, hyphal delivery of S-ions to the host plant has previously been demonstrated (Cooper & Tinker, 1978), AM-facilitated uptake of S-ions would be highly unlikely under most circumstances due to the high diffusion rate of these ions in soil solutions (Marschner & Dell, 1994).

1.8.6. Micronutrients: AM-facilitated uptake of free ion Zn^{+2} and Cu^{+2}, both relatively mobile ions in the soil solution, has been demonstrated in vines (Bavaresco & Fogher, 1992; Gildon & Tinker, 1983; Biricolti *et al.*, 1997; Petgen *et al.*, 1998).

1.8.7. Ferrous iron uptake (Fe): AM fungi are known to improve iron uptake by vines (Bavaresco & Fogher, 1996a). It was found that AM associations, especially with *G. mosseae*, increase the concentration of iron in vines (Bavaresco & Fogher, 1992). An increase in the chlorophyll levels is usually associated with an increase in iron uptake, subsequently reducing chlorosis of leaves. Compared to more tolerant rootstocks, a decrease in lime-induced chlorosis was found with AM-improved Fe uptake in lime-susceptible rootstocks (Bavaresco & Fogher, 1996a). However, other workers have found that AM fungi did not reduce grapevine susceptibility to lime, rather, a reduction in iron content was demonstrated, which accompanied chlorosis (Biricolti *et al.,* 1997).

1.9. AM-IMPROVED GROWTH

Various trials on AM-facilitated growth improvements showed dramatic increases in growth of vines (Possingham & Obbink, 1971; Menge *et al.*, 1983; Biricolti *et al.*, 1997). AM-improved growth is normally measured as total cane length or cane mass (dry weight) and the increased growth is predominantly ascribed to an increased uptake of P (Bolan, 1991), and to a lesser extent to other nutrients. Other studies also support an AM-facilitated growth improvement via an increased uptake of nutrients (Karagiannidis *et al.,* 1995; Biricolti *et al.*, 1997). On the contrary, AM root colonisation is normally associated with decreased root growth (Marschner & Dell, 1994; Bavaresco & Fogher, 1996a). In fact, a decreased root:shoot ratio is common (Hayman & Mosse, 1971). This might partially be ascribed to competition between the fungus and the root for photosynthetically derived products.

Suppression of growth is often consequential of root pathogen infection. Whilst favouring the colonisation of roots by other symbiotic microorganisms, AM fungi increase the tolerance of roots to soil-borne pathogens like nematodes (Schönbeck, 1979) and fungi (Bärtschi *et al.*, 1981), subsequently resulting in healthy vine growth. It was observed, that vine growth increased after inoculation with *G. mosseae*, which effectively eradicated replant setbacks that would have otherwise caused stunting of the vines (Waschkies *et al.,* 1994). The results of these studies

indicated that susceptibility of grapevines to causal agents of replant setbacks would be less likely if the mycorrhizal status of replant soil could be improved.

When vineyard soils are fumigated, it often results in the destruction of beneficial organisms including AM fungi (Menge *et al.*, 1978a; Menge *et al.*, 1983; Skinner *et al.,* 1988). Consequently the mycorrhizal status of the soil is reduced and the vines become stunted, clearly showing the role of AM fungi in suppressing root pathogenic infections and the dependency of vines on AM fungi in vineyard soils (Menge *et al.*, 1983). Apart from improved growth, the latter researchers observed increases in grape production in excess of 60%, following re-inoculation with AM fungi. Some AM fungal species are more effective than others in improving growth (Schubert *et al.,* 1988; Ravolanirina *et al.,* 1989; Biricolti *et al.*, 1997) but Karagiannidis *et al.* (1995) also found that rootstocks differ in growth response.

1.10. AM-FACILITATED PATHOGEN CONTROL

Pathogen infested agricultural soils are common and the subsequent infections have far-reaching consequences that often lead to loss of production of economically important crops. Young vines, in particular, are highly susceptible to setbacks resulting in poor vineyard establishment. This susceptibility is often exploited by rapidly invading pathogens shortly after fumigation has depleted the number of beneficial microorganisms (Menge *et al.*, 1978a; Menge *et al.,* 1983; Skinner *et al.*, 1988), often with adverse consequences. Such soils require additional reconditioning before it would be suitable for commercial crop production (Sances & Ingham, 1997). Reconditioning involves, amongst others, inoculation of vines with AM fungi, in which the fungus improve young vine survival by increasing resistance to soil-borne pathogens such as nematodes (Schönbeck, 1979) and fungi (Bärtschi *et al.*, 1981).

It is still highly disputable exactly how AM fungi facilitate resistance to pathogenic infection because the precise mechanisms involved are not well understood, and a number of hypotheses have been presented to explain the nature in which the AM fungus protects the host against pathogenic attack. A role for AM fungi in competition-driven pathogenic disease prevention or exclusion has been suggested (Waschkies *et al.*, 1994; Vigo *et al.,* 2000). This must-win contest involves two very similar, and yet distinct organisms, in terms of their role in the host, one being beneficial and the other a parasite, each of which is seeking to maximise its own growth and survival. Apparently the AM fungus competes with pathogenic fungi by competing for infection loci on the root surface of the host. In so doing, the fungus outperforms the pathogen by literally keeping it out of the host and subsequently lowers susceptibility of the host to pathogenic fungal attack. Thus, the effect on the number of infection loci is one mechanism through which AM fungi may achieve biological control over soil-borne diseases (Vigo *et al.,* 2000). Competition for carbon and other nutrient sources has also been suggested (Graham, 2001). Also, it appears that AM fungi either solely act as biological control agents (Vigo *et al.,* 2000), or may be engaged

in a combined effort with other biological control agents (Nemec, 1997) and in some cases even with other symbionts such as root nodulating bacteria (Dar *et al.*, 1997), to combat disease.

Used alone, *Trichoderma* is highly regarded as a biocontrol agent to trigger plant defense responses in economically important crops (Yedidia *et al.,* 1999). The induced systemic resistance mechanisms in the host are fascinating. Ultrathin sections from *Trichoderma*-treated roots revealed penetration of *Trichoderma* into the roots, limited mainly to the epidermis and outer cortex. Subsequent host reactions include, amongst others, strengthening of epidermal and cortical cell walls, deposition of newly formed barriers and a number of other induced changes, as revealed by electron microscopy and biochemical analysis (Yedidia *et al.*, 1999). However, if used in combination with AM fungi, an overall better performance is likely, but only under the conditions of compatibility and high survival rates. Out of four biological control agents, *Bacillus* and *Trichoderma* were the best survivors in a planting mix that was used as biocontrol agents in tomato plants (Nemec, 1997).

Future application of biological control agents should consider AM fungi, either as sole agent or included in commercial planting mixes with other biological control agents.

1.11. FUMIGANTS AND THEIR DETRIMENTAL CONSEQUENCES

Fumigation is a standard practice in agriculture and is common in viticulture. Pathogen infested vineyard soils are sometimes fumigated before planting (de Klerk, 1981) to a point where soil borne pathogens could be suppressed to commercially acceptable levels. However, fumigants may also be harmful to beneficial organisms including fungi (Menge *et al.*, 1978a; Menge *et al.,* 1983; Skinner *et al.*, 1988), and often to the extent to which both indigenous and applied fungi become non-detectable (Bendavid-Val *et al.*, 1997).

If applied at the commercial field dose, most fungicides are likely to effectively combat diseases, but in cases of over spilling, may also have side effects with detrimental consequences on AM fungi (Menge, 1982; Kjøller & Rosendahl, 2000; Thingstrup *et al.*, 2000). However, from studies elucidating the efficacy of several fungicides on AM fungi, it is also clear that the effects of different fungicides are not necessarily of the same magnitude (Kjøller & Rosendahl, 2000.). Benomyl, an agricultural fungicide, is particularly known for its deleterious effects on AM fungi and is particularly effective. However, it has been shown that this fungicide may sometimes have a fungistatic rather than a fungicidal effect on spore germination (Venedikian *et al.*, 1999). Nevertheless, the negative impact on the environment and detrimental side effects on AM fungi, necessitates an environmental friendly alternative to fungicides, as was suggested for methyl bromide fumigation (Thomas, 1996; Sances & Ingham, 1997). Methyl bromide fumigation would have been phased out in the United States by 2001, according to the Clean Air Act (Ristaino & Thomas, 1997). It was also suggested that previously fumigated soils that have been depleted of

AM fungi and other beneficial microbiota, require additional reconditioning before the soil would be suitable for commercial crop production.

Thus, until alternatives are found, agriculturalists need to consider the negative impact of fumigation on the environment, notably with regard to the indigenous AM fungal populations.

1.12. AM-FACILITATED DROUGHT RESISTANCE

Most research on the role of AM fungi in alleviating drought stress in plants focussed on comparative studies including, AM versus AM-free plants (Al-Karaki, 1998; Augé *et al.,* 2001), drought versus non-drought conditions (Subramanian *et al.*, 1997; Al-Karaki, 1998; Cruz *et al.*, 2000) and drought sensitive versus drought tolerant plant species (Al-Karaki, 1998).

It was found that AM plants are better able to sustain drought stress than AM-free plants (Subramanian *et al.,* 1997). Plants colonised by AM fungi, displayed higher water use efficiencies (Al-Karaki, 1998) and suffer less from drought stress (Cruz *et al.*, 2000) than AM-free plants. This reduction in drought stress (Safir & Nelsen, 1985) may therefore be as a result of increased rates in transpiration and photosynthesis after periods of water stress.

It was found that unfavourable, dry soil conditions lower the diffusion rates of nutrients, particularly of slowly diffusing nutrients such as P [Viets, 1972, quoted by Koide (1991)]. However, under these conditions AM fungi may still manage to facilitate P-uptake and the uptake of relatively mobile nutrients such as NO_3^-, resulting in an increase in plant biomass (Smith *et al.*, 1985; Tobar *et al.,* 1994). However, it must be noted that it is generally accepted that AM root colonisation does not function as low-resistance channels for transport of water (Sanders & Tinker, 1973). This may explain why AM root colonisation did not alter the ability of plants to extract water from soil even during extreme drought (Bryla & Duniway, 1997).

In addition to the above, AM fungi play an important role in improving soil aggregation to reduce erosion and increase the water retention capacity of soils (Sutton & Sheppard, 1976). Apparently, AM soil has more water stable aggregates and substantially higher extraradical hyphal densities than AM-free soils (Augé *et al.*, 2001). It also requires more time to dry to reach the same soil matrix potential than AM-free soil.

1.13. ENVIRONMENTAL FACTORS INFLUENCING VIABILITY AND SURVIVAL OF SPORES

The numbers of AM fungal spores in soil may be an indication of the potential level of root colonisation of plants in that soil (Schubert and Cravero, 1985). These spores are considered to be important structures for AM fungi, since they are essential for survival. The spores, which act as resting structures, are produced to survive harsh environmental conditions including amongst others, drought, heat, saline soils, pests and diseases.

A number of factors, some of which are beneficial to AM symbiosis, and others who might be detrimental, are briefly considered in the following subsections.

1.13.1. Seasonality, moisture and temperature: In previous studies it was observed that the abundance of AM fungal spores varied according to the season and more so, for viable than for nonviable spores (Guadarrama & Álvarez-sánchez, 1999; Muthukumar & Udaiyan, 1999). Although in some previous studies higher spore counts were made during the summer (Bhaskaran & Selvaraj, 1997), in more recent work spore numbers were found to be higher in the cold rainy seasons compared to other drier periods of the year (Schwob *et al.*, 1999). This indicates a possible adaptation of spores to conditions of moisture and lower temperatures. Also, spore viability was found to be significantly higher when spores were stored at low temperatures, between 5°C and 10°C, compared to spores stored under natural conditions with temperatures ranging between 10°C and 25°C during the winter and between 27°C and 38°C during the summer (Anwar & Jalaluddin 1997). In support of the latter study, low temperature also increased the ability of extraradical AM fungal hyphae to cause infections in soils that were slowly cooled (Addy *et al.*, 1998). Interestingly, winter survival of hyphae apparently is facilitated by the presence of roots, either by attachment, or by close proximity (Kabir *et al.*, 1997).

1.13.2. Association with roots, spores, seeds, bacteria, fungi and natural vectors: Spore germination and survival seems to depend on factors, other than the presence of a host, such as the presence of seeds (Rydlová & Vosátka, 2000), dead roots (Deal *et al.*, 1972; Tommerup & Abbott, 1981), bacteria (Bianciotto *et al.*, 2000), fungi (Fracchia *et al.*, 1998), small animals (Harinikumar & Bagyaraj, 1994; Reddell *et al.*, 1997) and even other spores (Muthukumar & Udaiyan, 1999).

Dead roots, in which spores are embedded or attach to, may serve as nutrient microsites, which favour germination (Deal *et al.*, 1972). This phenomenon should therefore be considered when vineyards are replanted, since spores within residual plant material in the soil, might be able to retain some viability and subsequently colonise newly established vines.

Sporulation within dead seeds was also recorded (Rydlová & Vosátka, 2000). During this process the spores become embedded in cavities within the seeds. The presence of these cavities, rather than organic matter released from the decomposing seeds, was believed to initiate sporulation.

Other AM fungal spores may occupy sporocarps or spores of AM fungi. Consequently, the dead spore structures then serve as microhabitats in which AM fungi survive (Muthukumar & Udaiyan, 1999). Species of *Acaulospora*, *Glomus* and *Scutellospora* have been shown to occupy spores of *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. The spore-in-spore syndrome suggests

that spores of AM fungi act, apart from their normal role in propagation, as microhabitats for others when dead.

Dead spores may also be colonised by other microorganisms such as bacteria (Bianciotto *et al.*, 2000). Surface ornamentation of these spores, such as pores, may increase susceptibility to bacterial colonisation. However, viable spores may also play host to bacteria and other microorganisms, including mycopathogenic fungi. AM fungal spores colonised by these pathogenic fungi are frequently encountered when spores are isolated directly from field soil. Such parasitised AM fungal spores, may have cavities on the surface of their walls, as a result of the activity of the pathogenic fungi (Lee & Koske, 1994). Saprophytic soil fungi, however, may stimulate AM fungal spore germination, indicating complex interactions between these fungi (Fracchia *et al.*, 1998).

Natural vectors, such as small mammal species, may play a potentially important role as dispersers of AM fungal spores in terrestrial habitats, providing that these spores are able to retain viability and colonise roots of host plants (Reddell *et al.,* 1997). It was found that feacal matter of these mammals contained a range of distinct AM fungal spore types. However, the most frequently encountered species belonged to the genus *Glomus*. It is believed that the majority of these spores were acquired incidentally through ingestion of soil during foraging activities.

1.13.3. Host root exudates: The establishment of a functional symbiosis between AM fungi and host plants involves a sequence of recognition events leading up to the morphological and physiological integration of the two symbionts (Giovannetti & Sbrana, 1998). Studies involving comparisons of AM vs AM-free plants have expanded our understanding of factors regulating this symbiosis (Nagahashi & Douds, 1997). Particularly, studies on root exudates contributed to our understanding of the pre-symbiotic phase, during which host-derived signals such as the release of critical metabolites, are necessary to trigger fungal growth and root colonisation (Buee, 2000). These studies have shown that a rather complex recognition process exists between the plant host and the germinating AM fungal spore. A root factor of unknown chemical composition has been implicated as a key plant signal in the development of the symbiosis.

In other studies it was demonstrated that host-derived signals are required to induce changes in hyphal growth patterns of germinating AM fungal spores (Nagahashi & Douds, 1999). However, other researchers have found that germination and growth of spores may occur in the absence of host-derived signals (Giovannetti, 2000). Some researchers are of the opinion that appressorium formation is a contact recognition event that does not require host-derived signals (Nagahashi & Douds, 1997). To conclude, it seems that several factors may be involved in recognition between the plant host and the AM fungus.

1.13.4. Patchy development of mycorrhizas: Soil is a heterogeneous environment in terms of nutrient availability. It was found that plant species might differ in the ability to exploit this heterogeneity, which in turn may affect the distribution of the plant species (Farley & Fitter, 1999). This phenomenon may also occur amongst AM fungal species, since it was found that these fungi are especially abundant at so-called microsites or nutrient patches (St John *et al.*, 1983; Hodge *et al.*, 2000; Tibbett, 2000). These soil nutrient patches represent areas that are rich in easily mineralised fractions of organic matter. Opposed to this, Nappi *et al.* (1985) observed that most AM fungal species associated with vines, occurred in nutrient deficient areas. Whatever the case may be, the mere existence of such soil nutrient patches has obvious environmental consequences on the occurrence and distribution of AM fungi. Soil nutrient patches may occur over a wide range of spatial and temporal scales (Farley & Fitter, 1999) and may include regular-distributed or irregular-distributed patches of differing sizes and nutrient concentration. Research on the ecological significance of AM fungi has mostly ignored the possible effects of soil nutrient patches on the distribution patterns of these fungi. It is likely that the abundance and species evenness of AM fungi are affected by their different nutrient acquisition abilities.

1.13.5. Influence of soil type on the occurrence of AM fungi: AM fungi are generally known to be associated with agricultural soils and one of the earliest recordings on AM fungal associations with grapevine roots was already made about a century ago by Stahl in 1900 (Possingham & Obbink, 1971). Today it is well known that AM fungi are naturally found in all vineyard soils, especially those that did not receive any fumigation treatment (Possingham & Obbink, 1971; Menge *et al.*, 1983). The type of soil does not seem to determine the presence of mycorrhizae because these associations occur in almost all types of soil (Hayman, 1982). However, some species may occur more commonly in certain types of soil than do others (Menge *et al.,* 1983; Nappi *et al.*, 1985; Schubert & Cravero, 1985; Karagiannidis *et al.,* 1997). According to the latter studies, species belonging to the genera *Glomus*, *Gigaspora*, *Scutellospora* and *Acaulospora* are well represented in vineyard soils.

1.13.6. Soil pH: It was found that soil pH has a significant impact on AM fungal populations (Abbott & Robson, 1991; Hamel *et al.,* 1997). Still, spores of these fungi are found in both acid (Clark, 1997) and alkaline soils (Weissenhorn & Leyval, 1996).

Spore formation is more commonly associated with soils of average to higher pH, but some fungal species are adapted to sporulate in acidic soils (Clark, 1997). For example, *Acaulospora* was found to be better adapted to low pH soils whereas *Gigaspora* spp. appeared to be more commonly associated with low pH soils than *Glomus* spp. (Clark, 1997). However, *Glomus manihotis* was shown to be particularly adapted to low pH soils (Hamel *et al.*, 1997). *Glomus monosporum* and *Glomus occultum* were found more often in soils with pH lower than 6.6

(Schubert & Cravero, 1985). However, most species are likely to occur in soils around and above a neutral pH (Schubert and Cravero, 1985).

The significance of soil pH for agriculture is undeniable, yet other soil properties, such as texture, density, porosity and organic content, are equally important and need to be considered when studying spore abundance.

1.13.7. Organic amendments in soil: Application of organic amendments, such as compost and manure, has a positive effect on spore numbers, as was proved by the addition of chicken litter/leaf compost and dairy cow manure/leaf compost to soil (Douds *et al.*, 1997). Similar results were obtained with other organic manure–amended mediums (Muthukumar & Udaiyan, 2000). A sharp decrease in spore numbers is likely in the absence of such organic material, as was demonstrated by the removal of the soil organic layer (Cuenca *et al.,* 1998).

Apparently, AM fungi have direct access to organic P as a result of the production of extracellular phosphatases by the extraradical hyphae (Koide & Kabir, 2000). The resultant inorganic P is then taken up and transported to the host. This hydrolyses of organic P was most noticeable in *G. intraradices*. Consequently, the presence of organic material in soil may prevent phosphate fixation that usually occurs as a result of phosphate adsorbing to alluminium complexes (Mengel, 1997).

1.13.8. Soil disturbances

Negative impacts: Investigations comparing tillage management systems have shown a negative impact on AM fungal spore populations by conventional tillage systems compared to no-tillage systems (Boddington & Dodd, 2000; Galvez *et al.,* 2001). It was shown that soil disturbance reduced the density and population of AM fungal spores, especially in the topsoil, where a significant reduction in spore density can be expected (Kabir *et al.,* 1998).

However damage, in the form of disruption or detachment, due to soil disturbances, is more severe on the integrity of the extraradical hyphal network of AM fungi, which in turn is expected to hamper AM-facilitated nutrient uptake, especially phosphate ions (Kabir *et al.*, 1998). Thus, agricultural tillage practices need to consider the negative impact of soil disturbance upon AM fungal spore populations and soil hyphal network integrity.

Damage prevention and control: Knowledge of the processes contributing to aggregate stability is required and is essential for the improvement in structure of degraded soils or prevention of degradation of structure in healthy soils (Wright *et al.,* 1999). Particularly, the protection of the integrity of the soil hyphal network is of extreme importance, because of its contribution to the stability of agricultural land. Such effects of AM fungi on structure and

aggregate stability has already been demonstrated (Bearden & Peterson, 2000), as well as the usefulness of AM inoculation to ensure successful establishment of plants in eroded soils (Estaún *et al.,* 1997).

Glomalin, an insoluble glycoprotein produced by AM fungal hyphae, was found to be essential for the aggregation and stabilisation of soils (Wright *et al.,* 1999). A high linear correlation between glomalin concentration in aggregates and aggregate stability was established. In no-tillage cropping systems, increases in both aggregate stability and glomalin concentration were observed. However, other studies found no significant correlation between soil aggregates and glomalin concentration, suggesting that aggregation and stability in certain soils may be governed by other interactions (Borie *et al.*, 2000).

It seems that reduced tillage and no-tillage management systems may be safe practices contributing to the stability of agricultural land (Borie *et al.*, 2000). Furthermore, the extraradical hyphal network and glomalin production by AM fungi are regarded as important considerations for the management of sustainable agriculture.

1.14. PURPOSE OF STUDY

The overall objective of this study was to test commercially available arbuscular mycorrhizal (AM) inocula in the field under conditions that would prevail on a typical farm. This entailed monitoring growth and drought stress resistance in a newly established commercial vineyard. In addition, xylem sap and leaf nutrient concentrations, as well as AM root colonisation were determined. These tests were conducted during the first two seasons after planting (Chapter 2). Due to the abundance of AM fungi in vineyard soils and since nutritional deficiencies should not occur in a commercial vineyard, it was important that effects of AM inoculation over the two seasons, should be determined in the presence of indigenous AM fungi and under adequate to high nutrition, notably with regard to P. The second part of the study was aimed at verifying the presence of AM fungi in the vineyard soil by quantifying and identifying AM spores using morphology as criterion (Chapter 3). In addition, a digital record (compact disc) was created, which may serve as a guide to identify AM fungi associated with grapevines, using morphology as criterion (Appendix 2).

1.15. LITERATURE CITED

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CHAPTER 2

HOST PLANT RESPONSE AND ROOT COLONISATION IN YOUNG COMMERCIAL GRAPEVINES FOLLOWING INOCULATION WITH ARBUSCULAR MYCORRHIZAL FUNGI: RESPONSES DURING THE FIRST AND SECOND SEASON

2.1. INTRODUCTION

In South Africa, young grapevines are often exposed to a number of environmental and physiological stress factors including drought, heat, saline soils, pests and diseases, resulting in poor vineyard establishment (van der Westhuizen, 1981). To minimise stress related to pests and diseases in young vines, some vineyard soils are fumigated before planting (de Klerk, 1981). However, vineyard establishment on fumigated soils can be variable and often results in loss of production, due in part to side effects of fumigants that lead to the destruction of beneficial soil organisms including fungi (Menge *et al.*, 1978a; Menge *et al.*, 1983; Skinner *et al.,* 1988). It is for these, as well as for health reasons that the use of fumigants in countries such as South Africa is currently being phased out (Koch *et al.*, 2002). Thus, to promote healthy vine growth and root development, especially during the first year of vineyard establishment, an environmentally friendly alternative to fumigation must be found. The inoculation of vine roots in unfumigated soils with arbuscular mycorrhizal (AM) fungi is such an alternative.

Various species of AM fungi positively affected growth and development of young, pot-grown vines under greenhouse conditions with sterilised soil (Menge *et al.*, 1983). Other pot trial studies have shown that AM fungi allow for greater uptake of nutrients, thus stimulating vine growth (Deal *et al.*, 1972; Karagiannidis *et al.*, 1995; Biricolti *et al.*, 1997). This increase in nutrient uptake by AM fungi is restricted to nutrients that are present at low concentrations and/or nutrients with low diffusion rates in soil, such as phosphorous (P) (Bolan *et al.*, 1987). Increased uptake of P was found to be the primary reason for increased growth in plants (Gianinazzi-Pearson & Gianinazzi, 1983). However, it was found by some workers that AM fungi do not enhance growth of plants under conditions of optimal soil nutrition, in particular P (Abbott & Robson, 1982).

In addition to the role of improving the uptake of P, AM fungi may also play an important role in the acquisition of readily mobile nutrients such as nitrate (NO_3^-), under conditions of drought (Smith *et al.*, 1985). Generally, under such conditions, the mobility of NO_3^- to the root surface is hindered. However, it was found that the external mycelium of AM fungi is actively involved in NO_3^- uptake and transport (Tobar *et al.*, 1994). AM-facilitated N nutrition may therefore be particularly beneficial to crops in arid and semi-arid agricultural soils, as is the case in South Africa. AM-facilitated uptake and transport of the readily mobile potassium ion, K^+, has also been demonstrated (Marschner & Dell, 1994), and it was found that AM fungal species might differ in K^+ uptake (Sieverding & Toro, 1988). Other studies showed a relatively low hyphal capacity for K^+ delivery (Marschner & Dell, 1994). Furthermore, it is known that AM fungi play an important role in plant water relations (Safir *et al.,* 1971; Hardie & Leyton, 1981; Sieverding, 1981) and may alleviate drought stress in vines.

The above-mentioned beneficial effects of AM fungi were demonstrated mainly during pot trials under controlled conditions, during which at least some of the plants were grown under suboptimal conditions. However, on a well-managed commercial farm, suboptimal conditions regarding plant

nutrition and water relations rarely exist and adequate soil P concentrations usually occur. High soil P concentrations are likely to inhibit AM fungal growth (Menge *et al.*, 1978b; Brundrett *et al.*, 1996). Despite this, positive effects of AM fungi may still be observed because AM fungi may develop a tolerance against high soil P (Brundrett *et al.*, 1996). Soil disturbance on the other hand, such as tillage, may destroy the delicate hyphal network formed by AM fungi in vineyard soils (Kabir *et al.*, 1998). These mycelial networks are formed by indigenous AM fungi known to naturally colonise grapevine roots (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Gebbing *et al.*, 1977; Menge *et al.*, 1983; Nappi *et al.,* 1985; Schubert & Cravero, 1985).

Thus, to have a significant effect on vine growth and survival in a vineyard, commercial AM fungi need to outperform these indigenous AM fungi present in vineyard soils (Abbott & Robson, 1982). Successful selection of AM fungal species must therefore be based on their performance in the field in unsterile soils and in the presence of indigenous AM fungi (Abbott & Robson, 1982; Menge, 1983; Schubert & Cravero, 1985).

The aim of the present study was to test commercially available AM inocula under field conditions that would prevail on a typical farm. This entailed measuring vine growth, nutrition, drought stress resistance and percentage root colonisation, over two consecutive seasons, from the onset of planting new commercial grapevines.

2.2. MATERIALS AND METHODS

2.2.1. Experiment layout: The study was carried out in a commercial vineyard planted on the farm Groenland, in the Stellenbosch Region. Merlot grafted on three rootstocks, i.e. 101-14 Mgt, 110 Richter (110 R) and 99 Richter (99 R), was used. These rootstocks were selected to accommodate different soil forms: 101-14 Mgt and 110 R on a Westleigh soil form, which was ridged and 99 R on an unridged Fernwood soil form (Soil Classification Working Group, 1991). The deeper layers of the Westleigh soil were characteristically drenched and consisted of recognisable plinthic material. The Fernwood soil predominantly consisted of layers of sand with the deeper layers visibly more leached than the top layers. Vines were planted in December 1998. Three similar randomised complete block designs were used to accommodate the different rootstocks. Five treatments (control, fungicide, Biocult®, *Glomus* sp. 1054 and Vaminoc®), replicated four times, were randomly allocated. An experimental unit (plot) consisted of 2 vine rows with 5 vines in each row and two buffer vines at each end of the experimental plot. Vines were planted 2.5 x 1.2 m apart. The dimensions of the planting holes were ca 300 x 300 x 300 mm in depth, width and length, respectively.

2.2.2. Treatments: The AM inocula applied during planting are listed below. All inocula contained healthy viable AM spores as determined microscopically (i.e. spores without visible damage and containing lipid droplets). The inocula were prepared according to the instructions of the manufacturers.

***1.* Biocult:** Biocult is a local product and manufactured by INVAM BIOCULT (Pty) Ltd., P.O BOX 261, Somerset West. Biocult contained amongst others, Phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zink (Zn), manganese (Mn), and boron (B), as well as *Glomus mosseae*, *Glomus intraradices, Glomus fasciculatum, Glomus etunicatum, Acaulosporae* spp. and *Trichoderma*. The spores of the individual AM species were supplied by the International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University. Each vine was treated with 50 mL of nutrient-rich Biocult. The inoculum was mixed with the soil in the planting hole.

***2. Glomus* sp. 1054:** *Glomus* sp. 1054 was originally isolated from the rhizospheres of 28-year-old, high yielding Golden Delicious' apple trees on seedling rootstocks at Elgin Experiment Farm, Elgin (S34° 08'; E019° 02'), which is located in an intermontane basin in the winter rainfall area of the Western Cape. A bulked inoculum of *G.* sp. 1054 that had been prepared earlier for use in a pot trial series (Wooldridge, 1999), had also been used in the current trial. Voucher samples of *G.* sp. 1054 for the preparation of the inoculum were obtained from INVAM, West Virginia University. Before the trial series commenced, Wooldridge (1999) prepared a sufficient supply of planting medium. Immediately before use the medium was sealed in plastic bags and sterilised by exposure to ionising radiation at a dose of 15 kGy. The individual spores of *G.* sp. 1054 were bulked in the irradiated medium in association with the roots of an alternating sequence of maize and grain sorghum host plants. Reference should be made to the latter study for procedural details concerning the preparation of the planting medium and inoculum. Microscopic examination of the batch of bulked inoculum that was obtained from Wooldridge (1999), showed that it contained an abundance of healthy, intact spores of *G.* sp. 1054 and no evidence of contamination was observed. A dose of 50 mL of inoculum was mixed with the soil in each planting hole. A further 50 mL sterilised Biocult (steam sterilised in an autoclave at 121°C, 100 kPa for 60 min) was added to the soil-inoculum mixture to ensure that all vines received the same amount of nutrients.

***3. Vaminoc*:** Vaminoc is an imported product, manufactured in the UK by mbi (MircoBio Division), a division of the Agricultural Genetics Co Ltd. The inoculum contained spores of *G. mosseae*, *G. fasciculatum*, *Glomus caledonium* and *Glomus versiforme* in a clay-amended medium. Each planting hole received 5g of the Vaminoc inoculum that was mixed with the soil. A further 50 mL sterilised Biocult was added to the soil-inoculum mixture.

4. Fungicides: A suspension containing the systemic fungicide Benlate® WP (100 g/100 L H_2O; active ingredient: benomyl) and the contact fungicide Rovral Flo® SC (200 mL/100 L H_2O; active ingredient: iprodione), was used as secondary control to inhibit AM fungal growth (Kjøller & Rosendahl, 2000) in and around the vine roots. Benlate is a product (wettable powder) of E I Du Pont de Nemours & Co. (Inc.), Wilmington, Delaware 19898, USA. Rovral Flo is a product (suspension concentrate) of Rhône-Poulenc Agrichem SA (Pty), Onderstepoort, SA. A 50 mL volume of sterilised

Biocult was mixed with the soil in each planting hole and soil was added to fill the hole completely. The soil was subsequently drenched with a combination of the above-mentioned fungicides. Each vine received 2 L of mixed fungicides that covered an area of *ca* 300 mm radius around the vine.

***5. Control*:** A 50 mL volume of sterilised Biocult was mixed with the soil in each planting hole. Neither fungicides nor AM inocula were added.

2.2.3. Cultivation practices: Standard soil management practices were maintained, which can be summarised as follows:

Previous crop: Wine grapes were established in 1976 and were removed during winter in 1998, prior to the establishment of the current vineyard.

Tillage: Before planting, the soils were deeply ploughed to a depth of 800 mm. At this stage, P was added to meet the prescribed nutritional requirements. Lime was added to adjust the pH to *ca* 5.6.

Weed control: In the ridged vineyards (101-14 Mgt and 110 R), weeds were controlled with a full surface herbicide spray. Gramoxone® (paraquat) was sprayed at 5 L/ha during August of each year. Re-emerging summer weeds were hand-hoed. In the unridged vineyard (99 R), weeds were controlled by means of a winter cover crop, Triticale, commonly known as korog (a hybrid of wheat and rye), sowed at a density of 75 kg/ha. The cover crop was sprayed with Gramoxone before bud-break.

Irrigation: Irrigation was applied on a supplementary basis. The 99 R grapevines were irrigated with an overhead system (12 mm/h for 3h at a time), whereas 101-14 Mgt and 110 R grapevines received micro-irrigation (3 mm/h for 12h at a time).

Vine nutrition: During April 1999 and again during bud-break in September 1999, limestone ammonium nitrate (LAN) was applied at 75 kg/ha on all three rootstock plots. This was repeated the following year.

Pest and disease control: Pests and diseases were managed according to a standard program, spraying approximately every 2 weeks, throughout the seasons. Powdery mildew was controlled with the commercially available fungicides, Olymp® 100 EW (active ingredient: flusilazole) and Sabithane® 400 EC (active ingredients: dinocap and myclobutanil). Sulphur dust was also applied. Downy mildew was controlled with Sancozeb® 80 DP (active ingredient: mancozeb) and Curzate Pro® WP (active ingredients: cymoxanil and mancozeb).

2.2.4. Microscopic analyses: Root samples (*ca* 3 g sample for each of 2 randomly chosen vines per experimental unit), representative of each of the 4 replicates per treatment were taken in April 1999 and again in June 2000 (during the first and second season, respectively) and stored in 50% ethanol at room temperature until analysed. To prepare the samples for microscopic analysis, the roots were first subjected to clearing and staining procedures. Roots were cleared by autoclaving for 6 min in a 2.5% (w/v) KOH solution at 121°C. The alkaline solution was subsequently rinsed from the roots using distilled water after which it was acidified in a 1% (v/v) HCl solution for 16 h. Roots were then stained for 2 to 3 days at room temperature in a 0.05% solution of Aniline Blue [containing 0.5 g Aniline Blue, 50 mL 1% (v/v) HCl, 700 mL glycerol and 250 mL distilled H_2O]. The stained roots were destained in destain solution [containing 50 mL 1% (v/v) HCl, 700 mL glycerol and 250 mL distilled H_2O]. Stained roots were mounted in Polyvinyl-Lacto-Glycerol (PVLG) on slides by arranging 20 mm length root segments with fine forceps to accommodate 25 segments per slide. Mounted root segments were covered with cover slips. Four slides per sample were prepared. Percentage root colonisation by AM fungi was calculated as described in Brundrett *et al.* (1994). References should also be made to Appendix 2 of this thesis for procedural details concerning the preparation of root material and analytical techniques employed, as well as points of discussion supplementary to those outlined above.

2.2.5. Field and laboratory measurements: In December 1998, soil analyses were performed on composite soil samples obtained from eight sub-sampling sites from each rootstock plot that represented soil variation in the vineyard. The sub-samples (*ca* 500 g each) were taken over 0-150 mm, 150-300 mm, 300-600 mm and 600-900 mm depth increments, using a standard soil auger. Soil analyses were subsequently carried out on the composite samples in accordance with methods prescribed by The Non-affiliated Soil Analysis Work Committee (1990). By using a pressure bomb, the leaf water potential was regularly measured according to standard procedures (Scholander *et al.*, 1965). These analyses were carried out at two-week intervals from February 1999 until April 1999, during the first season and again from November 1999 until March 2000, during the second season.

In April 1999 and June 2000, during the first and second season, respectively, vigour was determined by measuring the total cane length per vine. Five selected vines per treatment plot were used to calculate mean cane length. Xylem sap samples were taken during the first and second season, in April 1999 and February 2000, respectively. Sap extraction was carried out in the field in the morning between 8 and 11 am. The sap was extracted by modifying the standard procedure for measuring the leaf water potential (Scholander *et al.*, 1965). Similar to the leaf water potential measurements, minimal pressure was exerted to slowly press sap from the tip of the petiole. The pressure differed slightly when different leaves were used, but on average a pressure of approximately -1300 kPa was sufficient for sap extraction. The pressure was then regulated to allow for a continuous flow of sap, which was subsequently collected using a Gilson pipette and 1.5 mL eppendorf tubes. *Circa* 90 µL sap was collected for each replicate sample. The time designated to each extraction varied in

accordance to the different leaves. While extraction was in progress, the extracted samples were kept on ice in a cooler bag. Subsequently, the sap was frozen at -20°C until the chemical analyses were conducted. The phosphate, nitrate and amino acid concentrations in the xylem sap samples of both the seasons were determined according to the methods of Murphy & Riley (1962), Nydahl (1976) and Rosen (1957), respectively. The leaf mineral concentrations were also determined. Leaves were sampled during the first and second season, in March 1999 and in November 1999, respectively, and slowly dried to constant mass in a fan oven at 70°C, milled and dry ashed in a microwave furnace. The residues were taken up in acidified, distilled water, diluted to 100 mL and analysed for P and K using a Varian Liberty 200, inductively coupled plasma atomic emission spectrometer. Nitrogen was determined on the milled plant material using a Leco Nitrogen Determinator.

2.2.6. Statistical procedure: Statgraphics version 7 and SAS version 6.12 packages were used for the statistical analyses of the data of both the 1998/99-season and 1999/00-season.

1. The percentage data (root colonisation data) were arcsine transformed (Zar, 1981). The influence of the factors and their interactions were tested with a two-way analysis of variance (2-way ANOVA) (Statgraphics version 7, 1993, Statgraphics Corporation, USA). Where the ANOVA revealed significant effects by the factors, the differences between treatments were separated using a post hoc least significant difference (LSD), multiple comparison test ($P \leq 0.05$). Data for each rootstock and season were analysed separately.

2. The rest of the data were analysed using SAS version 6.12 (SAS, 1990). The analyses were performed on observations made for two seasons, three rootstocks, with five different treatments. The influence of these factors and their interactions were tested with an ANOVA. Different ANOVA 's were conducted on the data. The split-plot principle was applied because successive observations were made on the same unit (rootstock).

(a) The seasons were analysed as a sub-plot factor (Little and Hills, 1978), to test if season differences and its interaction with other factors existed. This analysis was done for each rootstock separately and with rootstock as a factor in the ANOVA.

(b) The seasons were also analysed separately. This analysis was done for each rootstock separately and with rootstock as a factor. For the ANOVA with rootstock as a factor, treatments were used as a sub-plot factor.

Fisher's Least Significance Differences were calculated at the 5% significance level to compare season, rootstock and treatment means (Ott, 1993). Shapiro-Wilks's test was performed to test for non-normality (Shapiro and Wilk, 1965).

2.3. RESULTS AND DISCUSSION

There appear to be little or no interactions between the seasons (1998/99 and 1999/00) and the treatments according to the statistical analyses performed on observations made on leaf and xylem

sap nutrient data, as well as cane length data for each rootstock (Appendix 1, Table 1-22). Therefore one would expect the treatment to behave more or less the same in both seasons. For this reason the analyses were done for each season separately. The analyses were also done for each rootstock separately since the rootstocks were planted on different soil forms.

This study was preliminary in nature and of limited scope therefore, results must be interpreted in full recognition of a number of constraints. Principal amongst these was the fact that the inocula products had not been subjected to infectivity tests prior to planting. Nevertheless, such tests should be included in future trials of this nature because it may allow better interpretation of results. Also, no prior tests had been conducted on the plant material for the presence of AM infection. Although hot water treatment may be used to sterilise vine roots to control root pathogenic fungi, this practice does not always apply in viticulture and was not an option in this study since the effectiveness thereof on AM fungi has not been proven yet. In future, the mycorrhizal status of young vines obtained from nurseries should be determined to conclude whether AM root colonisation already occurred in the nursery. Furthermore, there was no indication whether the effects of inoculation observed would have persisted had it been possible to repeat the trial over a longer time period or had other inocula, rootstocks or plots been selected.

Results obtained showed that all vine roots had been colonised by AM fungi at similar levels, including the fungicide treatments and controls, ranging from 40% to 85% and 70% to 90%, during the 1998/99-season and 1999/00-season, respectively (Fig. 1 & 2). The increased level of infection during the second season was to be expected since it was believed that re-invasion had taken place (Menge, 1982). Infected control vines indicated natural root colonisation by AM fungi originating from the vineyard and/or nursery soils. This is in agreement with previous observations that AM fungi naturally colonise grapevine roots and that the vines in AM-infected vineyards show satisfactory and even vigorous growth (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Gebbing *et al.*, 1977; Menge *et al.*, 1983; Nappi *et al.*, 1985; Schubert & Cravero, 1985; Nikolaou *et al.*, 1994).

The AM root colonisation observed in the roots of vines treated with fungicides, may have been the result of indigenous AM fungi present in the vineyard, since it is known that the fungicidal effect eventually fades, allowing for the invasion and colonisation of vine roots by indigenous AM fungi present in the soil surrounding the vine roots (Menge, 1982). The fungicides, Rovral Flo (contact fungicide) and Benlate (systemic fungicide), were presumed to have inhibited the external and internal phases of fungal development, respectively. These fungicides are commonly used in agriculture and are known to inhibit AM fungal growth (Kjøller & Rosendahl, 2000). Benlate is particularly known for its deleterious effect. However, the immediate impact of the fungicides on fungal growth in and on the vine roots was not determined in this experiment. Thus, any discussion on the reinvasion of roots by AM fungi remains speculative.

It was likely that the presence of indigenous AM fungi would have a substantial impact on the vine's response to inoculation, regarding growth and other parameters such as water stress and nutrition. The implication was that, unless the inoculant AM fungi were superior to the indigenous AM fungi, no AM-facilitated improvements regarding growth, water stress and nutrition, would be expected since all vines would be susceptible to root colonisation regardless of the treatment applied.

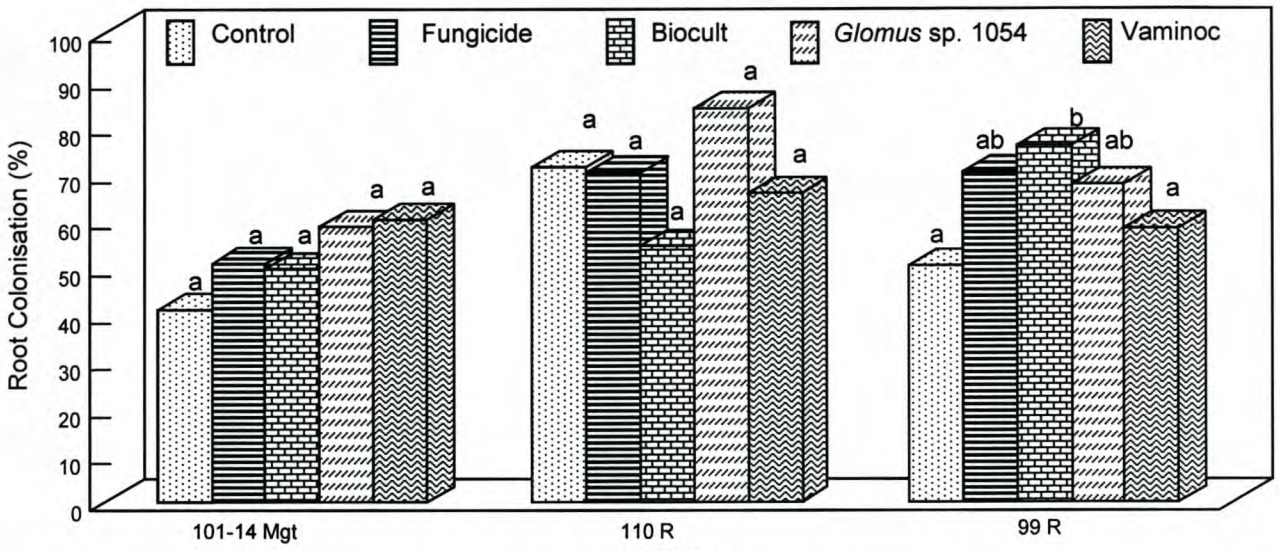

FIGURE 1

Maximum percentage of arbuscular mycorrhizal (AM) root colonisation of one-year-old Merlot grafted onto three different rootstocks (1998/99-season).

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

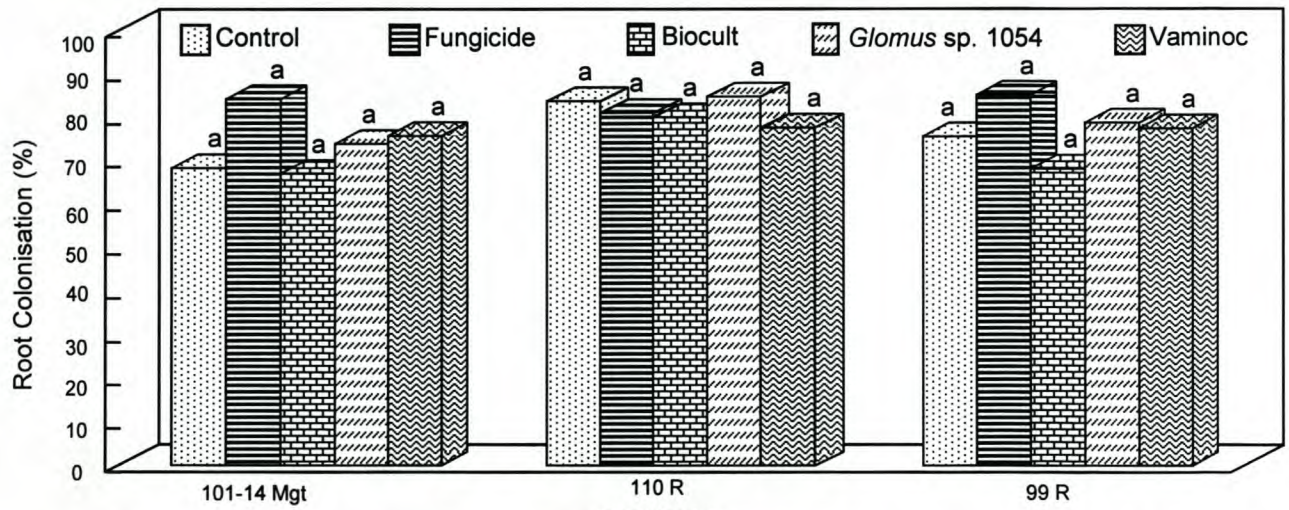

FIGURE 2

Maximum percentage of arbuscular mycorrhizal (AM) root colonisation of two-year-old Merlot grafted onto three different rootstocks (1999/00-season).

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

Except for the growth improvement observed in 110 R vines inoculated with *Glomus* sp. 1054 compared to the controls (Fig. 3) and for a concomitant increase in the percentage root colonisation for this treatment (although not significant) (Fig. 1), a general lack of positive growth responses were observed in the AM inoculated vines during the first season. The possibility of these vines going through an initial stage of delayed growth due to an imposed energy drain (Fitter, 1991; Graham & Eissenstat, 1998) on account of artificial AM inoculation has been ruled out, since an extended monitoring period of one year had not resulted in noticeable growth improvements compared to the controls (Fig. 4). The overall growth performance, independent of the treatments, was still exceptionally good. However, despite being one of the strongest growing rootstocks in South Africa (Carstens *et al.*, 1981), 99 R grafted grapevines exhibited severe growth retardation compared to the other two rootstocks (Fig. 3 & 4). Since the root colonisation levels of 99 R were similar to the other rootstocks (Fig. 1 & 2), the inability of 99 R to perform may be ascribed to other factors.

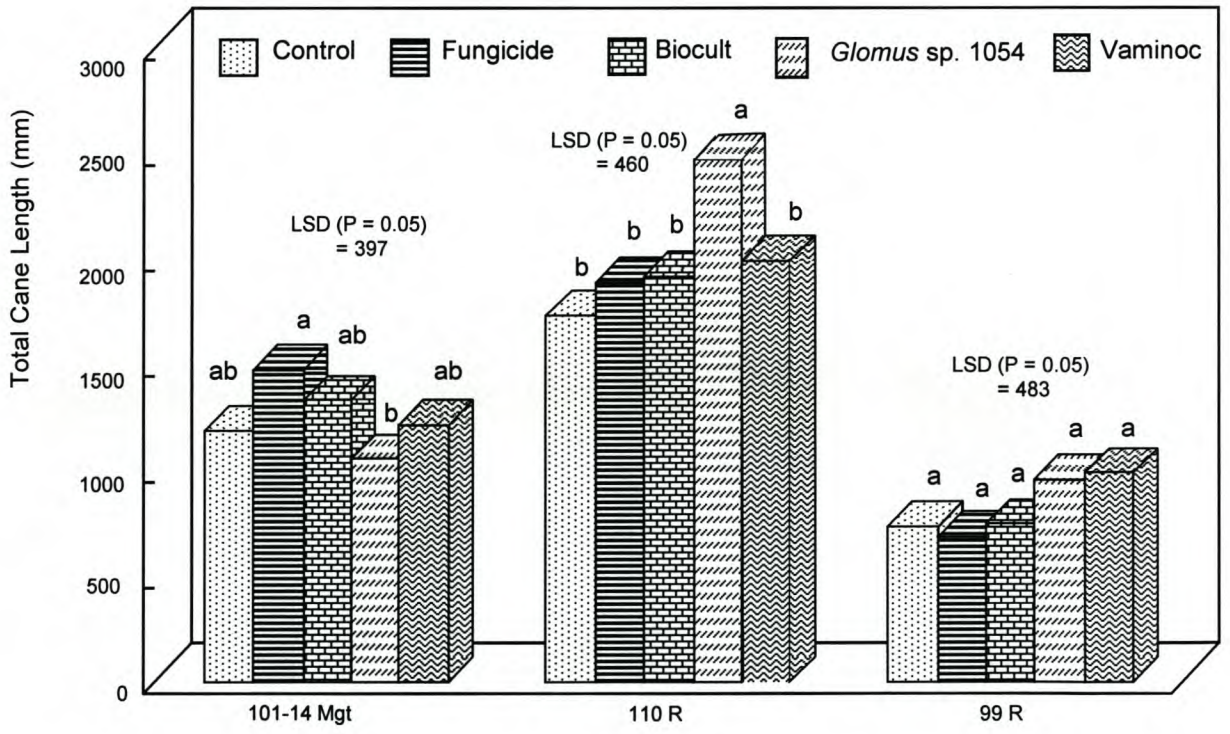

FIGURE 3

Total cane length (mm) per vine of one-year-old Merlot grafted onto three different rootstocks (1998/99-season).

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

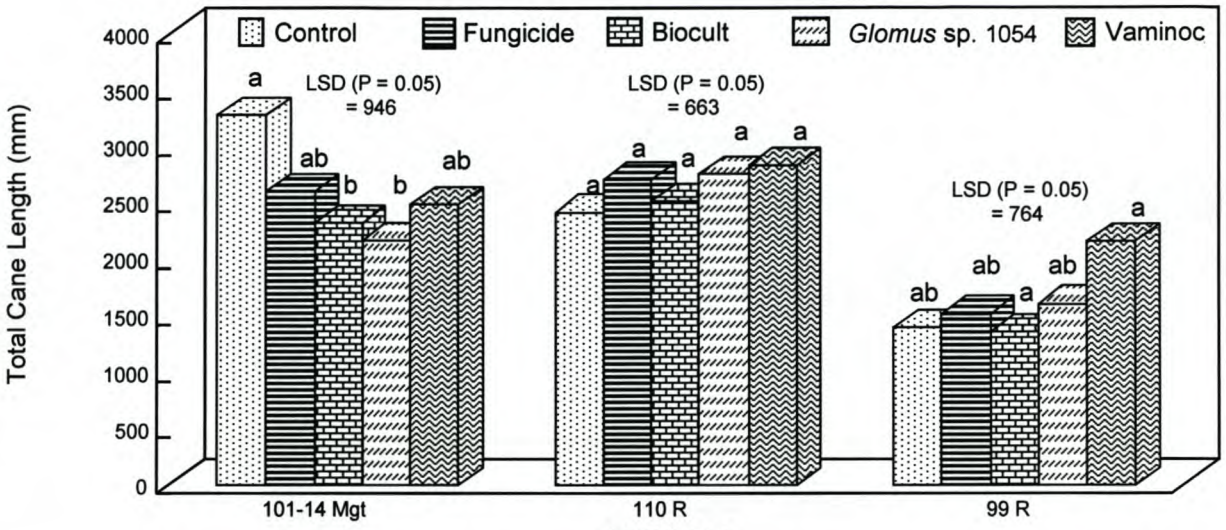

FIGURE 4

Total cane length (mm) per vine of two-year-old Merlot grafted onto three different rootstocks (1999/00-season).
a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

These factors may include: Fernwood (99 R) vs Westleigh (101-14 Mgt and 110 R) soil forms and disease susceptibility amongst the different rootstocks. For example, the Fernwood soil form is generally considered to be of lower potential than the Westleigh soil form, due to its sandy character (Saayman & van Huyssteen, 1981). Disease causing agents on the other hand, may have contributed to growth retardation of 99 R vines, since it is known that 99 R is susceptible to diseases such as *Phytophthora* and *Pythium* root rot, particularly in replant soils (Marais, 1981). However, no apparent root disease symptoms were visible.

In our study, the soil P concentrations were relatively high, ranging from 23 to 89 mg/kg P (Table 1), notably higher than the recommended phosphate fertilisation level for this vineyard [20 to 25 mg/kg P, based on fertilisation guidelines according to van Schoor *et al.* (2000)]. Although AM-induced uptake of P was found to be the primary reason for increased growth in plants (Gianinazzi-Pearson & Gianinazzi, 1983), AM fungi are unlikely to enhance growth of plants under conditions of optimal soil nutrition (Abbott & Robson, 1982). Nevertheless, in the present study, it was imperative to determine the vine's response under prevailing conditions of adequate to high soil P concentration, as would be expected on a typical commercial farm. Thus, the high soil P concentration may have contributed to the lack of AM-induced growth differences generally recorded (Fig. 3 & 4), but vines have been shown to benefit from AM root colonisation even when up to 40 mg/kg P was present in the soil (Schubert *et al.*, 1990). In the present study, such a benefit would have been masked in most cases by the presence of indigenous AM fungi originating from the vineyard soil.

TABLE 1
Soil P analyses of 101-14 Mgt, 110 R and 99 R rootstock plots (1998/99-season).

Soil depth (mm)	P (mg/kg)		
	101-14 Mgt	110 R	99 R
0-150	64	87	89
150-300	73	81	67
300-600	33	65	55
600-900	23	29	44

The data on xylem sap and leaf nutrient composition, obtained during the early stages of vine growth, are presented in Table 2. Little or no AM-induced increases, regarding xylem sap and leaf nutrient concentrations were observed. Results obtained during the second season were similar (Table 3). Since very little growth increases were obtained over the two seasons, the masking effects on vine nutrition were expected and may be attributed to root colonisation by indigenous AM fungi, originating from the vineyard and/or nursery soils.

Vines planted during extremely high temperatures are likely to experience severe water stress, which may result in replanting at considerable cost. In the current trial, vines were planted in mid-December and were at times exposed to high summer temperatures and even dry soil conditions. During the first season the vines exhibited severe water stress, above the norm of –1200 kPa, but the control vines tended to be more water stressed compared to the inoculated vines (Fig. 5). This tendency was not apparent during the second season (Fig. 6). Instead, the leaf water potential, although relatively high, continued to fluctuate variably, in which case all vines exhibited a similar degree of water stress. Inoculation with AM fungi therefore, did not seem to significantly alleviate water stress, although some studies have shown that AM root colonisation does alleviate water stress in plants (Safir *et al.*, 1971; Hardie & Leyton, 1981; Sieverding, 1981). Nevertheless, in the present study there were no setbacks in vine performance during periods of fluctuating or insufficient moisture over the two consecutive seasons (Fig. 5 & 6) and less than 1% dieback was recorded for the vines. Vine survival occurred irrespective of the late planting or the soil type in which the vines were grown.

TABLE 2
Xylem sap and leaf nutrient concentrations of one-year-old Merlot grafted onto 101-14 Mgt and 110 R (1998/99-season).

Rootstock*	Treatment	Xylem sap Nitrate µmol/L	Xylem sap Phosphate µmol/L	Xylem sap Amino acids mmol/L	Leaves N % w/w	Leaves P % w/w	Leaves K % w/w
101-14 Mgt	Control	227.2ab	305.6a	0.688a	2.91a	0.25a	1.22a
	Fungicide	111.2a	231.2a	0.424a	2.83a	0.25a	1.06a
	Biocult	311.2b	371.2a	0.432a	2.83a	0.22ab	1.14a
	Glomus sp. 1054	225.6ab	372.0a	0.488a	2.79a	0.23ab	1.09a
	Vaminoc	160.8ab	311.2a	0.632a	2.85a	0.20b	1.19a
	LSD (P = 0.05)	182.4	165.6	0.400	0.13	0.04	0.18
110 R	Control	105.6a	375.2a	0.144a	2.74a	0.41a	1.27a
	Fungicide	85.6a	263.2a	0.184a	2.85a	0.34a	1.22a
	Biocult	168.8a	273.6a	0.304a	2.81a	0.32a	1.16a
	Glomus sp. 1054	80.0a	264.0a	0.112a	2.75a	0.35a	1.29a
	Vaminoc	184.0a	336.8a	0.160a	3.14a	0.32a	1.20a
	LSD (P = 0.05)	221.6	232.0	0.232	0.61	0.13	0.23

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

* No tests were carried out on 99 R due to insufficient sap.

TABLE 3
Xylem sap and leaf nutrient concentrations of two-year-old Merlot grafted onto 101-14 Mgt and 110 R (1999/00-season).

Rootstock*	Treatment	Xylem sap Nitrate µmol/L	Xylem sap Phosphate µmol/L	Xylem sap Amino acids mmol/L	Leaves N % w/w	Leaves P % w/w	Leaves K % w/w
101-14 Mgt	Control	66.4a	258.4a	1.464a	3.70a	0.29a	1.44a
	Fungicide	92.8a	274.4a	1.688a	3.33a	0.27a	1.38ab
	Biocult	10.4a	398.4a	1.360a	3.60a	0.30a	1.43a
	Glomus sp. 1054	72.8a	284.8a	3.016a	3.30a	0.27a	1.28b
	Vaminoc	25.6a	327.2a	2.072a	3.43a	0.27a	1.41ab
	LSD (P = 0.05)	140.8	260.0	1.728	0.63	0.04	0.13
110 R	Control	148.0a	1087.2a	4.152a	3.56a	0.42a	1.24a
	Fungicide	72.0ab	760.8a	2.112a	3.64a	0.44a	1.25a
	Biocult	44.0b	494.4a	2.456a	3.61a	0.43a	1.24a
	Glomus sp. 1054	43.2b	902.4a	2.096a	3.43a	0.41a	1.21a
	Vaminoc	52.8b	655.2a	1.816a	3.30a	0.39a	1.20a
	LSD (P = 0.05)	82.4	748.8	2.768	0.39	0.09	0.07

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

* No tests were carried out on 99 R due to insufficient sap.

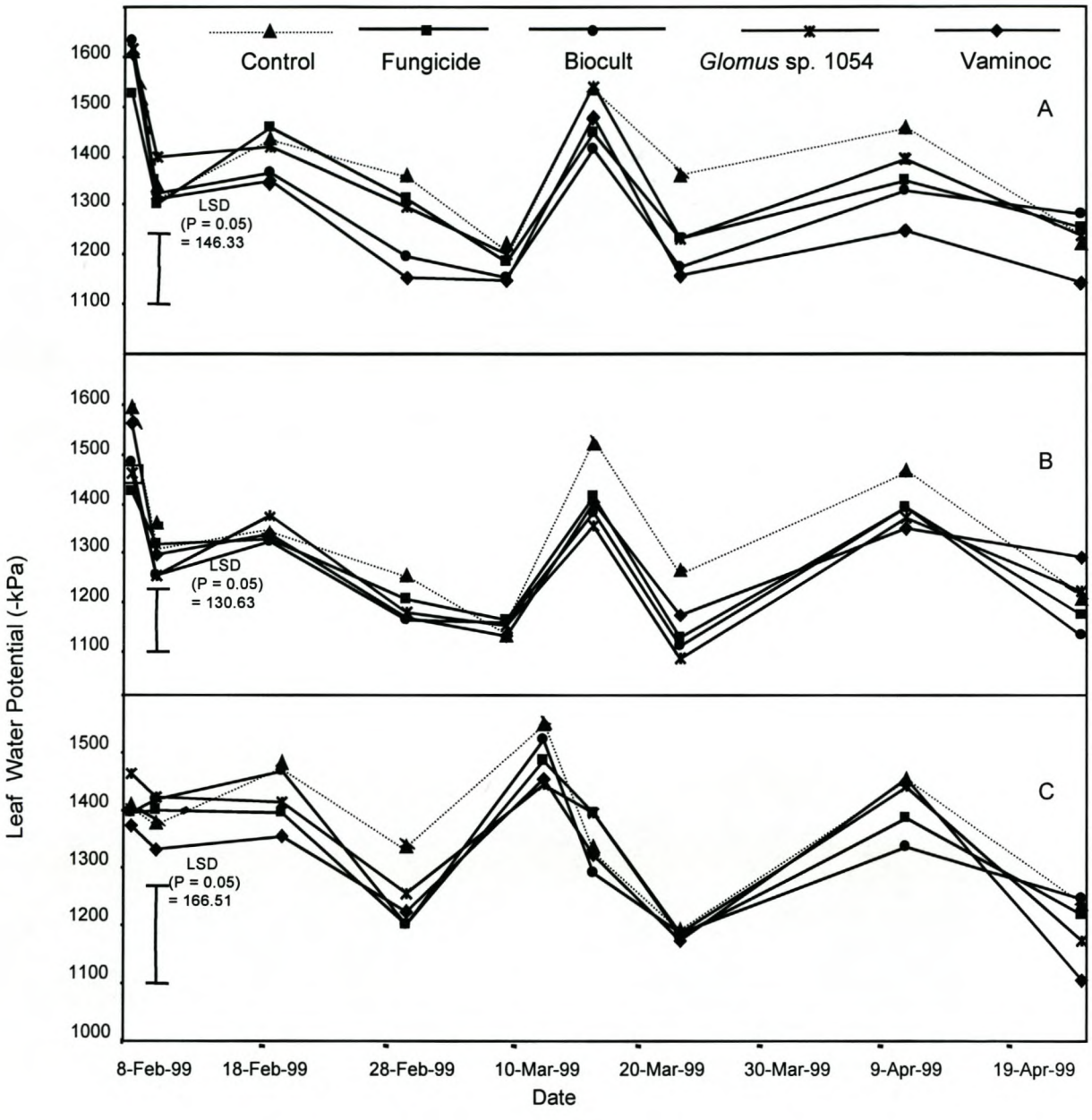

FIGURE 5

Leaf water potential of one-year-old Merlot grafted onto rootstocks 101-14 Mgt (**A**), 110 R (**B**) and 99 R (**C**) (1998/99-season).

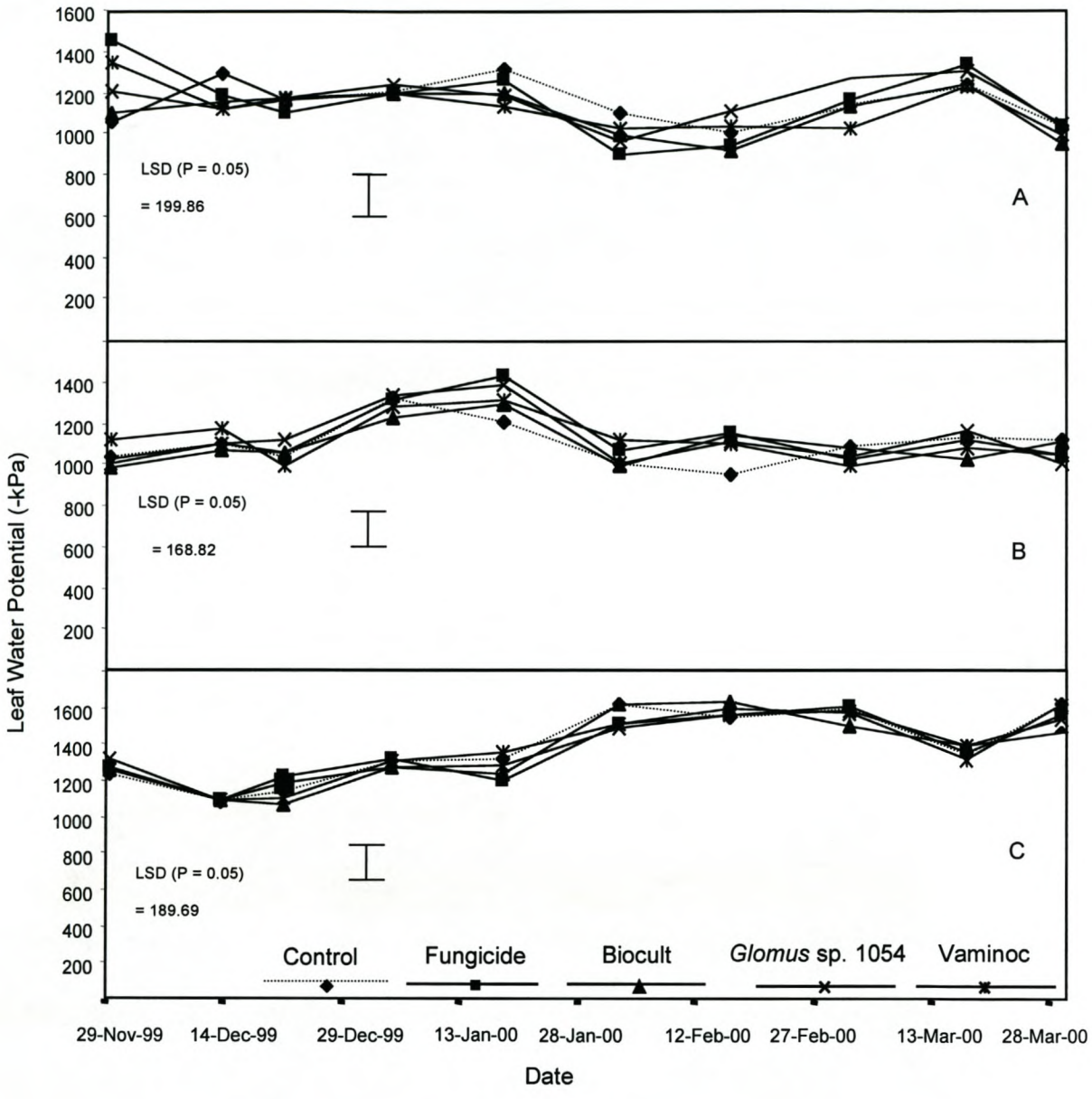

FIGURE 6

Leaf water potential of two-year-old Merlot grafted onto rootstocks 101-14 Mgt (**A**), 110 R (**B**) and 99 R (**C**) (1999/00-season).

2.4. CONCLUSIONS

The commercially available inocula, applied during planting generally had no significant effect on vine growth, nutrition and drought stress resistance, as well as root colonisation in the commercial vineyard. This may be ascribed to the presence of indigenous AM fungi originating from the vineyard and/or nursery the vines were obtained from.

Field inoculation with commercial AM fungal strains may therefore be of little additional benefit to farmers if effects of inoculation are masked by compatible indigenous AM fungi, already present at adequate concentrations in vineyard soils. Large-scale inoculation can then become a costly affair. It is therefore essential for farmers to first assess the mycorrhizal status of their soils and nursery vines before inoculation with AM fungi can commence. Failure to do so may result in unnecessary expenditure.

In view of the tentativeness of the evidence provided and the limited scope of this trial, the above-mentioned findings must be considered to be of a preliminary nature, requiring further substantiation. To obtain additional information on the mycorrhizal status of the vineyard investigated in the present study, the diversity of the AM fungal taxa occurring naturally in this soil will be the focus of a subsequent investigation.

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CHAPTER 3

THE OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN INOCULATED AND UNINOCULATED RHIZOSPHERE SOILS OF TWO-YEAR-OLD COMMERCIAL GRAPEVINES

3.1. INTRODUCTION

Arbuscular mycorrhizal fungi are known to naturally colonise grapevine roots to form an intricate association with the host (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Nappi *et al.*, 1985; Schubert & Cravero, 1985) and due to the abundance of these fungi in vineyards, they are generally believed to contribute naturally to the optimum performance of grapevines (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Nappi *et al.*, 1985; Schubert *et al.*, 1988; Bavaresco & Fogher, 1992; Waschkies *et al.*, 1993; Karagiannidis *et al.*, 1995; Karagiannidis *et al.*, 1997). However, some agricultural soils, particularly those deficient in mycorrhizas, due in part to side effects of practices such as fumigation, require reconditioning to render it suitable for optimum crop performance. From time to time, artificial inoculation with selected arbuscular mycorrhizal (AM) fungi is used to reintroduce these fungi to such soils (Abbott & Robson, 1982; Menge, 1983). It is for this reason that commercial AM fungal strains have been available to farmers abroad for nearly two decades (Menge, 1983). However, large-scale inoculation of cultivated crops in South Africa only emerged recently (M. Venter, personal communication, 2000) and there is little known about the mycorrhizal status of SA vineyard soils.

In a previous study, where the performance of young grapevines was measured after artificial AM inoculation (Chapter 2), it was found that vine roots were adequately colonised with indigenous populations of AM fungi. Consequently, no significant improvement in the performance of the vines could be detected, since it was believed that these fungi masked the effects of inoculation.

Since little is known about the mycorrhizal status of this particular vineyard, it was the objective of the present study to obtain additional information on the occurrence of AM fungi in the inoculated and uninoculated rhizosphere soils of the vines. Therefore, AM fungal spores in the rhizosphere soil of the vines were quantified and the fungi associated with the inoculated and uninoculated vine roots were identified using diagnostic structural features of the fungus, including spore morphology and root colonisation patterns in trap pot cultures, as criteria.

3.2. MATERIALS AND METHODS

Experiment layout and experiment procedures were described previously in Chapter 2 of this study. Briefly, the study was carried out in a commercial Merlot vineyard, grafted on three different rootstocks. The first two, i.e. 101-14 Mgt and 110 Richter (110 R), were planted on a ridged Westleigh soil and the third, 99 Richter (99 R), on an unridged Fernwood soil (Soil Classification Working group, 1991). Five treatments were applied at the time of planting. Three of the treatments (Biocult®, *Glomus* sp. 1054 and Vaminoc®) each involved inoculation with different AM inocula. The fourth treatment involved sterilisation of soil by a combination of fungicides, Benlate® WP and Rovral Flo® SC, in order to establish a control not affected by soil fungi. The fifth treatment (control) received neither fungicides not AM inocula. All treatments except the one where nutrient-rich Biocult was used

as AM inoculum, received 50 mL per vine of sterilised Biocult (steam sterilised in an autoclave at 121°C, 100 kPa, for 60 minutes) to ensure that all vines received the same amount of nutrients.

3.2.1 Sampling procedures and establishment of trap pot cultures: In June 2000, soil analyses were performed on composite soil samples obtained from eight sub-sampling sites from each rootstock plot that represented soil variation in the vineyard. The sub-samples (*ca* 500 g each) were taken over 0-150 mm, 150-300 mm, 300-600 mm and 600-900 mm depth increments, using a standard soil auger. Soil analyses were subsequently carried out on the composite samples in accordance with methods prescribed by The Non-affiliated Soil Analysis Work Committee (1990). In addition, rhizosphere soil and roots at each replication per treatment were sampled in winter during June 2000 (Chapter 2). Roots were stored in 50% ethanol at room temperature until analysed. Each of the rhizosphere soil samples (*ca* 1.5 kg) was divided into two sub-samples. The one sub-sample (*ca* 1 kg) was air-dried before it was subjected to microscopic analyses.

The other sub-sample (*ca* 500 g) was subjected to cold shock treatments for approximately 6 weeks at 0°C to enhance germination of spores dormant at the time of field collection (Vimard *et al.*, 1999). To obtain spores of sufficient quality (healthy, intact spores) required for the accurate identification of AM fungi (Walker, 1992), trap pot cultures were prepared using the 500 g sub-sample and incubated under glass house conditions according to the method described in Brundrett *et al.* (1996). Each sample was mixed with 500 g sterilised sand (gamma-irradiated at a minimum absorbed dose of 20 kGy per kg soil) and transferred to a free-draining pot. Surface sterilised grain sorghum seeds [treated with 5% (v/v) hypochlorite solution for 20 min] were sown onto these pots, covered with a thin layer of sand and watered to field capacity. No nutrients were added to the potting mixture and sufficient tap water was supplied via a splash-free drip irrigation system each day to permit limited drainage from each pot. All possible precausions were taken to minimise the likelihood of contamination. The pH (KCl) of the potting mixture was *ca* 5.9. Pot plants were allowed to reach maximal growth (after eight to twelve weeks) at which time roots and soils were sampled for microscopic examination.

3.2.2. Microscopic analyses of roots and spores: The field-collected vine roots, as well as the sorghum roots from the trap pot cultures were used to identify root colonisation patterns and diagnostic structural features of the AM fungi.

Spores in the air-dried sub-samples were separated from the soil using wet sieving and sucrose gradient centrifugation (Brundrett *et al.*, 1994; Appendix 2). Briefly, each sub-sample was mixed in a substantial volume of water (*ca* 2 L for each 100 g sieved sample) and decanted through a series of sieves after allowing heavy particles to settle for 15-20 seconds. This washing and decanting process was repeated until the water was clear. Roots and coarse debris were collected on a coarse (750 µm) screen, while spores were captured on a series of finer screens (50 µm, 100 µm and 250 µm fractions). The spores were washed onto pre-wetted filter paper in a Buchner funnel before vacuum

filtration and then enumerated using a stereo microscope (Nikon SMZ -10A). Counts were expressed as the total number of spores per 100 g of dry soil and included both dead and viable spores.

The same method was used to separate AM fungal spores from the soil in the trap pot cultures. These spores were then selected and grouped according to morphological types using dissecting microscopy. Subsequently, semi-permanent microscope slide preparations of the spores were made. Spores were stained and mounted in Melzer's reagent mixed 1:1 (v/v) with Polyvinyl-Lacto-Glycerol (PVLG), as explained in Appendix 2. Using a compound microscope, the spores were then identified according to the methods described in Brundrett *et al.* (1996) and the INVAM website (Morton, 2001). Figure 1 A & B summarizes the criteria used to identify AM fungi.

3.2.3. Statistical procedure: The data were analysed with SAS version 6.12 (SAS, 1990). The influence of the factors and their interactions were tested with a two-way analysis of variance (2-way ANOVA). The analysis was done for each rootstock separately and with rootstock as a factor. For the ANOVA with rootstock as a factor, treatments were used as a sub-plot factor. Fisher's Least Significance Differences (LSD) were calculated at the 5% significance level to compare treatment means. Shapiro-Wilks's test was performed to test for non-normality (Shapiro and Wilk, 1965).

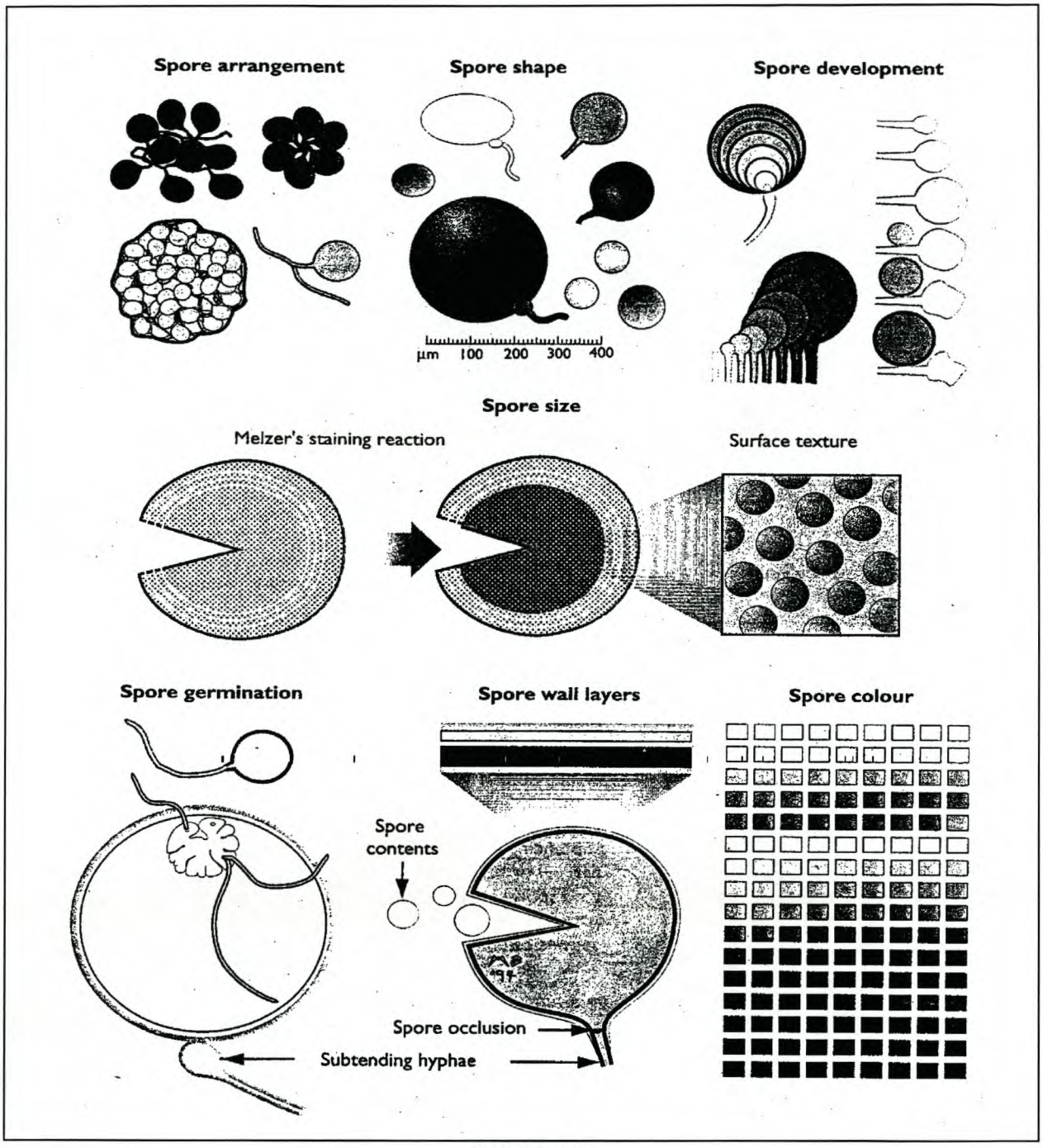

FIGURE 1 A

Taxonomic criteria used to identify Glomalean fungi include various aspects of spore structure, as well as details of spore production, external hyphae and mycorrhizal associations within roots. Important features include spore colour, surface texture, size, shape, subtending hyphae, contents, wall layers and germination processes. Details of spore wall structures may be examined only after making microscope preparations of squashed spores and comparing unstained spores to those stained with Melzer's reagent (Brundrett *et al.*, 1996).

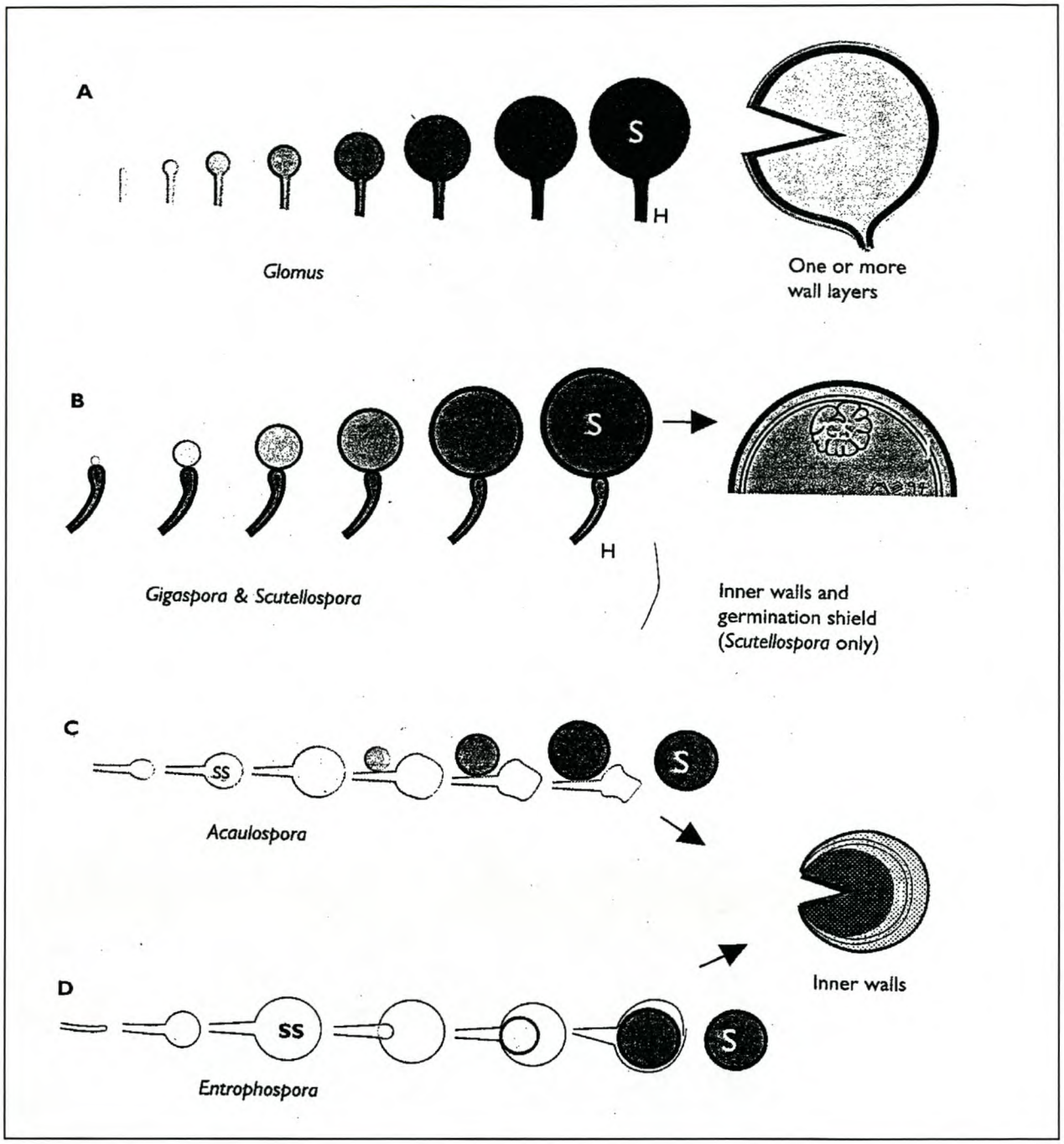

FIGURE 1 B

Developmental processes during spore formation by different genera of Glomalean fungi. **A**. *Glomus* spores are relatively simple structures that form from hyphal swellings, which develop thickened walls that may be multi-layered. **B.** *Scutellospora* and *Gigaspora* spores form on a swollen subtending hypha. *Scutellospora* spores develop various inner wall layers and have a germination shield. **C, D**. *Acaulospora* and *Entrophospora* spores are produced at the base of or within a sporiferous saccule which collapses after transferring its contents into the developing spores. These spores develop multiple wall layers and may have complex ornamentation (Brundrett *et al.*, 1996).

3.3. RESULTS AND DISCUSSION

From the results obtained previously during the 1998/99-season, it was evident that AM fungal strains other than those included in the inocula were colonising the vine roots (Chapter 2). This resulted in a relatively high level of AM root colonisation, which ranged between 40% and 85% during the first season (Chapter 2, Fig. 1). During the second season higher levels of root colonisation were obtained, ranging between 70% and 90% (Chapter 2, Fig. 2).

The mode by means of which the AM fungi spread through the root cortex, were typically that of the *Paris*- and *Arum*-types (Gallaud, 1905), which were, respectively, characterised by the formation of noticeable intracellular coils in the cortical tissue and a relatively parallel spreading of intercellular hyphae, respectively. Growth of the co-existing fungal species in the roots did not seem to have been affected by the prevailing high levels of soil P, up to 80 mg/kg P (Table 1). Furthermore, this soil P-level was much higher than the level of about 7 mg/kg P, above which inhibition of fungal development in roots can be expected (Brundrett *et al.*, 1996). This tolerance of AM fungi to relatively high levels of soil P is also in agreement with other studies (Plenchette *et al.*, 1983; Schubert *et al.*, 1990).

In the present study, 18 AM fungal species were obtained, of which 15 were positively identified on the basis of spore morphology and root colonisation patterns (Table 2). These species were present in the rhizosphere soils of both the inoculated and uninoculated (control) grapevines. There was considerable overlap in the species composition of the different treatments of each rootstock. Seven of the 18 species occurred at all the sampling sites, namely: *Acaulospora spinosa, Gigaspora gigantea, Glomus mosseae, Glomus sinuosum, Scutellospora calospora, Scutellospora dipurpurascens* and *Scutellospora fulgida*. One species, *Gigaspora decipiens,* occurred in the soil of all the treatments of rootstock 101-14 Mgt, but was not detected in the other soils. This may indicate a specific association between this rootstock and *G. decipiens*, as all vines were obtained from the same nursery. The rest of the species mentioned in Table 2, were not associated with a specific treatment. Most of the AM fungal species detected belonged to the genera *Glomus, Acaulospora, Scutellospora* and *Gigaspora,* and are similar to those obtained from soil of a vineyard located in northern Greece (Karagiannidis & Nikolaou, 1999).

TABLE 1
Soil P analyses of 101-14 Mgt, 110 R and 99 R rootstock plots (1999/00-season).

Soil depth (mm)	P (mg/kg)		
	101-14 Mgt	110 R	99 R
0-150	74	68	63
150-300	80	61	67
300-600	27	31	61
600-900	15	6	50

In the present study, spores of *Acaulospora spinosa* and small-spored species of the genus *Glomus* listed in Table 2, accounted for the major proportion of the total volume of spores per sampling site. This is in agreement with other work, in which it was found that members of the genus *Glomus* commonly occur in field samples collected from agricultural soils (Karagiannidis *et al.*, 1997; Karagiannidis & Nikolaou, 1999). Although large spores of *Gigaspora gigantea* and *Scutellospora* species were frequently encountered at the various sampling sites, along with auxiliary cells formed by them, these spores accounted only for a minor proportion (*ca* 5%) of the total volume of spores per sampling site.

TABLE 2
AM fungal species identified in the rhizosphere soils of 101-14 Mgt, 110 R and 99 R rootstock blocks (1999/00-season)

AM fungal species: (+) Present or (-) absent at the different treatments (T)	101-14 Mgt					110 R					99 R				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Acaulospora scrobiculata	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Acaulospora spinosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gigaspora sp.	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Gigaspora gigantea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gigaspora decipiens	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Glomus clavisporum	-	-	+	-	-	+	-	+	+	+	+	+	-	+	+
Glomus etunicatum	+	-	+	-	-	+	-	+	-	+	+	-	+	-	-
Glomus intraradices	+	+	+	-	-	-	-	+	+	-	+	+	+	-	+
Glomus mosseae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glomus sinuosum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glomus tortuosum	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Sclerocystis sp.	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-
Scutellospora sp.	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Scutellospora calospora	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scutellopspora cerradensis	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Scutellospora dipurpurascens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scutellospora erithropa	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Scutellospora fulgida	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

T1 = Control (uninoculated vines)
T2 = Fungicide treatment
T3 = Biocult
T4 = *Glomus* sp. 1054
T5 = Vaminoc

In a study by Franke-Snyder *et al.* (2001), where a range of agricultural soils and crops were investigated, *Gigaspora gigantea* was found to be present at higher spore concentration levels. It accounted for more than 60% of the total spore volume. The lower spore numbers of *Gigaspora gigantea* in the present study may be partially explained in terms of shifts induced in AM fungal communities due to the application of nitrogen fertiliser. This was demonstrated elsewhere in fertilisation experiments, where increased nitrogen application was associated with the displacement of large-spored *Scutellospora* and *Gigaspora* species by small-spored *Glomus* species (Egerton-Warburton & Allen, 2000). The nitrogen application level was about 60 kg/ha/yr, which is comparable to the 75 kg/ha/yr applied in this study.

It is important to note that only 5 out of the 18 species isolated from the vineyard soil (Table 2) were included in the original inocula (Chapter 2, p 54). The rest of the species were indigenous to the vineyard and/or originated from the nursery the vines were obtained from. Due to the close similarity of morphological features between isolates of the same species (Abbott & Robson, 1982), it was impossible, using the methods employed in this study, to distinguish with certainty whether *Glomus etunicatum*, *Glomus intraradices*, *Glomus mosseae* and *Acaulospora spinosa*, detected at the inoculated sites, could have originated from the Biocult and Vaminoc inocula (Chapter 2). These species also occurred in the uninoculated soils, and similar levels of AM root colonisation were observed in the uninoculated and inoculated vine roots (Chapter 2, Fig. 1 & 2).

Therefore, it can be assumed that the use of these species did not result in higher levels of root colonisation. The other AM species present in the inocula, which can be positively identified using the criteria in this study, i.e. *Glomus caledonium, Glomus fasciculatum* and *Glomus versiforme*, were either outperformed by the indigenous AM fungal species, or the vine roots were preferably susceptible for infection by indigenous AM fungi compared to these fungal species. As stated in Chapter 2, a better interpretation of the results would have been possible if the infectivity potential of the AM fungi in the inocula were measured prior to field inoculation.

High spore concentrations, ranging between 1000 and 3779 spores/100 g dry soil, were observed during June 2000 (Table 3). Since spore concentrations are often higher during wet, rainy seasons than during dry seasons (Allen *et al.*, 1998; Schwob *et al.*, 1999; Picone, 2000), a decrease in the spore concentration was likely during the dry summer months normally experienced in the study area. The pH-values (KCl) of soil originating from the spore sampling sites ranged between 5.63 and 6.10 (Table 3) and did not seem to have had a negative impact on spore abundance. It is known that most AM fungal species occur in soils around and above a neutral pH (Schubert and Cravero, 1985).

Spore numbers in soils may also indicate the ability of those soils to support infection of plant roots by AM fungi (Schubert & Cravero, 1985). The high spore numbers correlated well with the relatively high level of root colonisation, between 70% and 90% (Chapter 2, Fig. 2). At the various sampling sites of

each treatment per rootstock block, there was no indication of a significant increase in spore numbers as a direct result of inoculation with commercial AM fungal species. Both the inoculated and uninoculated rhizosphere soils contained similar levels of spore concentrations (Table 3). The fungicide treatment resulted in a significant increase in the number of spores in the rhizosphere soils of 101-14 Mgt vines compared to the rhizosphere soils that received the commercial inocula, Biocult and Vaminoc (Table 3). The application of fungicides may have resulted in a reduction in hyperparasites, predators, or competing plant parasites, which could have encouraged rapid growth and dispersion of AM fungi (Menge, 1982).

The high spore concentration recorded for the 99 R grapevines ranged between 2242 to 3779 spores/100 g dry soil, in comparison to 101-14 Mgt and 110 R grapevines where it never exceeded 2000 spores/100 g dry soil. This may be ascribed to the presence of cover crops (Triticale) that were sown annually between rows of the 99 R vines. The cover crop may have facilitated a vast network of AM fungal hyphae and spores interconnecting the roots of the cover crops and vines (Graves *et al.*, 1997). In contrast, no such network was possible in the ridged soils of the 101-14 Mgt and 110 R vines, due to the absence of cover crops and the annual removal of weeds at these sites (Chapter 2).

TABLE 3
Spore concentrations of AM fungi and pH-values measured at different soil plots of 101-14 Mgt, 110 R and 99 R rootstock cultivars (1999/00-season).

Treatment	Spore Count (/100 g dry soil)	pH (KCl)
101-14 Mgt		
Control	1296 ab	5.80 a
Fungicide	1458 a	5.78 a
Biocult	1000 b	5.83 a
Glomus sp. 1054	1234 ab	5.90 a
Vaminoc	1013 b	5.93 a
LSD (P = 0.05)	416	0.25
110 R		
Control	1254 a	5.75 a
Fungicide	1317 a	5.75 a
Biocult	1075 a	5.70 a
Glomus sp. 1054	1925 a	5.85 a
Vaminoc	1887 a	5.63 a
LSD (P = 0.05)	973	0.26
99 R		
Control	2242 a	5.78 a
Fungicide	2838 a	5.90 a
Biocult	3004 a	6.05 a
Glomus sp. 1054	3779 a	6.10 a
Vaminoc	2712 a	5.95 a
LSD (P = 0.05)	1594	1.27

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

The sandy character of the 99 R plots is another factor, which might have contributed to higher spore abundance at this sampling site. Observations regarding higher spore concentrations in sandy soils in comparison to heavier soils, such as the soils in which the 101-14 Mgt and 110 R vines were planted, were also made in other studies (Bhardwaj *et al.*, 1997; B. Bowman, personal communication, 2000).

3.4. CONCLUSIONS

This study showed that a wide range of AM fungal species, producing an abundance of spores, might occur in a typical South African commercial vineyard. These AM fungal taxa are similar to those recorded in vineyards of other countries by other workers.

The results showed that the mycorrhizal status of soils should be assessed before field inoculation in vineyards is considered. In soils containing adequate concentrations of efficient AM fungi, inoculation is not required. It is equally important for farmers to get acquainted with the negative and positive influences that current vineyard practices may have on the diversity and abundance of these AM fungi. Nitrogen fertilization, amongst others, may cause a shift in the AM fungal population towards small-spored species, whereas the use of cover crops may facilitate the formation of root-hyphal networks and subsequent spore production.

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APPENDIX 1

ANALYSIS OF VARIANCE (ANOVA) PERFORMED ON OBSERVATIONS MADE FOR TWO SEASONS, THREE ROOTSTOCKS, WITH FIVE DIFFERENT TREATMENTS

Table 1
ANOVA of leaf nutrient measurements performed on 101-14 Mgt over two consecutive seasons (1998/99 and 1999/00-seasons).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.23068	0.1383	0.00404	0.0072	0.02358	0.1743
Treatment	4	0.08983	0.5124	0.00151	0.1029	0.02784	0.1172
Error (a)	12	0.10387	0.2236	0.00062	0.6272	0.01204	0.3785
Season	1	3.97530	<0.0001	0.02352	<0.0001	0.62500	<0.0001
Season*Treatment	4	0.03517	0.7288	0.00100	0.3036	0.00579	0.6918
Error (b)	15	0.06886	-	0.00075	-	0.01026	-
Corrected total	39	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 2
ANOVA of leaf nutrient measurements performed on 110 R over two consecutive seasons (1998/99 and 1999/00-seasons).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.00491	0.9877	0.02004	0.1463	0.06636	0.0308
Treatment	4	0.03237	0.8851	0.00422	0.7678	0.00561	0.8377
Error (a)	12	0.11544	0.3403	0.00929	0.0099	0.01593	0.1620
Season	1	4.23801	<0.0001	0.04970	0.0005	0.00000	0.9743
Season*Treatment	4	0.15374	0.2124	0.00365	0.2690	0.00727	0.5554
Error (b)	15	0.09286	-	0.00253	-	0.00932	-
Corrected total	39	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 3
ANOVA of leaf nutrient measurements performed on 99 R over two consecutive seasons (1998/99 and 1999/00-seasons).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.11111	0.0408	0.00380	0.1888	0.01397	0.3227
Treatment	4	0.06882	0.1148	0.00360	0.2000	0.00749	0.6118
Error (a)	12	0.02949	0.8916	0.00203	0.0001	0.01083	0.0237
Season	1	0.20164	0.0876	0.01849	<0.0001	0.01369	0.0704
Season*Treatment	4	0.05803	0.4571	0.00047	0.1543	0.00670	0.1708
Error (b)	15	0.06039	-	0.00024	-	0.00361	-
Corrected total	39	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 4
ANOVA of xylem nutrient measurements performed on 101-14 Mgt over two consecutive seasons (1998/99 and 1999/00-seasons).

Dependent Variable		Amino acids		Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.00068	0.0093	3	0.9765	0.8685	3	0.5244	0.8561
Treatment	4	0.00015	0.3231	4	3.1983	0.5604	4	1.6920	0.5348
Error (a)	12	0.00011	0.7166	12	4.1103	0.0048	12	2.0543	0.1364
Season	1	0.00302	0.0006	1	0.0262	0.8577	1	28.0749	0.0004
Season*Treatment	4	0.00015	0.4473	4	1.0739	0.3036	4	3.2582	0.0613
Error (b)	14	0.00016	-	11	0.7790	-	10	1.0153	-
Corrected total	38	-	-	35	-	-	34	-	-

P-values ≤ 0.05 indicate significantly different results

Table 5
ANOVA of xylem nutrient measurements performed on 110 R over two consecutive seasons (1998/99 and 1999/00-seasons)..

Dependent Variable	Amino acids			Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.0002	0.0361	3	5.6049	0.7780	3	0.6592	0.7145
Treatment	4	0.0003	0.4493	4	14.2607	0.4766	4	0.9113	0.6456
Error (a)	12	0.0003	0.4471	12	15.2621	0.5305	12	1.4292	0.7732
Season	1	0.0091	<0.0001	1	272.9852	0.0024	1	5.6418	0.1374
Season*Treatment	4	0.0002	0.4525	4	11.4651	0.5941	4	1.2404	0.6981
Error (b)	13	0.0002	-	9	15.7220	-	12	2.2269	-
Corrected total	37	-	-	33	-	-	36	-	-

P-values ≤ 0.05 indicate significantly different results

Table 6
ANOVA of cane length measurements performed on 101-14 Mgt, 110 R and 99 R over two consecutive seasons (1998/99 and 1999/00-seasons).

Dependent Variable: Cane length							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	30117.23	0.0014	673.53	0.8624	5063.71	0.3529
Treatment	4	4461.61	0.2700	4017.48	0.2720	8505.75	0.1572
Error (a)	12	3022.31	0.1344	2734.97	0.2143	4233.95	0.6667
Season	1	176669.14	<0.0001	30619.62	0.0009	79816.36	0.0017
Season*Treatment	4	3984.10	0.0951	1127.07	0.6470	4025.09	0.5805
Error (b)	15	1653.19	-	1781.93	-	5453.85	-
Corrected total	39	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 7
ANOVA of xylem sap nutrient measurements performed on 101-14 Mg (1998/99-season).

Dependent Variable	Amino acids			Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.000025	0.1089	3	0.3437	0.8759	3	1.5345	0.5368
Treatment	4	0.000008	0.5451	4	2.0552	0.3168	4	3.2949	0.2323
Error	11	0.000010	-	10	1.5185	-	11	2.0044	-
Corrected total	18		-	17	-	-	18	-	-

P-values ≤ 0.05 indicate significantly different results

Table 8
ANOVA of xylem sap nutrient measurements performed on 110 R (1998/99-season).

Dependent Variable	Amino acids			Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.000008	0.1028	3	2.1644	0.5591	3	0.7202	0.8789
Treatment	4	0.000002	0.5471	4	1.2299	0.7958	4	1.4632	0.7692
Error	10	0.000003	-	10	2.9796	-	12	3.2365	-
Corrected total	17		-	17	-	-	19	-	-

P-values ≤ 0.05 indicate significantly different results

Table 9
ANOVA of xylem sap nutrient measurements performed on 101-14 Mgt (1999/00-season).

Dependent Variable	Amino acids			Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.001062	0.0138	3	2.5860	0.5787	3	0.70096	0.5462
Treatment	4	0.000283	0.2806	4	2.0248	0.7101	4	0.73073	0.5606
Error	12	0.000196	-	10	3.7492	-	8	0.91954	-
Corrected total	19	-	-	17	-	-	15	-	-

P-values ≤ 0.05 indicate significantly different results

Table 10
ANOVA of xylem sap nutrient measurements performed on 110 R (1999/00-season).

Dependent Variable	Amino acids			Phosphate			Nitrate		
Source	DF	Mean Square	P-value	DF	Mean Square	P-value	DF	Mean Square	P-value
Block	3	0.000470	0.4545	3	27.2722	0.4227	3	0.29494	0.4980
Treatment	4	0.000548	0.4053	4	20.7387	0.5595	4	1.20362	0.0551
Error	12	0.000503	-	8	26.0252	-	9	0.34456	-
Corrected total	19	-	-	15	-	-	16	-	-

P-values ≤ 0.05 indicate significantly different results

Table 11
ANOVA of cane length measurements performed on 101-14 Mgt, 110 R and 99 R (1998/99-season).

Dependent Variable: Cane length							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	9320.81	0.0003	3976.18	0.0252	288.40	0.8289
Treatment	4	1001.63	0.2607	3107.67	0.0413	763.71	0.5599
Error	12	663.40	-	890.756	-	980.36	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 12
ANOVA of cane length measurements performed on 101-14 Mgt, 110 R and 99 R (1999/00-season).

Dependent Variable: Cane length							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	23423.29	0.0086	1977.30	0.3996	4064.57	0.2292
Treatment	4	7444.08	0.1626	1211.54	0.6355	4137.60	0.2177
Error	12	3768.68	-	1853.83	-	2455.82	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 13
ANOVA of leaf nutrient measurements performed on 101-14 Mgt (1998/99-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.0377	0.0153	0.0028	0.0430	0.0171	0.3471
Treatment	4	0.0089	0.3498	0.0018	0.1172	0.0181	0.3302
Error	12	0.0072	-	0.0008	-	0.0141	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 14
ANOVA of leaf nutrient measurements performed on 110 R (1998/99-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.0276	0.9125	0.0086	0.3623	0.0669	0.0731
Treatment	4	0.1080	0.6200	0.0062	0.5260	0.0111	0.7397
Error	12	0.1592	-	0.0074	-	0.0223	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 15
ANOVA of leaf nutrient measurements performed on 99 R (1998/99-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.0608	0.0061	0.0016	0.2618	0.0103	0.2353
Treatment	4	0.0077	0.5143	0.0028	0.0871	0.0046	0.5864
Error	12	0.0089	-	0.0011	-	0.0063	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 16
ANOVA of leaf nutrient measurements performed on 101-14 Mgt (1999/00-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.2490	0.2707	0.0015	0.1420	0.0199	0.0944
Treatment	4	0.1162	0.6138	0.0007	0.4253	0.0155	0.1446
Error	12	0.1687	-	0.0007	-	0.0074	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 17
ANOVA of leaf nutrient measurements performed on 110 R (1999/00-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.0129	0.8917	0.0172	0.0204	0.0113	0.0176
Treatment	4	0.0781	0.3486	0.0017	0.7641	0.0018	0.5482
Error	12	0.0634	-	0.0036	-	0.0023	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 18
ANOVA of leaf nutrient measurements performed on 99 R (1999/00-season).

Dependent Variable		N		P		K	
Source	DF	Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	0.1941	0.0610	0.0027	0.1214	0.0065	0.5261
Treatment	4	0.1192	0.1615	0.0013	0.3982	0.0095	0.3802
Error	12	0.0601	-	0.0011	-	0.0083	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 19
ANOVA of leaf water potential measurements performed on 101-14 Mgt, 110 R and 99 R (1998/99-season).

Dependent Variable: Leaf Water Potential							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	8581.48	0.5040	109476.48	0.0001	17465.88	0.2994
Treatment	4	61926.88	0.0003	38596.32	0.0021	16900.56	0.3157
Day	8	363417.81	0.0001	325867.81	0.0001	213962.41	0.0001
Day*Treatment	32	8048.67	0.8417	6861.48	0.7804	6143.11	0.9977
Error	132	10924.76	-	8705.84	-	14144.10	-
Corrected total	179	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 20
ANOVA of leaf water potential measurements performed on 101-14 Mgt, 110 R and 99 R (1999/00-season).

Dependent Variable: Leaf Water Potential							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	39088.17	0.1292	19944.33	0.2538	67254.82	0.0139
Treatment	4	24140.75	0.3200	10372.63	0.5840	3503.45	0.9428
Day	9	207613.67	<0.0001	225206.44	<0.0001	659244.40	<0.0001
Day*Treatment	36	25008.74	0.1990	13534.91	0.5856	8639.90	0.9951
Error	147	20377.28		14540.00		18355.80	
Corrected total	199	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 21
ANOVA of spore concentrations performed on 101-14 Mgt, 110 R and 99 R (1999/00-season).

Dependent Variable: Spore Concentration							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	427636.40	0.0106	1663776.33	0.0307	1665698.98	0.2509
Treatment	4	15139.63	0.1463	604827.13	0.2588	1255132.95	0.3708
Error	12	73073.19	-	398728.46	-	1069827.48	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

Table 22
ANOVA of pH measurements performed on 101-14 Mgt, 110 R and 99 R (1999/00-season).

Dependent Variable: pH							
Source	DF	101-14 Mgt		110 R		99 R	
		Mean Square	P-value	Mean Square	P-value	Mean Square	P-value
Block	3	1.1192	0.0001	0.0898	0.0674	0.7765	0.3696
Treatment	4	0.0168	0.6578	0.0270	0.4783	0.0655	0.9815
Error	12	0.0271	-	0.0290	-	0.6765	-
Corrected total	19	-	-	-	-	-	-

P-values ≤ 0.05 indicate significantly different results

APPENDIX 2

ARBUSCULAR MYCORRHIZAL FUNGAL STRUCTURES ASSOCIATED WITH ROOTS OF NEWLY ESTABLISHED GRAPEVINES