AN INVESTIGATION OF SOILBORNE FUNGI ASSOCIATED WITH ROOTS AND CROWNS OF NURSERY GRAPEVINES

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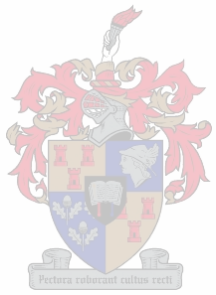

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DECLARATION

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

Signature: Date:

SUMMARY

AN INVESTIGATION OF SOILBORNE FUNGI ASSOCIATED WITH ROOTS AND CROWNS OF NURSERY GRAPEVINES

Soilborne diseases of grapevines represent a complex problem with limited information available, both locally and internationally. Previous research in South Africa indicated that *Phytophthora* and *Pythium* spp. were the most widespread and devastating pathogens in grapevine nurseries and vineyards in the Western Cape province. The local grapevine industry is currently expanding; new cultivars, methods and agricultural chemicals are being used which can affect soilborne pathogens. It has therefore become necessary to re-assess the status of soilborne pathogens in nurseries, since information in this regard is crucial for the development of disease management practices for the expanding local grapevine industry.

Soilborne fungal genera associated with roots and crowns of declining nursery grapevines were assessed in surveys conducted at three different grapevine nurseries in the Western Cape province. *Cylindrocarpon*, *Fusarium*, *Pythium*, and *Rhizoctonia* spp. were consistently isolated from roots and crowns of declining nursery grapevines. *Cylindrocladiella* spp. and *Phytophthora cinnamomi* were infrequently isolated from diseased roots, crowns and soil whereas *Pythium* spp. were abundant in most of the soils. Results suggest that the status of soilborne fungal pathogens in grapevine nurseries in the Western Cape province has changed over the last 30 years.

The DNA phylogeny and pathogenicity of the isolates of *Cylindrocladiella* were determined. Four species of *Cylindrocladiella* occur on grapevines in South Africa, namely *C. lageniformis*, *C. parva*, *C. peruviana*, as well as a new species, described in this study as *C. viticola*, which forms part of the *C. infestans* species complex. Pathogenicity trials were inconclusive.

Ten *Fusarium* spp. were isolated from roots and crowns of declining nursery grapevines, namely *F. acuminatum*, *F. anthophilum*, *F. chlamydosporum*, *F. equiseti*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. scirpi*, *F. semitectum* and *F. solani*. The dominant species was *F. oxysporum*, followed by *F. proliferatum* and *F. solani*. In pathogenicity trials *F. oxysporum* and *F. solani* significantly reduced root volume, root dry mass, length of new shoots, stem diameter and number of leaves, but increased the percentage of chlorotic leaves and root rot severity. *Fusarium proliferatum* also caused a significant

reduction in new shoot growth, number of leaves and increased root rot severity compared to the controls. *Fusarium solani* seems to be more virulent than *F. oxysporum*, followed by *F. proliferatum*. This is the first report of *F. oxysporum*, *F. proliferatum* and *F. solani* as pathogens of grapevines in South Africa, and the first report of *F. proliferatum* as a pathogen of grapevines in the world.

Phytophthora cinnamomi was isolated at low frequencies from declined grapevines, although present in the rhizosphere soil. It is possible that the extensive use of downy mildew chemicals in grapevine nurseries may protect grapevines from infection by *P. cinnamomi*. The effect of chemicals used to combat downy mildew on Phytophthora root rot of nursery grapevines was evaluated in a glasshouse. There was very little discernable effect of the chemicals tested relative to the control plants for the parameters measured and it was concluded that the inoculation technique needed refinement. However, plants treated with phosphorous acid tended to be taller and have more leaves, greater stem diameter and root volume than controls or plants treated with the other chemicals. The data obtained in this study are not conclusive, but indicated certain trends that more glasshouse trials and field trials would resolve.

Results presented in this thesis indicate that a major shift has occurred in the status of soilborne fungi associated with roots and crowns of grapevines in nurseries in the Western Cape since the 1970s when *Phytophthora* and *Pythium* were predominant. The prevalence and role of soilborne fungi need to be determined so that new appropriate disease management strategies can be developed to limit losses in grapevine nurseries and ensure the sustainable production of healthy plants for the grapevine industry.

OPSOMMING

'N ONDERSOEK NA GRONDGEDRAAGDE SWAMME GEASSOSIEER MET WORTELS EN KRONE VAN WINGERD IN KWEKERYE

Grondgedraagde siektes van wingerd is 'n komplekse probleem waaroor min inligting, beide plaaslik en internasionaal, beskikbaar is. Vorige navorsing in Suid-Afrika het aangedui dat swamme van die genera *Phytophthora* en *Pythium* die mees algemene en vernietigende grondgedraagde patogene in kwekerye en wingerde in die Wes-Kaap provinsie is. Die plaaslike wingerdbedryf brei huidiglik uit; nuwe kultivars, metodes en landbouchemikalieë word gebruik wat 'n invloed kan hê op grondgedraagde patogene. Gevolglik het dit noodsaaklik geword om die status van grondgedraagde patogene in wingerdkwekerye weer te bepaal, aangesien inligting in hierdie verband noodsaaklik is vir die ontwikkeling van siekte bestuurspraktyke vir die ontwikkelende plaaslike wingerdbedryf.

Grondgedraagde swamgenera geassosieer met wortels en krone van terugsterwende wingerd in kwekerye is bepaal in opnames wat by drie verskillende wingerdkwekerye in die Wes-Kaap provinsie uitgevoer is. *Cylindrocarpon*, *Fusarium*, *Pythium*, en *Rhizoctonia* spp. is konstant vanuit wortels en krone van terugsterwende wingerdplante in kwekery geïsoleer, *Cylindrocladiella* spp. en *Phytophthora cinnamomi* is ongereeld vanuit siek wortels, krone en grond geïsoleer, terwyl *Pythium* spp. algemeen in meeste gronde voorgekom het. Resultate dui daarop dat die status van grondgedraagde swampatogene in wingerdkwekerye in die Wes-Kaap provinsie oor die laaste 30 jaar verander het.

Die DNA filogenie en patogenisiteit van die isolate van *Cylindrocladiella* is bepaal. Vier spesies van *Cylindrocladiella* kom voor op wingerd in Suid-Afrika, naamlik *C. lageniformis*, *C. parva*, *C. peruviana*, sowel as 'n nuwe spesie, wat in hierdie studie as *C. viticola* aangedui is en wat deel is van die *C. infestans* spesie kompleks. Patogenisiteits proewe was onvoldoende om die patogeniese status van die swamme te bepaal.

Tien *Fusarium* spp. is vanuit wortels en krone van terugsterwende wingerdplante in kwekery geïsoleer, naamlik *F. acuminatum*, *F. anthophilum*, *F. chlamydosporum*, *F. equiseti*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. scirpi*, *F. semitectum* en *F. solani*. Die dominante spesies was *F. oxysporum*, gevolg deur *F. proliferatum* en *F. solani*. In pathogenisteitsproewe het *F. oxysporum* en *F. solani* gelei tot 'n betekenisvolle laer wortelvolume, droë massa van wortels, lengte en droë massa van nuwe groei en aantal blare, maar het die persentasie chlorotiese blare en graad van wortelvrot verhoog. *Fusarium*

proliferatum het ook gelei tot 'n betekenisvolle afname in lengte en massa van nuwe groei, aantal blare en 'n verhoogde graad van wortelvrot in vergelyking met die kontrole behandelings. Dit wil voorkom asof *Fusarium solani* meer virulent is as *F. oxysporum*, gevolg deur *F. proliferatum*. Hierdie is die eerste aanmelding van *F. oxysporum*, *F. proliferatum* en *F. solani* as patogene van wingerd in Suid-Afrika, en die eerste aanmelding van *F. proliferatum* as 'n patogeen van wingerd in die wêreld.

Phytophthora cinnamomi is konstant teen lae frekwensies vanuit terugsterwende wingerd in kwekerye geïsoleer, alhoewel dit in risosfeer gronde teenwoordig was. Dit is moontlik dat die ekstensiewe gebruik van chemikalieë teen donsskimmel in wingerdkwekerye die wingerdplante kan beskerm teen infeksie deur *P. cinnamomi*. Die effek van chemikalieë wat gebruik word teen donsskimmel op Phytophthora wortelverrotting van wingerd in kwekerye, is 'n glashuis geëvalueer. Die chemikalieë wat gestoets is, het vir die gemete parameters, tot baie min onderskeibare effek gelei relatief tot die kontrole plante, en daar is afgelei dat die inokulasie tegniek verbetering benodig. Plante wat met fosforiensuur behandel is, het egter geneig om langer te wees met meer blare, 'n groter stamdeursnee en wortelvolume as kontrole plante of plante behandel met ander chemikalieë. Data verkry vanuit die hierdie studie was onvoldoende, maar sekere neigings is aangedui wat deur verdere glashuis- en veldproewe verklaar sal word.

Resultate wat in hierdie tesis weergegee is, het aangedui dat 'n algehele verskuiwing in die status van grondgedraagde swamme geassosieer met wortels en krone van wingerd in kwekerye vanaf die 1970s, toe *Phytophthora* en *Pythium* die dominante genera was, plaasgevind het. Die voorkoms en rol van grondgedraagde swamme moet bepaal word, sodat nuwe voldoende siektebestuurspraktyke ontwikkel kan word om verliese in wingerdkwekerye te beperk en sodoende die volhoubare produksie van gesonde plante vir die wingerdbedryf te verseker.

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And much as Wine has play'd the Infidel
And robb'd me of my Robe of Honour – well
I often wonder what the Vintners buy
One half so precious as the Goods they sell

– Omar Khayyám

CONTENTS

1. AN OVERVIEW OF SOILBORNE DISEASES OF GRAPEVINES, WITH SPECIFIC REFERENCE TO PHYTOPHTHORA ROOT ROT

INTRODUCTION

The cultivation and production of wine and table grapes (*Vitis* spp.) for both local and export industries in the Western Cape province of South Africa, is one of the country's major agricultural industries. Although grapevines are planted extensively in the Western Cape province where a Mediterranean climate prevails, the industry is sizable in the Orange River region (Northern Cape) and is expanding to parts of Mpumalanga as well as the Northern Province (G. Holz pers. comm.). There has been a 10% increase over the past ten years in the land area planted to wine grapes. At the end of November 2001, 106 331 ha (Western Cape = 90 532 ha; Orange River region = 15 799 ha) were cultivated in comparison with 91 942 ha in 1991 (Western Cape = 81 079 ha; Orange River region = 10 863 ha in 1991) (Anonymous, 2002). The increased vineyard plantings coincided with the opening of trade on international markets.

In South Africa vineyards are established using plants produced in specialised local grapevine field nurseries that have to adhere strictly to high standards imposed by plant improvement legislation (Anonymous, 2002). The nurseries are therefore fundamental to maintaining the industry and supporting expansion of it. Currently there are 54 registered grapevine nurseries in the Western Cape province of which a total of 40 nurseries produces both grafted wine and table grape plants, 10 produce wine grape plants only and three produce table grape plants only (Anonymous, 2003). In 2001, 39.3 million grapevines were produced in nurseries, of which only 17.8 million (45.29%) were considered suitable for commercial use (Anonymous, 2002). Losses of grapevine propagation material in nurseries are therefore annually estimated at more or less 55% of the total number of cuttings grafted at the beginning of the season. The percentage of these losses that can be ascribed to diseases and insect damage is uncertain (N. Engelbrecht pers. comm.). Thus, huge savings could be made and the nursery industry could become more economical if a higher percentage of the nursery material could be used. Additionally, it is of crucial importance that grapevine material provided to the farmer is of good physical, physiological and phytosanitary quality, since weaker vines are more susceptible to stress and subsequent infection by various stress-related pathogens once planted in the

vineyard (Stamp, 2001). Clearly any improvement in nursery grapevine health would realise profits for nurserymen and long-term benefits for farmers.

In South Africa, grapevines cultivated in nurseries and vineyards are affected by various aboveground, as well as soilborne diseases caused by a large number of fungal, bacterial and viral pathogens (Marais, 1981). Although pest damage is also highly significant, it is beyond the scope of this study, which will concentrate on fungal diseases only.

Fungal pathogens can attack aboveground plant parts as well as roots and crowns. A list of aboveground fungal diseases of grapevines, including postharvest diseases, reported in the Western Cape province, is given in Table 1. Although the survival structures and infective propagules of some of these fungi may overwinter in plant debris on the soil surface, in general these pathogens do not affect grapevine roots, are not considered soilborne fungi and will therefore not be discussed further. Thus, true soilborne pathogens of grapevines are those that cause root rot, crown and collar rot and wilt.

The effects of soilborne pathogens are often greatly underestimated and consequently not sufficiently studied. Grapevines are no exception to this, and soilborne diseases of grapevines have not been studied extensively in recent years in South Africa, or other grape-producing areas in the world. However, to date the most comprehensive study on root pathogens of grapevines in South Africa was carried out by Dr. P.G. Marais in the 1970's and early 1980's. A diversity of fungi from several genera have been reported (Table 2). Soilborne fungal pathogens associated with the death and decline of grapevines have also been reported as a threat to the production, cultivation and preservation of high quality grapevines in other countries (Chiarappa, 1959; McGechan, 1966; Agnihothrudu, 1968; Bumbieris, 1970, 1972; Van der Merwe, Joubert & Matthee, 1972; Marais, 1979, 1980; Mansilla, Pintos & Salinero, 1993; Latorre, Wilcox & Banados, 1997). However, many of these reports were made 30 or more years ago and little recent information is available.

Increasing costs, high interest rates and the need for quick returns on investments have led to the need for rapid and reliable establishment of vineyards that will come into production soon after planting. This has caused a demand for high quality planting stock and expansion of the nursery industry. In view of the current losses reported by the nurseries and the possible gains that can be made by managing soilborne diseases it is necessary to reassess the status, cause and extent of losses attributable to soilborne pathogens. This is therefore the main aim of this thesis

and background knowledge on soilborne diseases and pathogens of grapevines is essential to this study.

SOILBORNE FUNGAL DISEASES REPORTED FROM GRAPEVINES

Armillaria root rot

Armillaria (Fr.) Staude species are soilborne basidiomycetous fungi that are voracious wood rotting pathogens, which affect a wide range of trees and shrubs throughout the world (Gregory, Rishbeth & Shaw, 1991). Armillaria root rot of grapevines is caused by the fungus *Armillaria mellea* (Vahl:Fr.) P. Karst., commonly known as the honey fungus. The fungus has a broad host range and is considered to originate in the Northern Hemisphere, although in South Africa it has been recorded on proteas in Kirstenbosch Gardens as well as being found in the Gardens in central Cape Town (Denman *et al.*, 2000; Coetzee *et al.*, 2001, 2003). In grapes, infection occurs mostly through root contact with diseased material. Airborne basidiospores are thought to be insignificant (Raabe, 1990). Therefore, unless vineyards are established on previously infected sites or in areas where trees may have been infected, this disease is unlikely to occur. Since the introduction of molecular tools as an aid to identification and taxonomic relationships of species, the taxonomy of *Armillaria* has undergone many changes. It is therefore now necessary to re-assess the taxonomy of *Armillaria* spp. associated with grapevines.

Symptoms. Infected grapevines show severe wilting and can die quickly, or may decline slowly, characterised by stunting of plants, lack of vigor, reduced foliage size and leaves may be dark-green and eventually vines die (Marais, 1981; Raabe, 1990). It has been reported that stunted grapevines can live through the growing season but then die during the dormant period (Raabe, 1990). Disease usually spreads along the rows of the vineyard where the pathogen can spread easily from vine to vine, but occasionally may traverse the row and form dead patches, especially if the rows are planted close together.

Signs. The fungus produces a white mat between the bark and the hardwood and during cooler periods at the beginning of winter, basidiocarps may be seen, although they are rarely produced (Raabe, 1979). Infected roots become soft and have a typical fungal, although not sour, odour (Marais, 1981). Guillaumin, Mercier and Dubos (1982) reported that signs of *Armillaria*

disease are similar to infection by *Rosellinia necatrix* Prill. (anamorph *Dematophora necatrix* R. Hartig) and mycelium is visible in the roots and collar.

Nurseries. Armillaria root rot of grapevines has not been reported in nurseries.

Vineyards. This disease is a serious problem in France, but in most other grape-producing areas it is less of a problem (Guillaumin *et al.*, 1982; Raabe, 1990). Although Marais (1981) reported that *A. mellea* occurs very sporadically in South Africa, it has not yet been isolated from vineyards in South Africa. This disease may be confused with Dematophora root rot (caused by *R. necatrix*) (Pearson & Goheen, 1988).

Black foot

Species of *Cylindrocarpon*, causal agents of black foot of grapevine, are known soil inhabitants, where they live saprobic, or as weak pathogens, often on roots of herbaceous woody plants (Brayford, 1993). On grapevines, two species of *Cylindrocarpon* have been associated with the disease known as black foot, namely *Cylindrocarpon destructans* (Zinssm.) Scholten and *Cylindrocarpon obtusisporum* Wollenw. (Maluta & Larignon, 1991), but DNA phylogeny of isolates of this genus occurring on grapevines have indicated various species to be pathogenic to grapevines (F. Halleen, pers. comm.) This disease occurs commonly in nurseries and younger grapevines in the Western Cape province (Fourie, Halleen & Volkmann, 2000; Fourie & Halleen, 2001; Halleen, Crous & Petrini, 2003), as well as other major grape producing areas like California, USA (Scheck *et al.*, 1998), France (Maluta & Larignon, 1991), Italy (Grasso, 1984), Portugal (Rego *et al.*, 2000), and Sicily (Grasso & Magnano di San Lio, 1975).

Symptoms. Typical symptoms include stunting, black discolouration of wood, and gum inclusions of xylem vessels (Grasso & Magnano di San Lio, 1975).

Signs. No signs of this pathogen is visible on infected grapevine tissue.

***Cylindrocladiella* spp. associated with declining grapevines**

The genus *Cylindrocladiella* Boesew., a hypocrealean genus, was proposed by Boesewinkel (Boesewinkel, 1982) to accommodate small-spored species of *Cylindrocladium* Morgan. This decision was based on the fact that species of *Cylindrocladiella* had different conidiophore branching patterns, conidial shapes, dimensions, cultural characteristics and teleomorphs than *Cylindrocladium*. In South Africa, five species of *Cylindrocladiella* have been reported as being phytopathogenic to, or saprobic on various hosts. *Cylindrocladiella camelliae* (Venkataram. & C.S.V. Ram) Boesew. has been associated with cutting rot of *Eucalyptus* spp., *C. elegans* Crous & M.J. Wingfield with roots of *Arachis hypogaea* L. and *Eucalyptus* leaf litter, *C. lageniformis* Crous, M.J. Wingf. & Alfenas with soil and roots of *Vitis vinifera* L., *C. parva* (P.J. Anderson) Boesew. with soil, *Eucalyptus* cuttings, and roots of *Fragaria* sp., *Persea americana* Mill., *Pinus* spp., *Prunus* sp. and *V. vinifera*, and *C. peruviana* (Bat., J.L. Bezerra & M.M.P. Herrera) Boesew. with root and cutting rot of *Acacia mearnsii* De Wild., *Eucalyptus* spp., *Protea* sp. and *V. vinifera* (Crous & Wingfield, 1993; Victor *et al.* 1998; Crous, 2002). However, pathogenicity was never confirmed for any of these species on grapevines. Isolates of *C. lageniformis*, *C. parva* and *C. peruviana* have over the past ten years been isolated from mature grapevines, cuttings and grafted grape seedlings (Lamprecht & Crous, unpubl. data), but Koch's postulates have never been proven.

Dematophora root rot (Rosellinia root rot)

Dematophora root rot is named after the anamorph stage of the pathogen, *D. necatrix*, because this is the most frequently encountered form. The teleomorph stage of this pathogen is the well-known ascomycete fungus *R. necatrix*. However, the latter takes several years to form, perithecia are uncommon and hence this stage is not frequently encountered (Raabe, 1990). Disease caused by this pathogen is usually very serious on both grapevines and deciduous fruit trees, particularly in temperate regions although it has been reported from the Mediterranean climates. Infection is thought to take place by root contact with diseased material and conidia produced on synnemata are not considered an important source of inoculum (Raabe, 1990).

Symptoms. Grapevines infected with Dematophora root rot may die very rapidly, or slowly during one season, or continue into the next year (Raabe, 1990). Usually, the grapevines

bear an extremely large crop the year before they die (Pearson & Goheen, 1988). On grapevines that die rapidly, the leaves usually remain attached, but on grapevines that die gradually the tendrils and leaves are stunted (Pearson & Goheen, 1988). Wilting of grapevines also occurs frequently (Pearson & Goheen, 1988). Infected roots (large and small) turn dark, become soft and the bark (root cortex) sloughs off (Marais, 1981). The wood of the crown is weakened and grapevines can easily be pulled from the soils because the roots are rotted away.

Signs. The fungus grows on the roots and crown area of the grapevine and is visible as a fine, white fluffy growth (Marais, 1981). The hyphae penetrate the wood and under moist soil conditions may even be evident on roots and collars as a fine mycelial mass (Guillaumin *et al.*, 1982; Pearson & Goheen, 1988). Confirmation of the pathogen may be made by microscopic examination of the hyphae, which have characteristic bulbous swellings on one side of each septum. Hyphae may also form tight aggregates of mycelium similar to sclerotial masses and these structures may be important in survival in the absence of root material.

Nurseries. This pathogen has been reported from nursery grapevines (Marais, 1979, 1981; Veghelyi 1991). In the Western Cape, Marais (1979) isolated *R. necatrix* only from grapevine nurseries on poorly drained soils, and concluded that it is not a major soilborne disease of grapevines in South Africa (0.7% of fungal isolates obtained). However, with the current expansion of the grapevine industry and the replacement of many apple and fruit orchards with vineyards, this disease may become more important in the future.

Vineyards. In South Africa, *R. necatrix* has not yet been reported from vineyards, although this pathogen causes serious problems in France and other European countries (Guillaumin *et al.*, 1982; Véghelyi, 1991; Véghelyi, Balogh & Polyák, 1995). This disease has also been reported from a vineyard in Japan (Kanadani, Date & Nasu, 1998). From 1976-1994, Véghelyi *et al.* (1995) collected 451 grape root samples from replanted vineyards, nurseries and soil of culled plantations. *Rosellinia necatrix* was the most common fungus on root debris (71.6 % of isolates obtained), and it was present in most of the field plots tested (Véghelyi *et al.*, 1995). Véghelyi (1991) also reported *R. necatrix* as one of the main causal agents of fruit tree decline in Hungary, and that it is common in soils from fruit and vineyards that are established soon after wood clearance. In Hungary the frequency of *R. necatrix* was independent of the type, structure and pH value of the soil (Véghelyi *et al.*, 1995), and it was also found on all fruit and grapevine

varieties, irrespective of soil type (Véghelyi, 1991). Sztejnberg and Madar (1980) determined the host range of *D. necatrix* in greenhouses as well as the field. Pathogenicity was determined on the grapevine rootstocks 41-B, 110 Richter, and SO_4, 1613 and Salt Creek. *Dematophora necatrix* was pathogenic to all the grapevine rootstocks, with the Salt Creek and 1613 rootstocks being the least susceptible (Sztejnberg & Madar, 1980).

Diseases on grapevines caused by *Fusarium* spp.

In general, the *Fusarium* species associated with grapevines are poorly recorded and often not identified to species level. In spite of this a few *Fusarium* species have been associated with grapevines. In Brazil, *Fusarium* spp. cited as "*F. equiseti, F. moniliforme* var. *anthophilum*, *F. moniliforme* var. *subglutinans* (*Gibberella fujikuroi* var. *subglutinans*), *F. oxysporum* and *F. solani*" were identified in a root and soil environment (Andrade, 1993). *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. has also been reported from Australia (Highet & Nair, 1995), California (Omer, Granett & Wakeman, 1999), Pennsylvania (Gugino, Travis & Stewart, 2001) and South Africa (Marais, 1979; Ferreira, Matthee & Thomas, 1989), and *Fusarium* spp. cited as "*Fusarium solani* and *Fusarium moniliforme*" were also found on grapevines in Egypt (Mahrous, 2001). The above mentioned species represent root infecting fungi that cause root rot and grapevine decline or may be opportunistic secondary root pathogens. However, one of the most important *Fusarium* species associated with grapevines that causes a very serious disease (Fusarium wilt) is *F. oxysporum* f.sp. *herbemontis* (Tochetto) n. comb. Syn. *F. oxysporum* Schl. var. *herbemontis* Tochetto. This pathogen was first reported in 1965 (Gordon, 1965) and caused serious problems in Brazil (Andrade, 1993).

Symptoms of root rot and vine decline. Roots sampled from declining grapevines showed rotting of epidermal tissue, and pink discolouration of cortical as well as vascular tissues (Highet & Nair, 1995). Necrosis of cortical tissue, along with browning and tylosis of the xylem was also observed in grapevine roots infected by *F. oxysporum* (Highet & Nair, 1995). Root necrosis and a reduction of feeder roots were observed and delayed, weak growth was also typical of decline (Mahrous, 2001).

Symptoms of Fusarium wilt. Visual symptoms on grapevines infected with the Fusarium wilt pathogen may first appear at flowering as yellow spots on the margin of leaves

(Andrade *et al.*, 1995). These spots expand into yellow streaks and leaves eventually die. Leaves of plants that are severely diseased may drop, while root systems may decay and exhibit brown vascular discolouration (Andrade *et al.*, 1995).

Signs. Signs of the pathogens are not usually witnessed for root rot, vine decline or Fusarium wilt.

Nurseries. No information is available on the effect of root rot and grapevine decline, or wilt, caused *Fusarium* spp. on grapevines in nurseries.

Vineyards.

Root rot and vine decline. In South Africa, Marais (1979) isolated three distinct groups of *Fusarium* from the rootstock cultivar Jacques, but in tests the isolates were not pathogenic to grapevines and were not identified further (Marais, 1979). Ferreira *et al.* (1989) sampled grapevines with dieback symptoms from 22 different localities in the Western Cape Province and made isolations from the diseased trunk-area. Fifteen different fungal genera were isolated, and six genera were pathogenic to vines (Ferreira *et al.*, 1989). Ferreira *et al.* 1989 reported that *Fusarium oxysporum* was the third most common fungus isolated but pathogenicity studies were not carried out.

In a preliminary survey conducted at Videira Experimental Station/EPAGRI in Brazil the high mortality rate of grapevines was attributed to *Fusarium* spp., predominantly, cited as "*F. solani*", followed by "*F. oxysporum*, *F. equiseti*, *F. moniliforme* var. *anthophilum* and *F. moniliforme* var. *subglutinans* (Teleomorph = *Gibberella fujikuroi* var. *subglutinans*)" (Andrade, 1993). The highest population levels occurred from September to February and for *F. equiseti*, *F. moniliforme* var. *anthophilum* and *F. moniliforme* var. *subglutinans* the highest infection level observed was during September (Andrade, 1993).

Grapevine decline in Egypt was always associated with root rot (Mahrous, 2001). *Fusarium oxysporum* and *Fusarium solani* (Mart.) Appel & Wollenw. Emend. Snyd. & Hans. were also among the fungi most commonly isolated from roots of grapevines suffering from replant problems in the New York state, USA (Deal, Mai & Boothroyd, 1972).

Fusarium wilt. Fusarium oxysporum f.sp. *herbemontis* was found to be the most important pathogen on grapevines, causing extensive plant death in some regions of Santa Catarina, Brazil (Andrade, 1993). Resistance of various grapevine cultivars to Fusarium wilt was tested by Andrade *et al.* (1995) in natural infected fields, as well as under controlled conditions. Hybrids of *Vitis riparia* M x *V. berlandieri* PI, as well as *V. riparia* x *V. rupestris* Sch. were susceptible to Fusarium wilt. Hybrids of *V. berlandieri* x *V. rupestris* gave intermediate resistance, while the cultivar Paulsen 1103, as well as the cultivar Issabella, and some hybrids of *V. vinifera* x *V. rotundifolia* were resistant.

Macrophomina root rot of grapevines

This disease is caused by *Macrophomina phaseolina* (Tassi) Goid. (subdivision Deuteromycotina, form-class Coelomycetes) which is the anamorphic stage of the ascomycete fungus *Orbilia obscura* Ghosh, Mukherji & Basak. However, this teleomorphic stage is still unconfirmed (Ghosh *et al.*, 1964).

Symptoms. According to Marais (1981) infected grapevines exhibited an overall weak growth. Leaves start to yellow and develop necrotic regions on the leaf margin or between veins (Marais, 1981). Roots turn dark brown and feeder roots become almost completely absent, while some roots can become very brittle (Marais, 1981). Black spots are visible in the vascular tissue when the grapevine is cut through transversely above the crown area (Marais, 1981).

Signs. *Macrophomina phaseolina* has a wide host range, and although microsclerotia are sometimes visible on host tissue (Mihail, 1992), no information about signs of this pathogen on grapevines are available.

Nurseries. No information is available on *M. phaseolina* associated with grapevines in nurseries internationally in general. Furthermore, in spite of the extensive work carried out in nurseries by Marais (1980), he never isolated *M. phaseolina* from nursery grapevines in the Western Cape province of South Africa.

Vineyards. In South Africa, Marais (1979) isolated *M. phaseolina* infrequently (6.2% of isolates obtained) from diseased grapevines, and only from grapevines grafted onto the rootstock

101-14 Mgt in regions with a high summer temperature (Marais, 1981). Marais (1979) confirmed that *M. phaseolina* is pathogenic to the rootstock 101-14 Mgt.

Phymatotrichum root rot

Phymatotrichum root rot is caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar (syn. *Ozonium auricomum* Lk., *Ozonium omnivorum* Shear, *Phymatotrichopsis omnivora* (Duggar) Hennebert). This fungus belongs to the subdivision Deuteromycotina, order Moniliales, and the family Moniliaceae (Lyda & Kenerley, 1992). Although there is currently no evidence for the existence of a teleomorphic stage of this fungus, *Hydnum omnivorum* Shear (Shear 1925) and *Treichispora brinkmanii* (Bres.) Rogers & Jackson (Baniecki & Bloss, 1969) have been indicated as possible teleomorphic forms for this fungus. *Phymatotrichum omnivorum* has a very wide host range; more than 2000 species of tap-rooted plants can be affected by this fungus (Lyda & Kenerley, 1992). To date, it has not been reported in South Africa and is on the country's list of quarantine organisms (E. Carstens pers. comm.).

Symptoms. This disease often occurs in a circular pattern in a vineyard, where infected vines can suddenly wilt and die during the early summer or midsummer (Pearson & Goheen, 1988). Leaves of vines that die rapidly turn brown and brittle and stay rigidly attached to the dead plant (Pearson & Goheen, 1988). Before the grapevines start showing symptoms of wilt, the leaves on some of the infected grapevines turn yellow or red, and interveinal yellow spots and irregular necrotic areas on leaf blades and margins are visible (Pearson & Goheen, 1988). By the time that the first foliar symptoms appear, many of the roots have already rotted (Pearson & Goheen, 1988).

Signs. Thick or thin strands of mycelium of the fungus can usually be seen on the surface of infected roots. The root cortex can easily be separated from the inner cylinder (Pearson & Goheen, 1988).

Nurseries. No information is available on the effect of *P. omnivorum* on grapevines in nurseries.

Vineyards. This disease, also known as Texas root rot, occurs on grapes in the southwestern United States (Texas, New Mexico, Arizona, southern Nevada, and southeastern California) (Pearson & Goheen, 1988).

Phytophthora root and crown rot

Phytophthora spp. are classified as fungal-like organisms or pseudofungi. They belong to the Kingdom Chromista, class Oomycetes. Different species of *Phytophthora* de Bary have been reported as pathogens of grapevines in various countries around the world, namely *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cinnamomi* Rands, *P. cryptogea* Pethybr. & Laff., *P. drechsleri* Tucker and *P. nicotianae* Breda De Haan (McGechan, 1966; Agnihothrudu, 1968; Bumbieris, 1970, 1972; Van der Merwe, Joubert & Matthee, 1972; Marais, 1979, 1980; Mansilla *et al.*, 1993; Latorre *et al.*, 1997). These reports usually indicated that *P. cinnamomi* was the most important and devastating *Phytophthora* species on grapevines.

Symptoms. The first aboveground evidence of infection is usually a discolouration of the foliage of the affected grapevine (Van der Merwe *et al.*, 1972; Latorre *et al.*, 1997). Foliage is sparse and often yellowish (Latorre *et al.*, 1997). Delayed, weak and unthrifty growth characterises affected grapevines (Chiarappa, 1959; Bumbieris, 1972). On many occasions, affected plants are stunted (Latorre *et al.*, 1997). The most striking symptom is the sudden dieback of affected grapevines (Van der Merwe *et al.*, 1972). This dieback can take place in the nursery or on older grapevines, usually up to an age of seven years (Van der Merwe *et al.*, 1972). Infected grapevines can exhibit low fruit yields (Latorre *et al.*, 1997), but in some cases grapevines can keep their grapes for most of the season (Chiarappa, 1959). Canes of infected plants do not mature properly at the end of the growing season and can be killed back by early frost (Chiarappa, 1959). Roots of grapevines affected by *P. cinnamomi* are usually almost dead and in many cases rotting of the crown occurs (Van der Merwe *et al.*, 1972). Abundant necrosis of large roots, abnormal branching of roots and reduction of feeder roots due to necrosis are associated with declined grapevines (Chiarappa, 1959). Grapevines growing on their own roots (not grafted onto rootstock cultivars) are most seriously affected (Latorre *et al.*, 1997).

Signs. Signs of the pathogen are usually not visible to the naked eye.

Nurseries. Marais (1980) indicated that *Phytophthora* spp. were, after various *Pythium* spp., the most common fungal pathogens isolated from the roots and rhizosphere soils of dead and dying grapevines in nurseries in the Western Cape. The various *Phytophthora* spp. isolated were *P. cactorum*, *P. cinnamomi*, *P. cryptogea*, *P. megasperma* Drechsler, and *P. nicotianae*, comprising 23.5% of the total number of isolates obtained from nurseries. Of the different *Phytophthora* spp. isolated, *P. cinnamomi* was isolated most frequently. During inoculation studies, *P. cinnamomi* and *P. nicotianae* caused severe root rot and death, while *P. cactorum* and *P. cryptogea* caused rotting of the fine feeder roots and reduction in root mass but not death of the plants. *Phytophthora megasperma* was nonpathogenic. Marais (1980) concluded that *P. cinnamomi* is the most pathogenic root rot fungus in grapevine nurseries in the Western Cape province, South Africa.

Vineyards. *Phytophthora cinnamomi* is a pathogen with a very wide range of different plant species (Zentmyer, 1980; Erwin & Ribeiro, 1996). Von Broembsen (1984) reported that *P. cinnamomi* was most frequently isolated from grapevines and proteas, of the cultivated plants tested in the South Western Cape region of South Africa.

Phytophthora cinnamomi is an important pathogen that causes rotting of roots and collars of grapevines grafted onto 99 Richter rootstocks. It was first noted as such in 1972 (Van der Merwe *et al.*, 1972) even though it was known as a serious pathogen for four decades prior to that (Van der Merwe *et al.*, 1972). In the Western Cape the most commonly obtained *Phytophthora* spp. was *P. cinnamomi* (27.49%), followed by *P. cactorum* (0.25%), *P. nicotianae* (0.19%) and *P. cryptogea* (0.15%) (Marais, 1979). All four *Phytophthora* spp. were shown to be pathogenic to certain grapevine rootstocks, with *P. cinnamomi* appearing to be the most aggressive species (Marais, 1979). Marais (1988) reported that the majority of losses due to *P. cinnamomi* occurred in young vineyards, but in some instances, grapevines of up to 10 years old were also killed.

In California, Chiarappa (1959) reported that a decline of unknown cause had occurred for several years in various irrigated vineyards in the San Joaquin Valley of California. More than half of the 1131 isolates obtained from grapevine roots and naturally infested soils of these California vineyards were Pythiaceous, and in total, about 32% were various unnamed species of *Phytophthora* (Chiarappa, 1959).

In Australia, McGechan (1966) consistently isolated *P. cinnamomi* from root and crown rots of two-year-old rooted cuttings of the rootstock *Vitis rupestris* Scheele cv. Rupestris du Lot.

Phytophthora cinnamomi was also isolated from phylloxera-resistant rootstocks, as well as from the roots and collars of affected mature grapevines (McGechan, 1966). Shortly after that, Bumbieris (1970) reported grapevine decline in South Australia and mentioned that several *Pythium* spp. and a *Phytophthora* sp. had been isolated from soil and diseased grapevine roots. Nematodes were also found in association with these grapevines (Bumbieris, 1970). Bumbieris (1972) investigated the role of various pythiaceous fungi associated with grapevine decline in South Australia, and reported that the grapevine cultivars most affected by this disease were Doradillo, Gordo, Grenache and Pedro.

In Chile, Latorre *et al.* (1997) reported that nearly 50 000 ha of table grapes were cultivated on their own roots and many of these were in a state of decline. Grapevines with crown or root rots were usually found singly or in small groups and were associated with either excessive irrigation or poor soil drainage (Latorre *et al.*, 1997). *Phytophthora cinnamomi*, *P. cryptogea*, and *P. drechsleri* were isolated from diseased root and crown tissues of various table grape cultivars, with *P. cryptogea* being isolated most frequently (Latorre *et al.*, 1997).

Agnihothrudu (1968) reported that death of mature grapevines had been observed in several vineyards in an around Hyderabad, in India. He showed that *P. cinnamomi* was invariably present in grapevines examined in the laboratory. Likewise, Mansilla *et al.* (1993) discovered that *P. cinnamomi* was responsible for decline, stunting and chlorosis of the grape cultivar Albariño in two villages in the province of Pontevedra, Spain.

Clearly of all the known *Phytophthora* species reported on grapevines to date, *P. cinnamomi* is the most important species causing root and collar rot, and decline and sudden death of grapevines. First records suggest that Phytophthora diseases were first reported in the 1960's, but by the 1970's more reports at different localities in the world were being made. There appears to be a dearth of information between the 1980's and late 1990's and current knowledge of this pathogen on grapevines appears to be scant, with particularly little reported from Northern Hemisphere grape producing countries.

Root rot caused by *Pythium* spp.

In the grapevine industry it is commonly known that *Pythium* species are fairly abundantly associated with grapevines (Bumbieris, 1972; Marais, 1979, 1980; Uthkede & Vielvoye, 1984), although the species identities and effect they have on the host are not

extensively documented. Various *Pythium* (*Pm.*) spp. that have been recorded on diseased grapevines include *Pm. aphanidermatum* (Edson) Fitzpatrick (Bumbieris, 1972; Marais, 1979, 1980), *Pm. irregulare* complex (Bumbieris, 1972; Marais, 1979, 1980), *Pm. rostratum* complex (Marais, 1979, 1980), *Pm. sylvaticum* complex (Marais, 1979, 1980) and *Pm. ultimum* Trow (Bumbieris, 1972; Marais, 1979, 1980; Uthkede & Vielvoye, 1984). *Pythium irregulare* Buisman and *Pm. ultimum* are common soil-inhabiting species with a very wide host range and almost universal distribution. Both have a broad-range temperature tolerance (Van der Plaats-Niterink, 1980). In contrast, *Pm. aphanidermatium* is a warm climate pathogen with optimal temperatures for growth at 30 – 35 °C. *Pythium sylvaticum* W.A. Campb. & J.W. Hendrix was the first heterothallic *Pythium* species recognised (Campbell & Hendrix, 1967) and also produces toxins (Van der Plaats-Niterink, 1980). It grows quite rapidly, across a wide range of temperatures from 5 – 40 °C, with optimal growth occurring at 25 °C. *Pythium rostratum* Butler is characterised by its slow growth rate and intercalary often catenulate oogonia. It is found in a range of environments from wet to very dry and is considered a weak pathogen (Van der Plaats-Niterink, 1980).

Symptoms. Leaves of grapevines that are affected by *Pythium* spp. turn yellow-green and sometimes develop yellow spots during the mid-summer (Marais, 1981). Browning of leaf margins occurs and shoot growth is reduced so that the grapevines appear stunted (Marais, 1981). These symptoms indicate water stress that can be attributed to roots rendered non-functional by *Pythium* infection. Grapevines between the age of three and five years remain weak and unthrifty but do not die. They yield fewer and smaller grape bunches than normal (Marais, 1981). Many of the medium-sized (fine roots) roots of infected plants are discoloured black and almost no side-rootlets are present. When washed and carefully examined under good lighting a streaky brown to black discolouration is visible inside affected vines (Marais, 1981).

Signs. Signs of *Pythium* infection cannot be seen with the naked eye. Root clearing and staining may, however, reveal oospores embedded in diseased tissues. If infected roots are incubated in pond water over night sporangia may develop from the roots (Van der Plaats-Niterink, 1980).

Nurseries. *Pythium* spp. most frequently isolated from diseased nursery grapevines were *Pm. ultimum*, *Pm. aphanidermatum*, *Pm. sylvaticum*, *Pm. irregulare* and *Pm. rostratum*, comprising 36.4% of the total number of isolates obtained. *Pythium ultimum* was the most aggressive species, although the various *Pythium* spp. tested caused rotting of the fine feeder roots and reduction in root mass but not death (Marais, 1980). However, Utkhede and Vielvoye (1984) reported that during pathogenicity trials, *Pm. ultimum* caused severe root rot and death of grapevines under greenhouse conditions and large lesions on excised twig vines *in vitro*.

Vineyards. In South Africa, Marais (1979) obtained 1409 *Pythium* isolates out of a total of 7548 fungal isolates, with the predominant species being *Pm. ultimum* (10.88%), followed by *Pm. aphanidermatum* (3.05%), *Pm. sylvaticum* (2.66%) and *Pm. irregulare* (2.08%). All four *Pythium* spp. were pathogenic on grapevine rootstocks with *Pm. ultimum* being the most virulent species (Marais, 1979).

Bumbieris (1970) reported that several *Pythium* spp., as well as a *Phytophthora* species were involved in grapevine decline in South Australia. In British Columbia, Utkhede and Vielvoye (1984) found that *Pythium ultimum* was the most commonly isolated pathogen from grapevines (cv. Okanagan Riesling) that showed symptoms of decline.

Root rot of grapevines caused by *Rhizoctonia solani* Kühn

Although Marais (1979) reported that *R. solani* was commonly associated with declining grapevines on Ramsey rootstocks in South Africa and caused decreased root mass, Walker (1992) stated that *Rhizoctonia* DC. was not regarded as an important pathogen of grapevines around the world. However, Walker (1992) did demonstrate that depressed root mass and root rots, particularly of cv. Sultana, were caused by this pathogen. The pathogenic isolates were identified as *R. solani* AG 4 and AG 2-1, and there were a few isolates from an unknown AG type (Walker, 1994, 1997). Disease in the field was far more severe than that obtained through artificial inoculations in the glasshouse, and this was attributed to other soilborne pathogens and environmental effects in the field (Walker, 1992).

Symptoms. Marais (1981) described above-ground symptoms of grapevines (rootstock Ramsey) infected with *Rhizoctonia* as leaves turning light yellow and later brown as the plants

die. Leaves remain on the grapevine long after it has died (Marais, 1981). Symptoms on roots of the rootstock Ramsey are similar to those caused by *Phytophthora* spp. on 99 Richter. Infected grapevines can be recognised because they are stunted and display dieback (Walker, 1992). Grapevines die in patches in the field and roots were rotted and blackened when removed from the soil, and *R. solani* was consistently isolated from these plants (Walker, 1992).

Signs. There are no reports on signs of the disease, however by microscopic examination of infected roots the mycelium of the pathogen might be seen (Carling & Sumner, 1992). Also, if roots are cleared and then appropriately stained, mycelium of the pathogen maybe seen.

Nurseries. Walker (1992) associated *R. solani* with root rot and dieback of grapevine rootlings in a field nursery at Barmera, South Australia. Pathogenicity of *R. solani* to the rootstock cv. Ramsey was confirmed in the glasshouse. Walker (1992) also reported that root rot was observed on the cv. Sultana when infected with the pathogen, but root mass was not significantly lower.

Vineyards. In the Western Cape province of South Africa, Marais (1979) isolated *R. solani* infrequently from diseased vineyards. *Rhizoctonia solani* was shown to be pathogenic to *Vitis champini* var. Salt Creek [= Ramsey] (Walker, 1992). *Rhizoctonia solani* was, among others, isolated from diseased vineyards in Egypt (Mahrous, 2001). The grapevine cultivars tested under greenhouse and field conditions that were most susceptible to root rot were Early Superior, Fiesta, Flame seedless, Superior and Thompson seedless (Mahrous, 2001).

Diseases on grapevines caused by *Sclerotium rolfsii* Sacc.

Sclerotium rolfsii has been reported as an important pathogen of grapevines from South Africa only (Marais, 1979, 1981; Keyser & Ferreira, 1988). It appears that young grapevines and nursery grapevines are most susceptible to this pathogen, where it attacks roots and stems at the graft union at an early age (Marais, 1979; Keyser & Ferreira, 1988). It is also an important pathogen of grapevines in callus trays (Marais, 1979). The pathogen spreads through the soil as mycelium and can overwinter and survive for many seasons as sclerotia (Punja & Rahe, 1992).

Symptoms. In nursery grapevines, the fungus penetrates mainly through the graft-union and then girdles the plant resulting in eventual death (Keyser & Ferreira, 1988). In older and younger grapevines the bark is rotted and becomes loose and yellow-coloured on the inside (Marais, 1981). Leaves of affected grapevines are light yellow, shoots are stunted and overall vigor is impeded (Marais, 1981).

Signs. On older grapevines the typical white fungal mass can be seen from the soil surface to more than one meter below the surface (Marais, 1981). During inoculation trials plants infected with *S. rolfsii* had a typical white fungal growth on the crown area and roots while bark and underlying tissue were soft and came off easily (Marais, 1980).

Nurseries. *Sclerotium rolfsii* was isolated infrequently (2.9% of fungal isolates obtained) from nursery material growing in open field nurseries (Marais, 1980), but Marais (1979) had indicated that it was a serious problem on grapevines in callus trays and nurseries. The latter was confirmed by Keyser and Ferreira (1988) who reported that *S. rolfsii* was responsible for tremendous losses in grapevine nurseries during the 1984/85 and 1985/86 seasons in the Wellington-area, Western Cape Province, South Africa.

Vineyards. Marais (1981) reported that *S. rolfsii* occurs seldom on older grapevines, as it is a bigger problem in grapevine nurseries. This is a commonly known trait of *S. rolfsii* from other industries (e.g. apples) where it causes southern blight (Jones & Aldwinckle, 1990).

Verticillium wilt

Verticillium wilt is caused by the fungus *Verticillium dahliae* Kleb, although a single report of this disease being caused by *Verticillium albo-atrum* Reinke & Berthold was made in Greece (Zachos & Panagopoulous, 1963 in Schnathorst & Goheen, 1977). This pathogen has a broad host range including cotton, lettuce, tomatoes and woody hosts such as pistachio (Pegg, 1974). *Verticillium dahliae* is the anamorph stage of the fungus, which taxonomically is placed in the *Hypocreales* (sub-family *Hypocreaceae*) (Ainsworth & Bisby, 1995). The teleomorphic stage of this fungus is unknown. Infection is thought to take place through the soilborne microsclerotia that germinate and penetrate roots, and a high level of inoculum is necessary for disease. The pathogen is not transmitted in cuttings propagated from infected mother material

(Thate, 1961 in Schnathorst & Goheen, 1991). Disease is therefore likely to occur only in areas where previous crops were heavily infected with the pathogen.

Symptoms. Grapevines do not show symptoms during the early part of the season, but some of the shoots start dying and the vascular elements in the wood of these grapevines discolour when the temperature rises and soil moisture declines (Pearson & Goheen, 1988; Canter-Visscher, 1970). Canter-Visscher (1970) reports that the first signs of infection are observed in the leaves, which wilt and discolour from green to a light yellow-green. According to Schnathorst and Goheen (1977), early symptoms included dead and dying grapevines as well as vascular discolouration of the wood. During the early summer the leaves on these declining shoots start to wilt and show marginal burning (Pearson & Goheen, 1988). By midsummer many grapevines collapse completely (Pearson & Goheen, 1988) or partially (Schnathorst & Goheen, 1977) where affected shoots are interspersed with healthy ones on alternate nodes. In other cases, all the canes on one side of the grapevine are affected, while those on the other half of the grapevine remained normal (Schnathorst & Goheen, 1977). The leaves may suddenly dry without being shed and turn a light grey-green (Canter-Visscher, 1970), or in other cases the leaves are shed and fruit clusters at the base of affected grapevines become dried out (Schnathorst & Goheen, 1977).

Signs. No visible signs of the pathogen have been reported. Schnathorst and Goheen (1977) maintained that the pathogen was more difficult to isolate from woody tissue of grapevines than from stem tissues from other hosts such as cotton, tomato and safflower.

Nurseries. The only information available on Verticillium wilt of grapevines in nurseries is a brief mention that *V. dahliae* was the causal agent of losses of container grown nursery grapevines in south-eastern Australia (M. Cole in Stephens *et al.*, 1992).

Vineyards. Verticillium wilt is not a very frequently recognised disease of grapevines but has been reported from California (Schnathorst & Goheen, 1977), France (Thate, 1961 in Schnathorst & Goheen, 1977), Germany (Thate, 1968 in Schnathorst & Goheen, 1977), Greece (Zachos & Panagopoulos, 1963 in Schnathorst & Goheen, 1977) Italy (Dalla Rosa, 1973 and D'Erole, 1970 in Schnathorst & Goheen, 1977), and New Zealand (Canter-Visscher, 1970). Schnathorst and Goheen (1977) maintained that the lack of detection could be due to the

difficulty in isolating the pathogen from grapevine wood. In general only a 40% recovery rate was obtained for isolations and it could take up to 8 weeks for the pathogen to emerge from plated material into the culture medium (Schnathorst & Goheen, 1977). In California it occurred more often in vineyards that had been established on soils where crops that are known to be susceptible to *Verticillium* Nees had been grown (Pearson & Goheen, 1988). The following cultivars were shown to be susceptible: Chardonnay, Chenin Blanc, French Colombard, Ganzin No. 1, Johannisberg Riesling, Petite Sirah, Pinot Blanc, Pinot Noir, Pinot St. George, and Semillon (Schnathorst & Goheen, 1977). The disease also occurred in own-rooted grapevines (Schnathorst & Goheen, 1977).

In New Zealand, Verticillium wilt was reported for the first time in 1969 from a two-year-old Rupestris St. George vineyard with an unthrifty appearance at Taradale, Napier (Canter-Vissher, 1970). This disease has not yet been reported on grapevines in South Africa.

Clearly research to date has identified a number of pathogenic fungal genera on *Vitis* spp. In South Africa, the different pathogens vary in importance to grape production. Thus the economical feasibility of conducting research on management of these pathogens and the diseases they cause, is not always justified. In general, however, *Phytophthora* diseases are very destructive and affect a large variety of crops. Consequently much effort and expense has been, and is continuously, spent on disease management tools and strategies that will reduce losses. Some of these tools and strategies have already been tested on Phytophthora root rot on grapevines and are successfully used, but new products and technologies are now available and need consideration. For this reason it is important to know what research has already been carried out on *Phytophthora* root rot on grapevines and what the current status is. Furthermore, it is pertinent to consider the biology and ecology of the organism since the preferred approach towards disease management involves integration of factors affecting disease. The latter aspect is reviewed here below.

PHYTOPHTHORA ROOT ROT OF GRAPEVINES

Disease cycle of *P. cinnamomi*

Phytophthora cinnamomi produces four asexual propagules namely sporangia, zoospores (released from the sporangia under favourable conditions), hyphal swellings and chlamydospores,

as well as sexual propagules called oospores (Zentmyer, 1980).

Roots are the primary plant organ infected by *P. cinnamomi* (Zentmyer, 1980). Exudates of grapevine roots vary between different grapevine rootstocks (Marais & Hattingh, 1985a). Motile zoospores of *P. cinnamomi* were chemotactically attracted to grapevine roots (Marais & Hattingh, 1985a). In this regard, Marais and Hattingh (1985a) showed that zoospores of *P. cinnamomi* were more attracted to roots of the susceptible rootstock 99 Richter than to roots of the more tolerant rootstock 143 B Mgt. Root exudates from 99 Richter contained higher concentrations of glutamic acid and arginine than root exudates from 143 B Mgt and the tolerant cultivar Jacquez (Marais & Hattingh, 1985a). The chemotactic index of zoospores of *P. cinnamomi* increased with increasing concentrations of glutamic acid, arginine and aspartic acid (Marais & Hattingh, 1985a). Using *in vitro* tests, Marais and De La Harpe (1982) determined how *P. cinnamomi* penetrated roots of the susceptible 99 Richter rootstock. Within a few minutes of contacting the root surface, the zoospores encysted and the cysts adhered to the root surface. The cysts then germinated and within 20 minutes after germination, scanning electron microscopy images showed the growth of germ tubes of *P. cinnamomi* over roots. The germ tube first appeared as a conical protuberance, where the cyst made contact with the root. It grew parallel to the root until it reached a site suitable for the germ tube to penetrate. Penetration of the root tissue occurred more often at the wall junction of epidermal cells than through the host wall, indicating that *P. cinnamomi* followed the path of least resistance to enter the host. Zentmyer (1980) observed that after penetration of the root, mycelium of *P. cinnamomi* started developing, and could be found throughout the root within 72 hours. During favourable conditions, namely plenty of moisture (Marais & De La Harpe, 1982) and warm temperatures (18-30°C), *P. cinnamomi* will form numerous sporangia containing zoospores on the surface of infected plant roots (Zentmyer, 1980). Within a short period of time a vast number of zoospores will be released from the sporangia (Zentmyer, 1980). This phase is very dangerous for the plants, because this cycle will continue to repeat if favourable conditions prevail, creating high disease pressure.

Phytophthora cinnamomi is also capable of forming chlamydospores. In the monograph of *P. cinnamomi* by Zentmyer (1980), the chlamydospores of *P. cinnamomi* are described as "a perennating walled spore which is slowly built up from a portion of the mycelium, more or less swollen with reserves, deliminated by a septum if terminal, or septa if intercalary, and with an extra inner, thickened wall layer." Chlamydospores are important survival units for *P.*

cinnamomi when there is no living host available, and it is also capable of surviving in dead root tissue or in soil for long periods of time (Zentmyer & Mircetich, 1966).

During sexual reproduction, *P. cinnamomi* forms oospores, either through interspecific crosses, or through intraspecific crosses (Zentmyer, 1980). If interspecific crosses take place, new hybrid species could emerge, and although this has been discovered in some species in Europe and the UK (Brasier, Cooke & Duncan, 1999) it has not yet been reported to occur in nature for *P. cinnamomi*. These oospores are extremely resistant to unfavourable environmental conditions and thus enable the fungus to survive in soil for years and also enhance the pathogen's ability to adapt through exchange of genetic material.

The two mating types of *P. cinnamomi* are known as the A1 and A2 mating types (Zentmyer, 1980). Both mating types of *P. cinnamomi* are known to occur in the Western Cape province, South Africa (Linde *et al.*, 1997), and both mating types are associated with grapevines in South Africa (S. Denman pers. comm.; Zentmyer, 1976).

Occurrence and spread of *P. cinnamomi* in vineyard soil

Phytophthora cinnamomi occurs widely in rivers and other sources of water throughout the Western Cape province (Von Broembsen, 1984b). Zoospores of *P. cinnamomi* are transported in free running groundwater, as well as in rivers, tributaries and headwaters throughout the South Western Cape, South Africa, which accounts for dispersal of the pathogen over longer distances (Von Broembsen, 1984b; Marais & Hattingh, 1985b). In vineyard soils, *P. cinnamomi* spreads mainly through water movement (Marais & Hattingh, 1985b).

Marais and Hattingh (1985b) determined the occurrence of *P. cinnamomi* in vineyards in the Western Cape province and its spread in a naturally infested vineyards soil. Furthermore, Marais (1988) did a quantitative analysis of *P. cinnamomi* in soils on a weekly basis over a period of one year. Lowest levels of the pathogen were recorded from April to June, followed by a sharp increase in soil populations in October that reached peak levels in November through to early January (Marais, 1988). Thus, it is clear that there are seasonal variation in inoculum potential and disease risk, and this must be taken into account in disease management strategies.

Marais and Hattingh (1985b), isolated *P. cinnamomi* from dead or dying grapevines grafted on different rootstocks in vineyards from 14 districts of the Western Cape province. Out

of 114 vineyards surveyed, *P. cinnamomi* was isolated from plant tissue from 69 of these vineyards, but it could only be recovered from soil surrounding infected grapevines in 11 vineyards only. The vertical distribution of the pathogen in a soil profile appeared to be related to soil form, and was mostly restricted to the upper part of the root zone of the grapevine. Marais and Hattingh (1985b) furthermore reported that in an Estcourt soil with an 8% slope, *P. cinnamomi* spread downhill in the first year at a rate of six meters in six months, after which it reached the Clovelly soil, and slowed down to a rate of six meters in 18 months. The reason for this slowing down of *P. cinnamomi* in the Clovelly soil was probably due to the fact that a water table or clay barrier did not exist in the Clovelly soil. The possibility that different microflora and vegetation types could affect the rate of dissemination, however, cannot be ignored. Marais and Hattingh (1985b) detected only slight lateral spread of *P. cinnmomi* in the soil, with almost no uphill movement.

When a new vineyard is planted, the indigenous flora that was previously situated on the particular soil must be taken into account, since naturally occurring *Proteaceae* is a well-known host of *P. cinnamomi* (Von Broembsen, 1984a), especially if the soil is an Estcourt soil with a clay barrier or perched water table (Marais & Hattingh, 1985b).

Management of *P. cinnamomi* on grapevines

The development of effective management strategies of Phytophthora root rot is especially difficult in the south western Cape, the area where grapevines are cultivated most extensively in South Africa. This is attributed to the fact that natural inoculum of *P. cinnamomi* from infected indigenous flora, as well as infested river water, is abundant (Von Broembsen, 1984a, 1984b), and because *P. cinnamomi* can spread from diseased to healthy grapevines in vineyards (Marais, 1979, 1980). The pathogen is furthermore widespread in vineyard and nursery soils in the Western Cape province (Marais, 1979, 1980). Therefore, an integrated approach to disease management is preferable, and producers should strive for disease avoidance through exclusion and prevention. In South Africa, Marais (1981) and Marais and Hattingh (1986) conducted research on the control of *P. cinnamomi* on grapevines. For the control of *Pythium* spp. on grapevines in South Africa, Marais (1981) prescribed the same control measures as those used for *P. cinnamomi*.

Soil treatments before plant. According to Marais (1981) soil fumigation with methyl bromide gives acceptable control of *P. cinnamomi* when applied at 125 g/m^2 underneath a plastic covering (250 µ). The plastic covering must remain on the soil surface for 72 h and a time span of at least six weeks must pass before grapevines can be planted (Marais, 1981). Since this process is very expensive and therefore impractical to fumigate a whole nursery, it is better to employ it in specific locations within nurseries where *P. cinnamomi* infestation occurs (Marais, 1981). Marais (1981) also concluded that dazomet (980 g a.i./kg Basamid GE, BASF) applied at 50 g/m^2 in the topsoil followed by an overhead application of water for one hour gives effective control of *P. cinnamomi*. Due to a phytotoxic effect, cuttings can only be planted three weeks after fungicide application.

Hot water treatment. According to Baker (1962), the basic principle of thermotherapy is that "parasitic microorganisms often are killed, or viruses inhibited at temperature-times only slightly injurious to the host". Von Broembsen and Marais (1978) reported that research was published as early as 1900 indicating hot water treatment as an effective way of eliminating phylloxera from rooted grapevines, and therefore investigated the use of hot water as a means of eradicating *P. cinnamomi* from grapevine material. They treated dormant rooted grapevines, actively growing vines, as well as canes with hot water at various temperatures for different times to determine the effect on *P. cinnamomi*. These dormant rooted grapevines were planted in a field nursery and no *P. cinnamomi* was isolated after eight months (Von Broembsen & Marais, 1978). It was concluded that hot water treatment of dormant, rooted grapevines at 50°C from 5 to 30 min eradicated *P. cinnamomi* and it was then strongly advocated that dormant rooted grapevines as well as dormant cuttings be treated with hot water at 50 °C for 15 min. Among the major advantages of hot water treatment, is that it is highly efficient, easy to conduct, economically viable, environmentally safe and it also reduces the amount of pesticides used drastically (Szegedi, 1995). However, if the treatment was not correctly applied there were losses incurred due to loss of viability.

Waite *et al.* (2001) determined the cause of losses of grapevine propagation material subjected to hot water treatment. Results indicated no single cause, but rather a number of contributing factors, like variety, collection date, and the position of cutting on the original cane. Goussard (1977) reported that callus formation was not inhibited at water temperatures of 50°C and below, for exposure times of up to 60 min. However, at temperatures of 55°C callus

formation was inhibited after exposure times of more than 10 min. Optimum callusing was obtained at 50°C for 30 min., which correlates well with the results obtained by Von Broembsen and Marais (1978).

The effect of hot water treatment on fungi occurring in apparently healthy grapevine cuttings were investigated by Crous *et al.* (2001), and also by Rooney and Gubler (2001), who investigated the effect of hot water treatment on *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams and *Phaeoacremonium inflatipes* W. Gams *et al.* from dormant grapevine wood. Crous *et al.* (2001) reported that hot water treatment of grapevine cuttings at 50°C effectively rid grapevines from fungi when compared against control treatments, but these results were contrary to the results obtained by Rooney and Gubler (2001).

Chemical treatment against *P. cinnamomi* before and after planting. Marais and Hattingh (1986) tested a variety of fungicides on 20 cm cuttings of 99 Richter rootstocks in the glasshouse and in field trials before and after planting, to determine their effect on Phytophthora root rot, caused by *P. cinnamomi*. In glasshouse trials, fourteen days after inoculation with *P. cinnamomi*, the following fungicides were applied as soil drenches in a 1-L volume of water, namely fenaminosulf (Bayer 5072WP) at 12 g a.i./m^2, metalaxyl (Ridomil 25WP, Syngenta) at 2 g a.i./ m^2, fosetyl-Al (Aliette WP, Aventis) at 16 g a.i./ m^2 and prothiocarb (Previcur 70EC) at 12 g a.i./m^2. Fosetyl-Al (Aliette WP, Aventis) was also applied as a foliar spray until runoff at 16 g a.i./ m^2. In field trials before planting the following fungicides were applied as soil drenches and irrigated with 25 mm of water, namely metalaxyl (Ridomil 25WP, Syngenta) at 2 g a.i./ m^2 and fosetyl-Al (Aliette WP, Aventis) at 16 g a.i./ m^2 while dazomet (Basamid GE, BASF), applied at 50 g/m^2were distributed and worked into the upper 20 cm of the soil. Methyl bromide (Meth-O-Gas) was applied as a gas underneath a black 0.25 mm polyethylene sheet that was removed four days later. In field trials eight weeks after planting, soil drenches were made with ethazol (Terrazole 24EC) at 5 g a.i./ m^2 and fosetyl-Al (Aliette WP, Aventis) at 2 g a.i./ m^2, as well as a foliar spray with fosetyl-Al (Aliette WP, Aventis) at 3.2 g a.i./m^2.

Results obtained from the glasshouse trials indicated that fosetyl-Al foliar spray and metalaxyl, applied as a soil drench, significantly reduced root rot of grapevines caused by *P. cinnamomi*. Ethazol, applied as a soil drench, did result in a decreased mortality of grapevines, but was less effective than fosetyl-Al and metalaxyl. With the fungicides tested before planting soil treatments with dazomet and methyl bromide (applied as a gas underneath a black, 0.25 mm

polyethelene sheet) significantly reduced mortality of grapevines, reduced root infection and increased root mass. Fosetyl-Al applied as a soil drench had no significant effect. With the fungicides tested after planting, fosetyl-Al (3.2 g a.i./L), applied as a foliar spray and metalaxyl (2 g a.i/L) applied as a soil drench, significantly reduced root rot and mortality of grapevines due to *P. cinnamomi*. The shoot length of grapevines also increased significantly with both of these fungicides. On the basis of these results, Marais and Hattingh (1986) recommended that grapevine nursery soils be treated with a drench of metalaxyl (2 g a.i./m^2) before planting and that planted grapevines should receive drench applications every five months, or monthly foliar applications of fosetyl-Al (3.2 g a.i./L), beginning when shoots are 10 cm long.

Susceptibility of grapevine rootstocks to *Phytophthora cinnamomi*. The use of resistant rootstock cultivars is a very important aspect of integrated disease management. Different grapevine rootstocks show different levels of resistance to *P. cinnamomi* (Marais, 1979). The rootstocks 105-Paulsen, 99 Richter and 110 Richter are more susceptible to *P. cinnamomi*, while Ramsey is moderately resistant and Jacquez, 101-14 Mgt and 143 B Mgt are considered resistant (Marais, 1988). Resistance was associated with root exudates (Marais & Hattingh, 1985a) (discussed previously).

Biological control. To date, little research has been carried out on biological control of pythiaceous fungi. Biological control has been defined by Baker and Cook (1974) as the reduction of inoculum density or disease producing activities of a pathogen in its active or dormant state by one or more organisms. It was shown that compounds released by mycorrhizal fungi can protect roots of *Pinus radiata* D. Don. against *P. cinnamomi* (Krupa & Nyland in Ribeiro, 1978). Some soils can naturally suppress *P. cinnamomi* (Baker & Cook, 1974) due to various factors such as the content of organic matter, high levels of calcium, favourable soil pH, a high population of soil microbes, and adequate levels of ammonium and nitrate, phosphate and magnesium. Soil amendments like alfalfa meal, cotton waste, soybean meal and wheat straw have also been reported to decrease the incidence of *P. cinnamomi* on avocado (Zentmyer, 1980; Gilpatrick in Ribeiro, 1980).

Other aspects of management. Cultural practices such as improved soil drainage and minimised irrigation are important. The effect of crop rotation on reduction of soilborne inoculum in nurseries as well as soil amendments with organic composts must still be

determined. There has also been interest in biofumigation to reduce the incidence of soilborne pathogens of grapevines (Stephens *et al.*, 1999). The concept of biofumigation is based upon the volatile gasses produced by certain plant species that may reduce soilborne pathogen populations (Stephens *et al.*, 1999). Regulations regarding the widespread use of fungicides are becoming more strict (Ragsdale, 2000), and therefore alternative methods of managing soilborne diseases of grapevines have to be developed.

CONCLUSION

This review emphasises the local and international importance of soilborne diseases to the sustainable development of the grapevine industry, as well as the potential economic impact of these diseases. In South Africa, no major work regarding soilborne diseases of grapevines in nurseries or vineyards have been conducted since the work conducted by Dr. P.G. Marais in the 1970s. With the current expansion of the local grapevine industry, the resulting increased demand for high quality healthy grapevine propagation material are placing nurserymen under great pressure. It is therefore of the utmost importance that the status of soilborne diseases in grapevine nurseries be re-assessed. The emergence and extensive use of new agricultural chemicals over the last years may also have an effect on the status of soilborne diseases of grapevines in nurseries, which further emphasises the importance of this project. Pathogenicity trials need to be conducted to determine the pathogenic status of the fungi obtained from nursery grapevines. Information obtained from this study will be essential for the development of sustainable disease management strategies for grapevine nurseries, which will have resulting financial benefits for the grapevine industry.

LITERATURE

Agnihothrudu, V. 1968. A root rot of grapes in Andhra Pradesh. *Current Science* 37: 292–294.

Ainsworth, G.C. & Bisby, G.R. 1995. *Dictionary of the Fungi.* Eigth edn. (eds. D.L. Hawksworth, P.M. Kirk, B.C. Sutton & D.N. Pegler), 616 pp. International Mycological Institute, CAB International, University Press, Cambridge.

Andrade, E.R. de. 1993. Ocorrência de espécies do gênero *Fusarium* em solo cultivado com videira (*Vitis* spp.) em Santa Catarina. [Occurrence of *Fusarium* species in grapevine (*Vitis* spp.) in Santa Catarina.] *Fitopatologia Brasileira* 18: 502–505 (*Abstract in Review of Plant Pathology* 74: 657).

Andrade, E.R. de., Dal Bó, M.A., Schuck, E. & Gallotti, G.J.M. 1995. Evaluation of grapevine (*Vitis* spp.) resistance to *Fusarium oxysporum* f.sp. *herbemontis* in Rio Do Peixe Valley, Santa Catarina State, Brazil. *Acta Horticulturae* 388: 65–69.

Anonymous. 2002. South African Wine Industry Statistics Report, 2002.

Anonymous. 2003. Vine improvement association report. *Wineland* February 2003: 72–73.

Baker, K.F. 1962. Thermotherapy of planting material. *Phytopathology* 52: 1244–1255.

Baker, K.F.& Cook, R.J. 1974. *Biological control of plant pathogens*. W.H. Freeman and Company: San Fransisco, USA. 433 pp.

Baniecki, J.F.& Bloss, H.E. (1969) The basidial stage of *Phymatotrichum omnivorum*. *Mycologia* 61: 1054–1059.

Boesewinkel, H.J. 1982. *Cylindrocladiella*, a new genus to accommodate *Cylindrocladium parvum* and other small-spored species of *Cylindrocladium*. *Canadian Journal of Botany* 60: 2288–2294.

Brasier, C.M., Cooke, D.E.L. & Duncan J.M. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridisation. *Proceedings of the National Academy of Sciences USA* 96: 5878–5883.

Brayford, D. 1993. Cylindrocarpon. In *Methods for Research on Soilborne Phytopathogenic Fungi* (eds. L.L. Singleton, J.D. Mihail & M. Rush), pp. 157–165. APS Press: St. Paul, USA.

Bumbieris, M. 1970. Effect of DBCP on pythiaceous fungi. *Plant Disease Reporter* 54: 622–624.

Bumbieris, M. 1972. Observations on some pythiaceous fungi associated with grapevine decline in South Australia. *Australian Journal of Agricultural Research* 23: 652–657.

Campbell, W.A. & Hendrix, F.F. Jr. 1967. A new heterothallic *Pythium* from southern United States. *Mycologia* 59: 274–278.

Canter-Visscher, T.W. 1970. Verticillium wilt of grapevine, a new record in New Zealand. *New Zealand Journal of Agricultural Research* 13: 359–361.

Carling, D.E. & Sumner, D.R. 1992. Rhizoctonia. In *Methods for Research on Soilborne Phytopathogenic Fungi* (eds. L.L. Singleton, J.D. Mihail & C.M. Rush), pp. 157–165. APS Press: St. Paul, USA.

Chiarappa, L. 1959. The root rot complex of *Vitis vinifera* in California. *Phytopathology* 49: 670–674.

Coetzee, M.P.A., Wingfield, B.D., Harrington, T.C., Steimel, J., Coutinho, T.A. & Wingfield, M.J. 2001. The root fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10: 387–396.

Coetzee, M.P.A., Wingfield, B.D., Roux, J., Crous, P.W., Denman, S., & Wingfield, M.J. 2003. Discovery of two northern hemisphere *Armillaria* species on *Proteaceae* in South Africa. *Plant Pathology* 52: 604–612.

Crous, P.W. 2002. *Taxonomy and pathology of* Cylindrocladium *(*Calonectria*) and allied genera*. APS Press: Minnesota, USA. 294 pp.

Crous, P.W. & Wingfield, M.J. 1993. A re-evaluation of *Cylindrocladiella*, and a comparison with morphological similar genera. *Mycological Research* 97: 433–448.

Crous, P.W. & Braun, U. 1996. Cercosporoid fungi from South Africa. *Mycotaxon* 57: 233–321.

Crous, P.W., Gams, W., Wingfield, M.J. & Van Wyk, P.S. 1996. *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia* 88: 786–796.

Crous, P.W., Swart, L. & Coertze, S. 2001. The effect of hot water treatment on fungi occurring in apparently healthy grapevine cuttings. *Phytopathologia Mediterranea* 40: Supplement S464–S466.

Deal, D.R., Mai, W.F. & Boothroyd, C.W. 1972. A survey of biotic relationships in grape replant situations. *Phytopathology* 62: 503–507.

Denman, S., Coetzee, M.P.A., Wingfield, B.D., Wingfield, M.J. & Crous, P.W. 2000. *Armillaria* root rot: A new disease of cut-flower proteas in South Africa. SAPPEX News 106.

Doidge, E.M. 1924. A preliminary checklist of plant diseases occurring in South Africa. *Botanical Survey of South Africa, Memoir* 6: 1–56.

Doidge, E.M. 1950. The South African fungi and lichens to the end of 1945. *Bothalia* 5: 1–1094.

Doidge, E.M. & Bottomley, A.M. 1931. A revised list of diseases occurring in South Africa. *Botanical Survey of South Africa, Memoir* 11: 1–78.

Doidge, E.M., Bottomley, A.M., Van der Plank, J.E. & Pauer, G.D. 1953. A revised list of plant diseases in South Africa. *Union of South Africa, Department of Agriculture, Science Bulletin* 346: 1–122.

Du Plessis, S.J. 1938. The dead-arm disease of the vine. *Farming in South Africa* 13: 79–80, 83.

Du Plessis, S.J. 1946. Die voorkoms en bestryding van roetdou op sultanastokke veroorsaak deur *Exosporium sultanae* nov. spec. *Annals of the University of Stellenbosch* 24 A (5): 1–32.

Du Plessis, S.J. 1947. *Wingerdsiektes in Suid-Afrika*. Pro Ecclesia Drukkery: Stellenbosch, Suid-Afrika. 222 pp.

Erwin, D.C. & Ribeiro, O.K. 1996. Phytophthora *diseases worldwide*. APS Press: Minnesota, USA. 562 pp.

Ferreira, J.H.S., Matthee, F.N. & Thomas, A.C. 1989. Fungi associated with dieback and pruning wounds of grapevines in South Africa. *South African Journal of Enology and Viticulture* 10: 62–66.

Fourie, P.H., Halleen, F. & Volkmann, A.S. 2000. Fungi associated with grape wood, root and trunk diseases: A summary of the 1999-2000 results from the diagnostic service at Nietvoorbij. In *Proceedings of the 2nd International Viticulture and Enology Congress*. 8-10 November 2000, Cape Town, South Africa. p. 12. (South African Society of Viticulture and Enology: Stellenbosch)

Fourie, P.H. & Halleen, F. 2001. Diagnose van swamsiektes en hul betrokkenheid by terugsterwing van jong wingerd. *Wynboer* 149: 19–23.

Ghosh, T., Mukherji, N. & Basak, M. 1964. On the occurrence of a new species of *Orbilia* Fr. *Jute Bulletin* 27: 134–141.

Gordon, W.L. 1965. Pathogenic strains of *Fusarium oxysporum*. *Canadian Journal of Botany* 43: 1309–1318.

Goussard, P.G. 1977. Effect of hot-water treatments on vine cuttings and one-year-old grafts. *Vitis* 16: 272–278.

Grasso, S. 1984. Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. [Infections of *Fusarium oxysporum* and *Cylindrocarpon destructans* associated with a decline of young grapevine plants in Sicily.] *Informatore Fitopatologico* 34: 59–63.

Grasso, S. & Magnano di San Lio, G. 1975. Infections of *Cylindrocarpon obtusisporum* on grapevines in Sicily. *Vitis* 14: 36–39.

Gregory, S.C., Rishbeth, J. & Shaw, C.G. 1991. Pathogenicity and virulence. In *Armillaria Root Disease* (eds. C.G. Shaw & G.A. Kile), pp. 76–87. Washington, USA: Forest Service, USDA. (Agriculture Handbook No. 691).

Gugino, B.K., Travis, J.W. & Stewart, E.L. 2001. Pathogenicity of *Fusarium* spp., *Cylindrocarpon* sp. and *Diplodina* sp. on grape roots. *Phytopathology* 91: S34.

Guillaumin, J.J., Mercier, S. & Dubos, B. 1982. Les pourridiés à *Armillaria* et *Rosellinia* en France sur vigne, abres fruitiers et cultures florales. I. Étiology et symptomatologie. [Root rots due to *Armillaria* and *Rosellinia* in France on grapevine, fruit trees and flower crops. I. Aetiology and symptoms.] *Agronomie* 2: 71–80.

Halleen, F., Crous, P.W. & Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australasian Plant Pathology* 32: 47–52.

Highet, A.S. & Nair, N.G. 1995. *Fusarium oxysporum* associated with grapevine decline in the Hunter Valley, NSW, Australia. *Australian Journal of Grape and Wine Research* 1: 48–50.

Jones, A.L. & Aldwinckle, H.S. 1990. Compendium of apple and pear diseases (eds. A.L. Jones & H.S. Aldwinckle), 100 pp. APS Press: Minnesota, USA.

Kanadani, G., Date, H. & Nasu, H. 1998. [Effect of fluazinam soil-drench on white root rot of grapevine] *Annals of the Phytopathological Society of Japan* 64: 139–141.

Keyser, A. & Ferreira, J.H.S. 1988. Chemical and biological control of *Sclerotium rolfsii* in grapevine nurseries. *South African Journal of Enology and Viticulture* 9: 43–44.

Latorre, B.A., Wilcox, W.F. & Banados, M.P. 1997. Crown and root rots of table grapes caused by *Phytophthora* spp. in Chile. *Vitis* 36: 195–197.

Linde, C., Drenth, A., Kemp, G.H.J., Wingfield, M.J. & Von Broembsen, S.L. 1997. Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* 87: 822–827.

Lyda, S.D. & Kenerley, C.M. 1992. Phymatotrichum. In *Methods for Research on Soilborne Phytopathogenic Fungi* (eds. L.L. Singleton, J.D. Mihail & C.M. Rush), pp. 145–148. APS Press: St. Paul, USA.

Mahrous, H.A.H. 2001. Reaction of different grapevine cultivars to infection with root rotting fungi and its control. *Phytopathologia Mediterranea* 40, Supplement, S484.

Maluta, D-R. & Larignon, P. 1991. Pied-noir: mieux vaut prévenir. *Viticulture* 11: 71–72.

Mansilla, J.P., Pintos, C. & Salinero, M.C. 1993. Isolation and identification of *Phytophthora cinnamomi* Rands. as a pathogen of grape in the province of Pontevedra. *Boletín de Sanidad Vegetal, Plagas* 19: 541–549.

Marais, P.G. 1979. Fungi associated with root rot in vineyards in the Western Cape. *Phytophylactica* 11: 65–68.

Marais, P.G. 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. *Phytophylactica* 12: 9–13.

Marais, P.G. 1981. Wingerdsiektes en abnormailiteite. In *Wingerdbou in Suid-Afrika*. (eds. J. Burger & J. Deist), pp. 384–432. CTP Press, Cape Town, South Africa.

Marais, P.G. 1988. Grapevine roots and soilborne fungi. In *The Grapevine Root and its Environment*. (eds. J.A. van Zyl), pp. 106–137. South Africa Department of Agriculture and Water Supply Technical Bulletin 215.

Marais, P.G. & De La Harpe, A.C. 1982. Penetration of 99 Richter grapevine roots by *Phytophthora cinnamomi*. *South African Journal of Enology and Viticulture* 3: 81–86.

Marais, P.G. & Hattingh, M.J. 1985a. Exudates from roots of grapevine rootstocks tolerant and susceptible to *Phytophthora cinnamomi*. *Phytophylactica* 17: 205–207.

Marais, P.G. & Hattingh, M.J. 1985b. Spread of *Phytophthora cinnamomi* in a naturally infested vineyard soil. *South African Journal of Enology and Viticulture* 6: 17–19.

Marais, P.G. & Hattingh, M.J. 1986. Reduction of root rot caused by *Phytophthora cinnamomi* in grapevines by chemical treatments. *Plant Disease* 70: 109–111.

Matthee, F.N. & Thomas, A.C. 1977. *Eutypa armeniacae*. Is this apricot pathogen the cause of dying arm in vines in South Africa? *Deciduous Fruit Grower* 27: 78–84.

McGechan, J.K. 1966. *Phytophthora cinnamomi* responsible for a root rot of grapevines. *Australian Journal of Science* 28: 345.

Mihail, J.D. 1992. Macrophomina. In *Methods for Research on Soilborne Phytopathogenic Fungi*. (eds. L.L. Singleton, J.D. Mihail & C.M. Rush), pp. 134–136. APS Press: Minnesota, USA.

Omer, A.D., Granett, J. & Wakeman, R.J. 1999. Pathogenicity of *Fusarium oxysporum* on different *Vitis* rootstocks. *Journal of Phytopathology* 47: 433–436.

Pearson, R.C. & Goheen, A.C. 1988. *Compendium of grape diseases* (eds. R.C. Pearson & A.C. Goheen), 93 pp. APS Press: Minnesota, USA.

Pegg, G.F. 1974. Verticillium diseases. *Review of Plant Pathology* 5: 157–182.

Punja, Z.K. & Rahe, J.E. 1992. Sclerotium. In *Methods for Research on Soilborne Phytopathogenic Fungi* (eds. L.L. Singleton, J.D. Mihail & C.M. Rush), pp. 166–170. APS Press: St. Paul, USA.

Putterill, V.A. 1923. Plant diseases in the Western Cape province. Some of the causes of wastage in export grapes. *Union of South Africa, Journal of the Department of Agriculture* 7: 332–336.

Raabe, R.D. 1979. Testing grape rootstocks for resistance to the oak root fungus. *California Plant Pathology* 46: 3–4.

Raabe, R.D. 1990. Diseases of native oaks in California. *Fremontia* 18: 64–67.

Ragsdale, N.N. 2000. The impact of the food quality protection act on the future of plant health management. *Annual Review of Phytopathology* 38: 577–596.

Rego, C., Oliveira, H., Carvalho, A. & Phillips, A.J.L. 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* 39: 76–79.

Ribeiro, O.K. 1978. *A sourcebook on the genus* Phytophthora. J. Cramer: Lehre, Germany. 420 pp.

Rooney, S.N. & Gubler, W.D. 2001. Effect of hot water treatments on eradication of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from dormant grapevine wood. *Phytopathologia Mediterranea* 40, Supplement S467–S472.

Scheck, H.J., Vasquez, S.J, Fogle, D. & Gubler, W.D. 1998. Grape growers report losses to black-foot and grapevine decline. *California Agriculture* 52: 19–23.

Schnathorst, W.C. & Goheen, A.C. 1977. A wilt disease of grapevines (*Vitis vinifera*) in California. *Plant Disease Reporter* 61: 909–913.

Shear, C.L. 1925. The life history of the texas root rot fungus, *Ozonium omnivorum* Shear. *Journal of Agricultural Research* 30: 475–477.

Stamp, J.A. 2001. The contribution of imperfections in nursery stock to the decline of young vines in California. *Phytopathologia Mediterranea* 40, Supplement S369–S375.

Stephens, P.M., Davoren, C.W. & Wicks, T. 1992. Effect of methyl bromide, metham sodium and the biofumigants Indian mustard and canola on the incidence of soilborne fungal pathogens and growth of grapevine nursery stock. *Australasian Plant Pathology* 28: 187–196.

Sztejnberg, A. & Madar, Z. 1980. Host range of *Dematophora necatrix*, the cause of White root rot disease in fruit trees. *Plant Disease* 64: 662–664.

Szegedi, E. 1995. A review of the use of thermotherapy in viticulture to eliminate pathogens and pests from propagating material. Extended summaries, 41st Hungarian Plant Protection Days. *Pesticide Science* 45: 285–286.

Utkhede, R.S. & Vielvoye, J. 1984. Cause and control of decline of grapevines in British Columbia. *Plant Disease* 68: 820–822.

Van der Merwe, J.J.H., Joubert, D.J. & Matthee, F.N. 1972. *Phytophthora cinnamomi* root rot of grapevines in the Western Cape. *Phytophylactica* 4: 133–136.

Van der Plaats-Niterink, A.J. 1980. Monograph of the genus *Pythium*. Studies in Mycology 21. Centraalbureau voor Schimmelcultures, Baarn. 242 pp.

Véghelyi, K. 1991. Berl. szerepe a gyümölczfák fiatalkori pusztulásában. *Növényvédelem* 27: 103–113 [The role of *Rosellinia necatrix* (Hart.) Berl. in early decline of fruit trees] *Rosellinia necatrix* (Hart.) (*Abstract in Review of Plant Pathology* 71: 348).

Véghelyi, K., Balogh, I. & Polyák, D. 1995. A szölö gyökérgombáinak elöfordulása és károsítása Magyarországon. [Root rot fungi on grapevine in Hungary.] *Horticultural Science* 27: 48–53.

Verbeek, W.A. 1977. Plant Pathology. In: *Annual Report of the Secretary for Agricultural Technical Services for the Period 1 July 1975 – 30 June 1976.* Pretoria: Government Printer.

Verwoerd, L. & Dippenaar, B.J. 1930. On the occurrence of a berry wilt and rot of grape (*Vitis vinifera*) caused by *Sphaeropsis malorum. Union of South Africa Department of Agriculture, Science Bulletin* 86: 1–16.

Victor, D., Crous, P.W., Janse, B.J.H., Van Zyl, W.H., Wingfield, M.J. & Alfenas, A.C. 1998. Systematic appraisal of species complexes within *Cylindrocladiella*. *Mycological Research* 102: 273–279.

Von Broembsen, S.L. & Marais, P.G. 1978. Eradication of *Phytophthora cinnamomi* from grapevine by hot water treatment. *Phytophylactica* 10: 25–27.

Von Broembsen, S. 1984a. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the south-western Cape Province. *Phytophylactica* 16: 221–225.

Von Broembsen, S.L. 1984b. Distribution of *Phytophthora cinnamomi* in rivers of the South Western Cape Province. *Phytophylactica* 16: 227–229.

Waite, H., Crocker, J., Fletcher, G., Wright, P. & De Laine, A. 2001. Implications of hot water treatment and other nursery practices in the production of quality grapevine planting material. *Phytopathologia Mediterranea* 40: Supplement S486.

Walker, G.E. 1992. Root rot of grapevine rootlings in South Australia caused by *Rhizoctonia solani*. *Australasian Plant Pathology* 21: 58–60.

Walker, G.E. 1994. Growth of grapevine rootlings in soil from a field nursery naturally infested with *Meloidogyne incognita* and *Rhizoctonia solani*. *South African Journal of Enology and Viticulture* 15: 26–32.

Walker, G.E. 1997. Effects of *Meloidogyne* spp. and *Rhizoctonia solani* on the growth of grapevine rootlings. *Journal of Nematology* 29: 190–198.

Zentmyer, G.A. 1976. Distribution of the A1 mating type of *Phytophthora cinnamomi*. *Phytopathology* 66: 701–703.

Zentmyer, G.A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monograph 10. The American Phytopathological Society, St. Paul, Minnesota. 96 pp.

Zentmyer, G.A. & Mircetich, S.M. 1966. Saprophytism and persistence in soil by *Phytophthora cinnamomi*. *Phytopathology* 56: 710–712.

Table 1. Above-ground fungal pathogens and diseases recorded on grapevines in South Africa

Pathogen	Disease	Reference
Aspergillus carbonarius	Storage rot	Doidge *et al.*, 1953
Aspergillus niger	Storage rot	Doidge *et al.*, 1953
Aureobasidium pullulans	Lead leaf spot	Du Plessis, 1947
Botryosphaeria obtusa	Sphaeropsis rot, berry wilt/rot	Verwoerd & Dippenaar, 1930
Botryosphaeria dothidea	Excoriose	Doidge *et al.*, 1953
Botryosphaeria ribis	Cane blight	Du Plessis, 1947
Botrytis cinerea	Botrytis rot	Putterill, 1923
Cladosporium baccae	Cladosporium rot	Doidge *et al.*, 1953
Coniella diplodiella	White rot	Verbeek, 1977
Elsinoë ampelina	Anthracnose	Doidge & Bottemley, 1931
Eutypa lata	Dying arm	Matthee & Thomas, 1977
Exosporium sultanae	Sooty mould	Du Plessis, 1946
Fusarium sp.	Fusarium rot (Postharvest)	Doidge *et al.*, 1953
Glomerella cingulata	Ripe rot	Gorter, 1982
Greeneria uvicola	Bitter rot	Du Plessis, 1947
Mycosphaerella personata	Cercospora leaf spot	Doidge, 1924
Penicillium aurantiogriseum	Penicillium rot	Doidge *et al.*, 1953
Penicillium elongatum	Penicillium rot	Doidge *et al.*, 1953
Penicillium expansum	Penicillium rot	Doidge *et al.*, 1953
Phaeomoniella chlamydospora	Petri decline	Crous *et al.*, 1996
Phaeoramularia dissiliensis	Cercospora leaf spot	Crous & Braun, 1996
Phomopsis viticola	Phomopsis cane and leaf spot	Du Plessis, 1938
Plasmopara viticola	Downy mildew	Doidge, 1950
Rhizopus stolonifer	Rhizopus rot	Doidge *et al.*, 1953
Uncinula necator	Powdery mildew	Doidge & Bottomley, 1931

Table 2. Soilborne fungal pathogens and diseases recorded on grapevines in South Africa

Pathogen	Disease	Reference
Cylindrocarpon destructans	Black foot	Fourie, Halleen & Volkmann, 2000
Macrophomina phaseolina	Root rot	Marais, 1979, 1981
Phytophthora cactorum	Root rot	Marais, 1979, 1980
Phytophthora cinnamomi	Root rot, decline	Marais, 1979, 1980, 1981
Phytophthora cryptogea	Root rot, decline	Marais, 1979, 1980
Phytophthora nicotianae	Root rot	Marais, 1979, 1980
Pythium aphanidermatum	Root rot	Marais, 1979, 1980
Pythium irregulare complex	Root rot	Marais, 1979, 1980
Pythium rostratum complex	Root rot	Marais, 1980
Pythium sylvaticum complex	Root rot	Marais, 1979, 1980
Pythium ultimum	Root rot	Marais, 1979, 1980
Rhizoctonia solani	Root rot	Marais, 1979, 1980
Rosellinia necatrix	Root rot	Marais, 1980, 1981
Sclerotium rolfsii	Root rot, decline	Marais, 1980; Keyser & Ferreira, 1988

2. FUNGAL GENERA ASSOCIATED WITH ROOTS AND CROWNS OF DISEASED NURSERY GRAPEVINES IN THE WESTERN CAPE PROVINCE

ABSTRACT

Research conducted on soilborne diseases of nursery grapevines in the Western Cape province in the 1970's demonstrated that *Phytophthora cinnamomi* was the most important fungal pathogen of nursery grapevines. No further research has been conducted on this aspect since, and a re-assessment of the status of soilborne fungal pathogens associated with diseased grapevines in nurseries was necessary. Declining nursery grapevines representing different rootstock/scion combinations and rhizosphere soil samples were collected from three commercial nurseries in the Western Cape province between February and April 2001 (survey one), November and December 2001 (survey two), and March and April 2002 (survey three). Isolations were made from diseased tissues excised from roots and crowns of symptomatic plants. The effect of surface disinfestation of diseased material on the incidence of fungal genera was also evaluated. Qualitative and quantitative assessments were made of pythiaceous fungi in the soil. Results indicated that *Cylindrocarpon*, *Fusarium*, *Pythium*, and *Rhizoctonia* spp. were consistently isolated from roots and crowns. Surface disinfestation significantly affected the frequency of isolation of *Cylindrocarpon*, *Fusarium* and *Rhizoctonia*. *Rhizoctonia* occurred at significantly ($P = 0.05$) higher incidences in crown tissue during survey two (summer), than survey three (autumn), while *Fusarium* occurred at significantly higher incidences in roots during surveys one and three (autumn) than during survey two (summer). *Phytophthora cinnamomi* was infrequently isolated from diseased roots, crowns and soil during the first survey, and not obtained at all during the second or third surveys. *Pythium* spp. were abundant in most of the soil samples. These results suggest that the status of soilborne fungal pathogens in grapevine nurseries in the Western Cape province has changed considerably over the last 30 years. It is therefore of the utmost importance that further surveys be conducted in representative areas of grapevine production in the Western Cape province, in order to determine which soilborne pathogens are important in the different areas.

INTRODUCTION

The grapevine industry is supplied with planting material by nurseries, where the vines are grafted and cultivated in field nurseries for a full growing season, after which it is uprooted and sold to farmers in different production areas (J. Wiese, Voor-Groenberg nurseries, Wellington, South Africa, pers. comm.). Soilborne diseases play a major role in losses sustained in grapevine nurseries (Marais, 1980; Keyser & Ferreira, 1988). A number of soilborne diseases have been recorded on nursery grapevines in many countries. In Australia, Pythium root rot (Stephens, Davoren & Wicks, 1992) and Rhizoctonia root rot (Walker, 1992, 1994, 1997) were reported in field nurseries. Vegehlyi (1991) reported Dematophora root rot (Rosellinia root rot) in Hungary, Fusarium root rot was documented in Egypt (Mahrous, 2001), black foot (*Cylindrocarpon* spp.) of nursery grapevines in Sicily (Grasso, 1975) and Verticillium wilt of nursery vines grown in containers in south eastern Australia (Cole in Stephens *et al.*, 1992). In South Africa, black foot, Dematophora root rot, Phytophthora root and crown rot, Pythium root rot, Rhizoctonia root rot and girdling and death of nursery vines caused by *Sclerotium rolfsii* Sacc. are regarded as important soilborne diseases of grapevines in nurseries (Marais, 1980, 1981; Keyser & Ferreira, 1988; Fourie, Halleen & Volkmann, 2000; Fourie & Halleen, 2001; Halleen, Crous & Petrini, 2003).

Black foot disease is commonly recorded on grapevines in the Western Cape province (Halleen *et al.*, 2003). The disease is caused by *Cylindrocarpon destructans* (Zinssm.) Scholten, and *Cylindrocarpon obtusisporium* Wollenw. (Grasso, 1975), although new information indicates that various other *Cylindrocarpon* spp. are also associated with this disease (F. Halleen, pers. comm.). Typical symptoms include stunting, black discoloration of wood, and gum inclusions of xylem vessels.

Dematophora root rot caused by *Rosellinia necatrix* Hart. Berl. (anamorph stage) has been reported by Marais (1981) and Veghelyi (1991) on nursery grapevines in South Africa and Hungary, respectively. The name of the disease Dematophora root rot is derived from the sexual stage (teleomorph) of the pathogen. Marais (1981) maintained that *R. necatrix* has only been found in grapevine nurseries in the Western Cape province on poorly drained soils. He concluded, however, that *R. necatrix* is not a major soilborne disease of grapevines in South Africa. *Rosellinia necatrix* is a well-known pathogen of fruit trees in the Western Cape and is also an important pathogen of fruit trees in Hungary (Veghelyi, 1991). With the current

expansion of the local grapevine industry and the replacement of many apple and fruit orchards with vineyards, this disease may become more important on grapevines in the future.

The sustainable production of nursery grapevines is hampered by these soilborne diseases. Previous research regarding soilborne diseases of grapevine nurseries in the Western Cape province was conducted in the 1970s (Marais, 1979, 1980). According to Marais (1980), Phytophthora root and crown rot are the most important soilborne diseases of grapevines in nurseries in the Western Cape province. In a survey carried out in the 1970s, approximately 25% of the total number of isolates obtained were *Phytophthora* species including *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cinnamomi* Rands, *P. cryptogea* Pethybr. & Laff., *P. megasperma* Drechsler and *P. nicotianae* Breda De Haan (Marais, 1980). *Phytophthora cinnamomi* was most frequently isolated and the most virulent root rot fungus in grapevine nurseries. Marais (1980) also reported that *Pythium* spp. were most frequently associated with diseased nursery grapevines and comprised 36.4 % of the total number of isolates obtained in the 1970s survey. *Pythium* spp. (*Pm.*) associated with diseased nursery grapevines included *Pm. aphanidermatum* (Edson) Fitzpatrick, *Pm. irregulare* complex, *Pm. rostratum* complex, *Pm. sylvaticum* complex, and *Pm. ultimum* Trow. Marais (1980) reported that these species, when tested, caused rotting of the fine feeder roots and reduction in root mass but not death, with *Pm. ultimum* being the most aggressive species.

Walker (1992) associated *Rhizoctonia solani* Kühn with root rot and dieback of grapevine rootlings in a field nursery at Barmera, South Australia. He confirmed pathogenicity of *R. solani* to the rootstock cv. Ramsey in a glasshouse, and reported root rot on cv. Sultana when it was inoculated with the pathogen, but showed that root mass of this cultivar was not significantly lower than that of the controls (Walker, 1992). Later on, Walker (1994, 1997) reported that the anastomosis groups (AG) of *R. solani* associated with nursery grapevines in Australia were mostly AG 4, but sometimes also AG 2-1, and that *R. solani* is often associated with *Meloidogyne incognita* (Kofoid & White) Chitwood, the common the root knot nematode occurring in grapevine nurseries.

In South Africa, Marais (1979) identified the *Rhizoctonia* sp. pathogenic to the rootstock *Vitis champini* var. Salt Creek (cv. Ramsey), as *R. solani*. Halleen *et al.* (2003) also obtained isolates of *R. solani* from grapevines in nurseries. There is, however, no indication as to what AG-types of *R. solani* are associated with grapevines in the Western Cape province. Halleen *et*

al. (2003) obtained no isolates of *R. solani* in nursery vines before they were planted in field nurseries, but after three months in the nursery, *R. solani* was found to be present.

Sclerotium rolfsii is another soilborne pathogen of vines that is highly virulent to plants in callus trays (Marais, 1980), and also in nurseries (Marais, 1980; Keyser & Ferreira, 1988). During the 1984/85 and 1985/86 seasons, *S. rolfsii* was responsible for severe losses sustained in grapevine nurseries near Wellington in the Western Cape province (Keyser & Ferreira, 1988).

Since the 1970s, the industry has expanded considerably and cultural conditions have changed. Therefore it is necessary to re-evaluate the status and importance of potential soilborne pathogens in grapevine nurseries. The aim of this research was to determine the potentially pathogenic fungal genera associated with nursery grapevines on grapevines grafted onto 2 different rootstocks in three selected grapevine nurseries in the Western Cape province, and to determine whether the occurrence of soilborne pathogens of nursery grapevines has changed since the 1970s. Fungal genera like *Pythium* are known pathogens of grapevines, resulting in a decreased feeder root mass, but not death of plants (Marais, 1979, 1980). Surface disinfestation of rotted feeder roots may kill pathogens residing in these roots, as well as watery lesions of crown tissue, but not much is known in this regard. Therefore it is important to determine whether there are any differences in the incidence of fungal genera obtained from surface disinfested tissue in comparison with tissue not disinfested.

MATERIALS AND METHODS

Three surveys [February – April 2001 (survey one), November – December 2001 (survey two), March – April 2002 (survey three)] were conducted at three grapevine nurseries in the Wellington production area of the Western Cape province. Grapevines collected during the first and third surveys were 5 – 7 months old (after planting) and 1-2 month old grapevines (after planting) were collected during the second survey. Grapevines exhibiting symptoms of decline, e.g. chlorotic leaves, stunted growth and dieback were sampled (Fig. 1). Plants were carefully uprooted and placed in polythene bags, which were put in a cool box and brought to the laboratory. A sample of the rhizosphere soil from around each plant was also taken (Table 1). The number of grapevines of each rootstock sampled during every survey at each nursery is given in Table 1.

Isolations from plants. All plants were washed in tap water to remove soil adhering to the roots. The roots of half of the plants were surface disinfested by dipping into a commercial bleach agent containing 1% sodium hypochlorite for 1 min, followed by 30 s in 70% ethanol and then washing them in water. Roots of the remaining plants were washed with tap water only, to rid roots of adhering soil. Diseased segments from roots (Fig. 2) and crowns (Fig. 3) were excised and plated onto various culturing media. The media used during the first survey was *Phytophthora* selective medium (PH medium) (Solel & Pinkas, 1982); *Pythium* selective medium (P medium = PH medium without hymexazol); potato dextrose agar (PDA) (Biolab Diagnostics; Midrand, South Africa); water agar (WA) (Agar Bacteriological, Biolab Diagnostics, Midrand, South Africa) containing 0.04g/L streptomycin sulphate, unamended WA, and *Fusarium oxysporum* selective medium (Komada's medium) (Komada, 1975). For surveys two and three, the same culturing media were used, but the WA amended with streptomycin sulphate was left out, and Komada's medium was replaced with selective *Fusarium* agar (Tio *et al.*, 1977) to isolate a wider range of *Fusarium* spp. During the first survey, five 60 mm Petri dishes per medium were used per plant to plate excised pieces of diseased tissue. Three pieces of root tissue and pieces of crown tissue were plated from each plant. During surveys two and three, only two pieces of root tissue and one piece of crown tissue was plated. The fungal colonies that developed were transferred to divided plates containing PDA (Biolab Diagnostics, Midrand, South Africa) in one half and carnation leaf agar (Fisher *et al.*, 1982) in the other half. The plates were incubated under near-ultraviolet and cool white lights with a 12-hour photoperiod for 3 weeks. Pure cultures were identified to generic level using the keys of Barnett and Hunter (1999).

Parameters measured. The percentage incidences of the different fungal genera isolated from crowns and roots were determined by calculating the number of tissue pieces yielding the fungal genera out of the total number of tissue pieces plated.

Soil assessments.

Qualitative assessment for pythiaceous fungi: soil baiting. The qualitative assessment method of Pegg (1977) was used to determine the presence of pythiaceous fungi in rhizosphere soils. Ten grams of soil were placed in a 90 mm diameter Petri dish and moistened with 10 mL carbon-filtered water. Five avocado leaf discs, cut with a 5 mm diam. cork borer, were floated

adaxial side facing upward on the water surface of each sample. After 72 h, four leaf discs were transferred onto PH medium, and the remaining leaf disc to P medium. The dishes were monitored after 72 h to determine the presence or absence of *Pythium* spp. and *Phytophthora* spp. in the nursery soils. The control treatment consisted of avocado leaf discs floated on carbon-filtered water only.

Quantitative assessment for pythiaceous fungi: soil dilutions. A soil dilution series was prepared for each soil sample by adding 10 g of soil per sample in 90 mL of sloppy agar (1 g Agar Bacteriological in 1 L water) to obtain the stock solution. Serial dilutions were made up to 10^{-4}. One mL of each dilution was plated onto PH medium, and P medium. Five plates for each dilution per medium for each soil were used. Plates were incubated for 72 h and the number of colony forming units (CFU's) per gram of soil was calculated.

Statistical analysis.

Isolations from plants. Data on the incidences of the different fungal genera isolated from roots and crowns of vines were subjected to factorial analysis of variance using SAS statistical software version 8.02 (SAS Institute Inc., Cary, NC, United States of America). Fischer's protected least significant difference (Snedecor & Cochran, 1989) was calculated at the 5% level of probability to compare differences between means. Main factors that were subjected to analyses of variance were (i) nurseries, (ii) rootstocks, (iii) surveys, and (iv) treatments (surface disinfestation and non-surface disinfestation). Data obtained from the different culturing media were pooled and not subjected to analyses of variance.

Soil assessments. Data obtained from qualitative and quantitative assessments were not statistically analysed.

RESULTS

Fungal genera obtained. Ten fungal genera potentially pathogenic to roots and crowns of grapevines, *viz. Botryosphaeria* Ces. & de Not., *Cylindrocarpon* Wollenw., *Cylindrocladiella* Boesew., *Fusarium* Link, *Macrophomina* Petr., *Phomopsis* (Sacc.) Bubák, *Phytophthora* de Bary, *Pythium* Pringsh., *Rhizoctonia* DC., and *Sclerotium* Tode, were isolated from diseased

grapevines. Other fungal genera *viz. Acremonium* Link, *Alternaria* Nees, *Aspergillus* Link, *Aureobasidium* Viala & G. Boyer, *Botrytis* P. Micheli ex Pers., *Coniella* Höhn, *Curvularia* Boedijn, *Gliocladium* Corda, *Hainesia* Ellis & Sacc., *Mortierella* Coem., *Mucor* Fresen., *Penicillium* Fr., *Periconia* Tode, *Pestalotia* De Not, *Pestalotiopsis* Steyaert, *Phoma* Sacc., *Rhizopus* Ehrenb., *Robillarda* Sacc., *Sporothrix* Hektoen & C.F. Perkins, *Stemphylium* Wallr., *Trichoderma* Pers., *Truncatella* Steyaert, as well as sterile fungi and basidiomycetes, were also isolated, but the data on these were pooled and are indicated as "other" in Tables.

The incidence of the different fungal genera could, however, not be compared against each other to determine which genera occurred most commonly in grapevine nurseries since different culturing media were used for isolations, which may promote the growth of some genera while being detrimental to the growth of others.

Nurseries. Fungal genera isolated from crowns and roots of grapevines at the three nurseries are given in Table 2. *Phytophthora* was not isolated from crowns of nursery grapevines, *Cylindrocladiella*, *Macrophomina*, and *Phomopsis* only from crowns of plants collected at nursery 3 and *Sclerotium* from crowns of plants from nursery 2. There were no significant differences in the incidences of *Botryosphaeria*, *Cylindrocarpon*, *Cylindrocladiella*, *Fusarium*, *Macrophomina*, *Phomopsis*, *Pythium*, and *Sclerotium* in crowns of grapevines at the three nurseries. *Rhizoctonia* was significantly more frequently isolated from grapevine crowns, and "other" significantly less frequently isolated from these tissues at nursery 3 than the other two nurseries (Table 2).

Cylindrocarpon, Fusarium and *Rhizoctonia* were potentially pathogenic fungal genera that were most frequently isolated from roots of diseased grapevines in nurseries. A higher number of fungal genera was recorded on grapevine roots at the three nurseries than on crowns (Table 2). *Phytophthora* was obtained from roots of plants at nursery 3 only, and *Sclerotium* only from roots of plants at nursery 2. Significant differences in the incidence of *Botryosphaeria*, *Cylindrocarpon*, *Fusarium*, *Macrophomina*, *Rhizoctonia*, *Sclerotium*, and "other" were recorded on roots of plants from the different nurseries. The highest incidences of *Botryosphaeria* and *Cylindrocarpon* were recorded on roots from nursery 1, and the highest incidences of *Fusarium*, *Macrophomina*, *Sclerotium*, and "other" on roots from nursery 2. Roots from plants collected at nursery 3 yielded the highest percentage *Rhizoctonia* isolates (Table 2).

Rootstocks. Fungal genera isolated from the two grapevine rootstocks are listed in Table 3. *Botryosphaeria*, *Cylindrocarpon*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Sclerotium* and "other" were recorded in crowns of both 99 Richter and 101-14 Mgt, whereas *Cylindrocladiella* and *Macrophomina* were obtained only from 101-14 Mgt crowns and *Phomopsis* and *Sclerotium* only from 99 Richter crowns. *Rhizoctonia* was significantly more frequently isolated from 101-14 Mgt and *Pythium* from 99 Richter crowns.

Nine potentially pathogenic fungal genera were isolated from the roots of 99 Richter plants, and six from roots of 101-14 Mgt (Table 3). *Cylindrocladiella* was not isolated from roots of these two rootstocks (Table 3). *Phomopsis*, *Pythium*, and *Sclerotium* were only obtained from 99 Richter roots and similarly to isolations from crown tissue, *Pythium* was significantly more frequently isolated from roots of 99 Richter than roots of 101-14 Mgt, and *Rhizoctonia* from roots of 101-14 Mgt than roots of 99 Richter (Table 3). A significant rootstock x treatment interaction was observed for the incidence of *Cylindrocarpon* (P = 0.0022) and *Fusarium* (P = 0.0056) isolates obtained from roots of declining grapevines (Table 4). A significant higher incidence of *Cylindrocarpon* was obtained from disinfested roots of the rootstock 99 Richter than non-disinfested roots, while no difference was observed between the incidence of *Cylindrocarpon* isolates from disinfested and non-disinfested roots of the rootstock 101-14 Mgt (Table 4). Non-disinfested roots of the rootstock 99 Richter yielded a significant higher incidence of *Fusarium* spp. than non-disinfested roots of the rootstock 101-14 Mgt, as well as disinfested 99 Richter and 101-14 Mgt roots (Table 4).

Surveys. There were no significant (P = 0.05) differences in the occurrence of *Botryosphaeria*, *Cylindrocarpon*, *Cylindrocladiella*, *Fusarium*, *Phomopsis*, *Phytophthora*, and *Pythium* obtained from the crown-area of grapevines during the three surveys (Table 5). No *Phytophthora* was obtained from the crown-area of grapevines during any survey (Table 5). However, significantly more isolates of *Rhizoctonia* and the non-pathogenic ("other") group were obtained from crown tissue of grapevines during the second (November–December 2001) survey than during the third (March–April 2002) survey (Table 5). *Cylindrocladiella*, *Phomopsis* and *Sclerotium* were only obtained from the crown-area of grapevines collected during the second survey (Table 5), while *Macrophomina* was only isolated from the crown-area of grapevines during the first survey (Table 5).

All fungal genera isolated from the crowns, except *Cylindrocladiella* were also obtained from the roots. *Botryosphaeria* isolates were obtained significantly more frequently from roots of diseased grapevines during the second compared to the first survey (Table 5). A significantly higher occurrence of isolates of *Cylindrocarpon*, *Rhizoctonia*, and the non-pathogenic ("other") group were obtained from roots of grapevines during the first and second surveys, than during the third survey (Table 5), while *Phomopsis* and *Sclerotium* were only obtained during the second survey, and isolates of *Phytophthora* only during the first survey. A significantly higher incidence of isolates of *Fusarium* was obtained during surveys one and three, than during survey two, while significantly more *Pythium* was obtained during survey two than during surveys one and three. The occurrence of *Macrophomina* in roots of grapevines was significantly higher during the first survey than during the second survey, while no isolates of *Macrophomina* was obtained during the third survey (Table 5).

Treatments. Surface disinfestation did not significantly affect the isolation of fungi from the crown tissue of diseased nursery grapevines (Table 6). The significant treatment x rootstock interactions recorded for the incidences of *Cylindrocarpon* (P = 0.0022) and *Fusarium* (P = 0.0056) on roots of diseased nursery grapevines have already been discussed (Table 4). *Rhizoctonia* and "other" were significantly (P = 0.05) more frequently isolated from non-disinfested than disinfested roots of diseased grapevine plants in nurseries.

Soil assessments.

Qualitative assessment for pythiaceous fungi: soil baiting. Phytophthora was only obtained from soil collected at nursery 3 during the first survey, whereas *Pythium* was frequently isolated from all soil samples collected during all three surveys (Table 7).

Quantitative assessment for pythiaceous fungi: soil dilutions. Using quantitative methods, *Phytophthora* was only isolated from soil collected at nursery 3 during the first survey, and *Pythium* from all soil samples collected during the three surveys (Table 8). At nursery 3, high numbers of colony forming units of *Phytophthora* were obtained from rhizosphere soils from the rootstock 99 Richter, while the rootstock 101-14 Mgt yielded lower numbers of colony forming units of *Phytophthora*.

Rhizosphere soils sampled from all rootstock-scion combinations during the second and third surveys yielded *Pythium* spp. (Table 8). Although the results obtained were not statistically analysed, it appears as if rhizosphere soils of grapevines with the rootstock 99 Richter yielded a higher number of colony forming units of *Pythium* than soils from the rootstock 101-14 Mgt during the second and third surveys (Table 8).

DISCUSSION

Potentially pathogenic fungal genera most commonly associated with crowns and roots of declining grapevines in nurseries were *Cylindrocarpon*, *Fusarium*, and *Rhizoctonia*, while known pathogenic genera like *Phytophthora* were isolated only occasionally. During this study *Phytophthora* was infrequently isolated and was obtained from the rootstock 99 Richter, but not from 101-14 Mgt. *Pythium* was more frequently obtained and recorded on 99 Richter than on 101-14 Mgt. Marais (1988) and Uys (1993) reported that the rootstock 99 Richter was more susceptible to *P. cinnamomi* than 101-14 Mgt. Species of *Phytophthora* and *Pythium* were identified by Marais (1980) as the most important soilborne pathogens of grapevines in nurseries in the Western Cape, with *P. cinnamomi* as the most important soilborne pathogen of grapevines in nurseries. Species from the genus *Phytophthora* and *Pythium* comprised 23.5% and 36.4 % of isolates obtained by Marais (1980) from diseased and declining nursery grapevines in the Western Cape. Results obtained in our study indicate that a dramatic shift in the status of soilborne pathogens may have occurred over the last 20–30 years, since *Phytophthora* was only obtained in our study at one nursery during one survey, and *Pythium* was isolated at low incidences during all surveys.

Phytophthora was obtained with the soil assessments only at the third nursery during the first survey, and almost never from diseased grapevines, while *Pythium* spp. were frequently present in nursery soils during all surveys at all nurseries. The incidence of *Pythium* spp. isolated from roots and crowns of diseased grapevines was also low. These results are contrary to the results obtained by Marais concerning the incidence of *Phytophthora* spp. and *Pythium* spp. in diseased nursery grapevines (Marais, 1980), as well as the population levels of *Phytophthora* in the soil (Marais & Hattingh, 1985). Marais and Hattingh (1985) reported that *P. cinnamomi* was always present in soil before symptoms developed, that not all grapevines in an infested area developed symptoms at the same time, and that grapevines died in patches. Within an infested

area, the fungus was only isolated from 2% of seemingly healthy grapevines (Marais & Hattingh, 1985). In our study, *Phytophthora* was seldom isolated from declining grapevines within an infested area. A possible explanation for this low occurrence of *Phytophthora* in nursery grapevines planted in a naturally infested nursery, may be due to the extensive use of chemicals against downy mildew of grapevines in nurseries, since some of the systemic chemicals applied to the foliage against downy mildew, especially the phosphonates, can be translocated downwards through the roots and thereby control Phytophthora root rot of grapevines (Marais & Hattingh, 1986). This hypothesis was tested in chapter 5 of this thesis.

Species from the genus *Cylindrocarpon* are well-known soilborne pathogens of grapevines causing black foot (Maluta & Larignon, 1991). This disease is common on grapevines in the Western Cape province, and Halleen *et al.* (2003) indicated that *Cylindrocarpon* was present in less than 1% of plants before being planted in nurseries (October), whereas 50% or more of the plants were infected in nurseries by the end of the season (June). This clearly shows the importance of nursery soils as a source of inoculum for this disease and demonstrates that nursery grapevines might already be infected when planted into commercial vineyards.

In this study, *Fusarium* was frequently isolated from crowns and roots of diseased nursery grapevines. The two *Fusarium* spp. most commonly associated with decline of grapevines were cited as "*Fusarium oxysporum* and *Fusarium solani*". *Fusarium oxysporum* caused both root rot and wilt, while *F. solani* caused root rot only (Lele *et al.*, 1978; Grasso, 1984; Andrade, 1993; Highet & Nair, 1995; Gugino, Travis & Stewart, 2001). Marais (1979) obtained three *Fusarium* spp. from diseased grapevines in vineyards in the Western Cape province, but did not identify these to species level. He also reported that these species were not pathogenic to the rootstocks Jacquez, 99 Richter and 101-14 Mgt (Marais, 1979). The *Fusarium* spp. obtained in the current surveys were identified and evaluated for pathogenicity in chapter 4 of this thesis.

Fusarium was also obtained at a significantly lower occurrence from roots during the second survey that during the first and third surveys, indicating that the level of infection of nursery grapevines with *Fusarium* increases as the season progresses. These results confirm the results obtained by Halleen *et al.* (2003), who also indicated an increase in the incidence of *Fusarium* spp. obtained from nursery grapevines as the season progresses. The increased incidence is probably due to the fact that *Fusarium* spp. are soilborne inhabitants and pathogens, that can accumulate and reproduce in grapevine roots over time, eventually leading to a higher

incidence of the pathogen. These results also suggest that *Fusarium* levels needs to be reduced early in the season, so as to prevent a high occurrence of *Fusarium* later in the season. Halleen *et al.* (2003) also indicated *Fusarium* spp. are already present, although at low incidences, in nursery grapevines prior to planting in the field nurseries. Management strategies should therefore focus on reducing *Fusarium* populations in both plant material and soil to decrease diseases in nursery grapevines.

Rhizoctonia also occurred at relative high incidences in declining grapevines, and significantly more *Rhizoctonia* was obtained from the rootstock 101-14 Mgt than from 99 Richter. Marais (1979) isolated *Rhizoctonia* from grapevines in vineyards, but not from grapevines in nurseries (Marais, 1980). Marais (1979) identified isolates of *Rhizoctonia* obtained from grapevines in the Western Cape province as *R. solani*, and reported that they were pathogenic to the rootstock *V. champini* var. Salt Creek. Later, Halleen *et al.* (2003) obtained isolates of *Rhizoctonia* from grapevines in nurseries in the Western Cape province and identified them also as *R. solani*. However, none of these authors gave any indication as to what AG-types of *R. solani* were associated with grapevines in the Western Cape province. In Australia, Walker (1992, 1994, 1997) reported the effect of *R. solani* and *Meloidogyne* spp. on grapevines in a field nursery. The isolates of *R. solani* in these studies were mostly *R. solani* AG 4, but also AG 2-1, as well as an unknown group. These *Rhizoctonia* isolates were confirmed as the causal agent of root rotting of grapevines in field nurseries.

Halleen *et al.* (2003) obtained no *Rhizoctonia* in nursery grapevines before they were planted in the field nurseries, but after three months in the nursery, *Rhizoctonia* was already present in nursery grapevines. Nursery soils can thus be seen as a primary source of inoculum for this pathogen. The characterisation of *Rhizoctonia* spp. and AG-types associated with grapevines in the Western Cape province as well as their relative virulence and incidence need to be addressed as a matter of urgency.

Some of the potentially pathogenic fungal genera occurred only sporadically in crowns and roots of nursery grapevines, namely *Botryosphaeria*, *Cylindrocladiella*, *Macrophomina*, *Phytophthora*, *Phomopsis* and *Sclerotium*, and will be discussed briefly below.

Species from the genus *Cylindrocladiella* are well-known pathogens of various crops like *Eucalyptus* spp., where they cause severe losses in nurseries (Crous, Phillips & Wingfield, 1991). *Cylindrocladiella* have also been isolated from the graft union of recently grafted green-shoot

grapevines that showed symptoms of decline (Lamprecht *et al.*, unpubl. data). In the present study *Cylindrocladiella* was only isolated from crown tissue of the 101-14 Mgt rootstock. The characterisation and pathogenicity of *Cylindrocladiella* spp. associated with grapevines in the Western Cape province are addressed in chapter 3 of this thesis.

Marais (1979) isolated *Macrophomina phaseolina* (Tassi) Goid. from grapevine roots in vineyards and confirmed the fungus to be pathogenic to the rootstock 101-14 Mgt, but did not report it in grapevine nurseries (Marais, 1980). Although *M. phaseolina* occurred at lower frequencies in vineyards than *Phytophthora* and *Pythium* spp., it occurred consistently in vineyards in the Western Cape (Marais, 1979). However, in our study, it occurred only during the first and second surveys in nurseries, on roots of 99 Richter and 101-14 Mgt, and crowns of 101-14 Mgt rootstocks. *Macrophomina* may therefore be of lesser importance in grapevine nurseries, but this needs to be confirmed in a more comprehensive survey of soilborne diseases of nursery grapevines.

Botryosphaeria and *Phomopsis* spp., especially *B. obtusa* (Schwein.) Shoemaker, *B. dothidea* (Moug.:Fr.) Ces. & De Not., *B. ribis* Grossenb. & Duggar, and *Phomopsis viticola* (Sacc.) Sacc. are well-known above-ground pathogens of grapevines in the Western Cape province and are responsible for stem- and trunk diseases (Verwoerd & Dippenaar, 1930; Doidge *et al.*, 1953; Du Plessis, 1938, 1947; Mostert *et al.*, 2001). The role of *Botryosphaeria* and *Phomopsis* as potential soilborne pathogens of grapevines is uncertain, but since they occurred at such low frequencies in roots and crowns of nursery grapevines, they can be regarded as having little importance as potential soilborne pathogens. The primary infection of rootstocks in this case occurs most probably in the rootstock mother blocks where *Botryosphaeria* and *Phomopsis* can enter the mother plants through wounds.

The *Sclerotium* isolates obtained in this study were identified as *Sclerotium rolfsii*. Marais (1980) reported *S. rolfsii* to be a problem in callus trays at the beginning of the grapevine season and that infection with this fungus can lead to death of grapevines. He, however, concluded that *S. rolfsii* was a lesser problem in grapevine nurseries. On the other hand Keyser and Ferreira (1988) reported that *S. rolfsii* caused significant losses in the 1984/85 and 1985/86 seasons in grapevine nurseries in the Western Cape province near the town of Wellington. In our survey, *S. rolfsii* was obtained from only one nursery, and at low incidences. These results indicate that *S. rolfsii* occurs infrequently, which confirms the results of Marais (1980). Under

certain conditions, however, *S. rolfsii* may become a more important nursery pathogen (Keyser & Ferreira, 1988).

Variation in the incidence of fungi between nurseries was observed for *Cylindrocarpon*, *Fusarium*, *Rhizoctonia*, as well as the non-pathogenic group, where some nurseries had higher incidences of these fungi than other nurseries. *Cylindrocladiella* and *Phytophthora* occurred only in the third nursery, while *Sclerotium rolfsii* occurred only in the second nursery. These results indicate the importance of a more comprehensive survey that needs to be conducted in the different production areas of grapevines in the Western Cape province, in order to determine what pathogens are important in the different production areas.

Results obtained from the surface disinfestation and non-surface disinfestation treatments indicated that both treatments needs to be included when isolating different fungal genera from grapevine roots, since different genera are isolated more frequently when applying different treatments. Rootstock crown tissue is less subjected to variance in the incidence of different genera and therefore does not need to be surface disinfested prior to isolation.

A shift in the status of soilborne pathogens in nurseries will have an important impact on the grapevine industry, since it implies that disease management strategies, as developed when *Phytophthora* and *Pythium* were the important pathogens, will no longer be effective in managing soilborne diseases in nurseries. Therefore it is of the utmost importance that further surveys be conducted in representative areas of grapevine production in the Western Cape province, in order to determine which soilborne pathogens are important in the different areas. These results are needed before any disease management strategies can be developed for the grapevine industry.

LITERATURE

Andrade, E.R. de. 1993. Ocorrência de espécies do gênero *Fusarium* em solo cultivado com videira (*Vitis* spp.) em Santa Catarina. [Occurrence of *Fusarium* species in grapevine (*Vitis* spp.) in Santa Catarina.] *Fitopatologia Brasileira* 18: 502–505 (*Abstract in Review of Plant Pathology* 74: 657).

Barnett, H.L. & Hunter, B.B. 1999. *Illustrated genera of imperfect fungi*. Fourth edn. APS Press: Minnesota, USA.

Crous, P.W., Phillips, A.J.L. & Wingfield, M.J. 1991. The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *Suid-Afrikaanse Bosboutydskrif* 157: 69–85.

Doidge, E.M., Bottomley, A.M., Van der Plank, J.E. & Pauer, G.D. 1953. A revised list of plant diseases in South Africa. *Union of South Africa, Department of Agriculture, Science Bulletin* 346: 1–122.

Du Plessis, S.J. 1938. The dead-arm disease of the vine. *Farming in South Africa* 13: 79–83.

Du Plessis, S.J. 1947. *Wingerdsiektes in Suid-Afrika*. Pro Ecclesia Drukkery: Stellenbosch, Suid-Afrika. 222 pp.

Fisher, N.L., Burgess, L.W., Toussoun, T.A. & Nelson, P.E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

Fourie, P.H., Halleen, F. & Volkmann, A.S. 2000. Fungi associated with grape wood, root and trunk diseases: A summary of the 1999-2000 results from the diagnostic service at Nietvoorbij. In *Proceedings of the 2nd International Viticulture and Enology Congress*. 8-10 November 2000, Cape Town, South Africa. p. 12.

Fourie, P.H. & Halleen, F. 2001. Diagnose van swamsiektes en hul betrokkenheid by terugsterwing van jong wingerd. *Wynboer* 149: 19–23.

Grasso, S. 1975. Infezioni di *Cylindrocarpon obtusisporium* su piante di vite in Sicilia. *Vitis* 14: 36–39.

Grasso, S. 1984. Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. [Infections of *Fusarium oxysporum* and *Cylindrocarpon destructans* associated with a decline of young grapevine plants in Sicily.] *Informatore Fitopatologico* 34: 59–63.

Gugino, B.K., Travis, J.W. & Stewart, E.L. 2001. Pathogenicity of *Fusarium* spp., *Cylindrocarpon* sp. and a *Diplodina* sp. on grape roots. *Phytopathology* 91, Supplement: S34.

Halleen, F., Crous, P.W. & Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australasian Plant Pathology* 32: 47–52.

Highet, A.S. & Nair, N.G. 1995. *Fusarium oxysporum* associated with grapevine decline in the Hunter Valley, NSW, Australia. *Australian Journal of Grape and Wine Research* 1: 48–50.

Keyser, H.A. & Ferreira, J.H.S. 1988. Chemical and biological control of *Sclerotium rolfsii* in grapevine nurseries. *South African Journal for Enology and Viticulture* 9: 43–44.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8: 114–125.

Lele, V.C., Durgapal, J.C., Agarwal, D.K. & Sethi, C.L. 1978. Crown and root gall of grape (*Vitis vinifera* L.) in Andhra Pradesh. *Current Science* 47: 280.

Mahrous, H.A.H. 2001. Reaction of different grapevine cultivars to infection with root rotting fungi and its control. *Phytopathologia Mediterranea* 40, Supplement: S484.

Maluta, D-R. & Larignon, P. 1991. Pied-noir: mieux vaut prévenir. *Viticulture* 11: 71–72.

Marais, P.G. 1979. Fungi associated with root rot in vineyards in the Western Cape. *Phytophylactica* 11: 65–68.

Marais, P.G. 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. *Phytophylactica* 12: 9–13.

Marais, P.G. 1981. Wingerdsiektes en abnormailiteite. In *Wingerdbou in Suid-Afrika* (eds. J. Burger & J. Deist), pp. 384–432. CTP Press, Cape Town, South Africa.

Marais, P.G. 1988. Grapevine roots and soilborne fungi. In *The Grapevine Root and its Environment*. (eds. J.A. van Zyl), pp. 106–137. South Africa Department of Agriculture and Water Supply Technical Bulletin 215.

Marais, P.G. & Hattingh, M.J. 1985. Spread of *Phytophthora cinnamomi* in a naturally infested vineyard soil. *South African Journal of Enology and Viticulture* 6: 17–19.

Marais, P.G. & Hattingh, M.J. 1986. Reduction of root rot caused by *Phytophthora cinnamomi* in grapevines by chemical treatments. *Plant Disease* 70: 109–111.

Mostert, L., Crous, P.W., Kang, J. & Phillips, A.J.L. 2001. Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia* 93: 146–167.

Pegg, K.G. 1977. Soil application of elemental sulphur as a control of *Phytophthora cinnamomi* root and heart rot of pineapple. *Australian Journal of Experimental Agriculture and Animal Husbandry* 17: 859–865.

Snedecor, G.W. & Cochran, W.G. 1989. *Statistical methods*. Eighth edn. Iowa State University Press: Iowa, USA. 503 pp.

Solel, Z, & Pinkas, Y. 1982. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. *Phytopathology* 74: 506–508.

Stephens, P.M., Davoren, C.W. & Wicks, T. 1992. Effect of methyl bromide, methan sodium and the biofumigants mustard and canola on the incidence of soilborne fungal pathogens and growth of grapevine nursery stock. *Australasian Plant Pathology* 28: 187–196.

Tio, M., Burgess, L.W., Nelson, P.E. & Toussoun, T.A. 1977. Techniques for the isolation, culture and preservation of the fusaria. *Australian Plant Pathological Society Newsletter* 6: 11–13.

Uys, D. 1993. Richter 99 disappoints with table grapes. *Deciduous Fruit Grower* 43: 309.

Véghelyi, K. 1991. *Rosellinia necatrix* (Hart.) Berl. szerepe a gyümölczfák fiatalkori pusztulásában. [The role of *Rosellinia necatrix* (Hart.) Berl. in early decline of fruit trees] *Növényvédelem* 27: 103–113 (*Abstract in Review of Plant Pathology* 71: 348).

Verwoerd, L. & Dippenaar, B.J. 1930. On the occurrence of a berry wilt and rot of grape (*Vitis vinifera*) caused by *Sphaeropsis malorum*. *Union of South Africa Department of Agriculture, Science Bulletin* 86: 1–16.

Walker, G.E. 1992. Root rot of grapevine rootlings in South Australia caused by *Rhizoctonia solani*. *Australasian Plant Pathology* 21: 58–60.

Walker, G.E. 1994. Growth of grapevine rootlings in soil from a field nursery naturally infested with *Meloidogyne incognita* and *Rhizoctonia solani*. *South African Journal for Enology and Viticulture* 15: 26–32.

Walker, G.E. 1997. Effects of *Meloidogyne* spp. and *Rhizoctonia solani* on the growth of grapevine rootlings. *Journal of Nematology* 29: 190–198.

Table 1. Number of grafted declining grapevines sampled from three nurseries in the Western Cape province

Rootstock	Survey	No. of samples [x]		
		Nursery 1	Nursery 2	Nursery 3
99 Richter	1	2	8	8
101-14 Mgt	1	4	8	15
99 Richter	2	24	18	20
101-14 Mgt	2	- [y]	16	20
99 Richter	3	20	16	16
101-14 Mgt	3	-	16	16

[x] Number of plants collected, with soil samples from the rhizosphere.

[y] Rootstock not available.

Table 2. Mean incidence of fungal genera in crowns and roots of diseased nursery vines at three nurseries

Nursery	Plant part	Incidence (%) of fungal genus [xyz]										
		Bot	***Cylin***	***Cyl***	***Fus***	***Mac***	***Pho***	***Phy***	***Pyt***	***Rhi***	***Scl***	**Other**
1	Crown	0.78 a	2.73 a	0.00 a	11.33 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 b	0.00 a	21.48 a
2		0.30 a	3.94 a	0.00 a	11.52 a	0.00 a	0.00 a	0.00 a	1.21 a	1.52 b	0.39 a	20.91 a
3		0.00 a	3.27 a	0.21 a	12.48 a	0.11 a	0.42 a	0.00 a	1.58 a	4.11 a	0.00 a	13.12 b
1	Roots	1.60 a	5.62 a	0.00 a	18.47 ab	0.40 ab	0.20 a	0.00 a	0.80 a	3.82 b	0.00 b	12.65 b
2		0.59 b	3.76 ab	0.00 a	22.89 a	1.29 a	0.00 a	0.00 a	1.76 a	5.87 ab	0.70 a	21.83 a
3		0.32 b	2.52 b	0.00 a	16.53 b	0.14 b	0.11 a	0.21 a	2.20 a	8.21 a	0.00 b	14.20 b

[x] Fungal genus: *Bot* = *Botryosphaeria*, *Cylin* = *Cylindrocarpon*, *Cyl* = *Cylindrocladiella*, *Fus* = *Fusarium*, *Mac* = *Macrophomina*, *Pho* = *Phomopsis*, *Phy* = *Phytophthora*, *Pyt* = *Pythium*, *Rhi* = *Rhizoctonia*, *Scl* = *Sclerotium*, Other = combined data for other fungal genera.

[y] Means within a column within a plant part followed by the same letter do not differ significantly ($P = 0.05$).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 3. Incidence of fungal genera occurring in crowns and roots of two rootstocks of nursery vines collected at three nurseries

Rootstock	Plant part	Incidence (%) of fungal genus [wxy]										
		Bot	*Cylin*	*Cyl*	*Fus*	*Mac*	*Pho*	*Phy*	*Pyt*	*Rhi*	*Scl*	Other
99 Richter	Crown	0.32 a	3.78 a	0.00 a	12.06 a	0.00 a	0.32 a	0.00 a	1.53 a	1.13 b	0.23 a	17.20 a
101-14 Mgt		0.23 a	2.74 a	0.23 a	11.68 a	0.11 a	0.00 a	0.00 a	0.46 b	4.00 a	0.00 a	18.08 a
99 Richter	Roots	0.67 a	- [z]	0.00 a	- [z]	0.45 a	0.15 a	0.15 a	2.29 a	5.08 b	0.45 a	16.52 a
101-14 Mgt		0.74 a	-	0.00 a	-	0.88 a	0.00 a	0.00 a	0.95 b	8.23 a	0.00 a	16.97 a

[w] Fungal genus: *Bot* = *Botryosphaeria*, *Cylin* = *Cylindrocarpon*, *Cyl* = *Cylindrocladiella*, *Fus* = *Fusarium*, *Mac* = *Macrophomina*, *Pho* = *Phomopsis*, *Phy* = *Phytophthora*, *Pyt* = *Pythium*, *Rhi* = *Rhizoctonia*, *Scl* = *Sclerotium*, Other = combined data for other fungal genera.

[x] Means within a column within a plant part followed by the same letter do not differ significantly ($P = 0.05$).

[y] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

[z] See Table 7 (rootstock x treatment interaction).

Table 4. Incidence of *Cylindrocarpon* and *Fusarium* isolates occurring in roots of different rootstocks of vines subjected to different treatments, taken from three nurseries

Treatment	Incidence (%) [xy]			
	Cylindrocarpon		*Fusarium*	
	99 Richter	**101-14 Mgt**	**99 Richter**	**101-14 Mgt**
Disinfested	6.47 a	2.77 b	13.68 c	16.74 bc
Not disinfested	1.74 b	3.28 b	26.67 a	19.46 b

[x] Means within a fungal genus followed by the same letter do not differ significantly ($P = 0.05$).

[y] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 5. Mean incidence of phytopathogenic fungi occurring in crowns and roots of nursery vines over different seasons in three nurseries

Survey (season)	Plant part	Incidence (%) of fungal genus [xyz]										
		Bot	*Cylin*	*Cyl*	*Fus*	*Mac*	*Pho*	*Phy*	*Pyt*	*Rhi*	*Scl*	Other
1 (Feb-Apr 2001)	Crown	0.00 a	6.29 a	0.00 a	10.27 a	0.33 a	0.00 a	0.00 a	1.66 a	2.32 ab	0.00 b	16.23 b
2 (Nov-Dec 2001)		0.41 a	2.86 b	0.20 a	11.97 a	0.00 a	0.41 a	0.00 a	1.63 a	3.47 a	0.66 a	24.01 a
3 (March-Apr 2002)		0.24 a	2.86 b	0.00 a	12.41 a	0.00 a	0.00 a	0.00 a	0.24 a	0.96 b	0.00 b	10.50 b
1 (Feb-Apr 2001)	Roots	0.00 b	4.72 a	0.00 a	24.79 a	2.78 a	0.00 a	0.42 a	1.18 b	9.17 a	0.00 a	21.60 a
2 (Nov-Dec 2001)		1.13 a	4.54 a	0.00 a	15.16 b	0.10 b	0.21 a	0.00 b	3.30 a	8.97 a	0.62 a	20.21 a
3 (March-Apr 2002)		0.60 ab	2.03 b	0.00 a	21.00 a	0.00 b	0.00 a	0.00 b	0.24 b	1.79 b	0.00 a	9.85 b

[x] Fungal genus: *Bot* = *Botryosphaeria*, *Cylin* = *Cylindrocarpon*, *Cyl* = *Cylindrocladiella*, *Fus* = *Fusarium*, *Mac* = *Macrophomina*, *Pho* = *Phomopsis*, *Phy* = *Phytophthora*, *Pyt* = *Pythium*, *Rhi* = *Rhizoctonia*, *Scl* = *Sclerotium*, Other = combined data for other fungal genera.

[y] Means within a column within a plant part followed by the same letter do not differ significantly (P = 0.05).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 6. Incidence of phytopathogenic fungi occurring in the crown-areas of vines subjected to different treatments

Treatment	Plant part	Incidence (%) of fungal genus [wxy]										
		Bot	***Cylin***	***Cyl***	***Fus***	***Mac***	***Pho***	***Phy***	***Pyt***	***Rhi***	***Scl***	**Other**
Disinfested	Crown	0.38 a	3.22 a	0.19 a	11.84 a	0.00 a	0.00 a	0.00 a	1.23 a	1.99 a	0.00 a	16.19 a
Not disinfested		0.19 a	3.48 a	0.00 a	11.97 a	0.09 a	0.38 a	0.00 a	0.94 a	2.63 a	0.19 a	18.92 a
Disinfested	Roots	0.80 a	- [z]	0.00 a	- [z]	0.41 a	0.09 a	0.12 a	1.73 a	4.53 b	0.53 a	12.84 b
Not disinfested		0.61 a	-	0.00 a	-	0.84 a	0.09 a	0.06 a	1.74 a	8.22 a	0.00 a	20.54 a

[w] Fungal genus: *Bot* = *Botryosphaeria*, *Cylin* = *Cylindrocarpon*, *Cyl* = *Cylindrocladiella*, *Fus* = *Fusarium*, *Mac* = *Macrophomina*, *Pho* = *Phomopsis*, *Phy* = *Phytophthora*, *Pyt* = *Pythium*, *Rhi* = *Rhizoctonia*, *Scl* = *Sclerotium*, Other = combined data for other fungal genera.

[x] Means within a column within a plant part followed by the same letter do not differ significantly ($P = 0.05$).

[y] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

[z] See Table 7 (rootstock x treatment interaction).

Table 7. A qualitative assessment of *Phytophthora* and *Pythium* in rhizosphere soils of various rootstocks of nursery grapevines collected from three nurseries during three surveys

Rootstock	Survey (season)	*Phytophthora* (%) [x]			*Pythium* (%) [x]		
		Nursery 1	Nursery 2	Nursery 3	Nursery 1	Nursery 2	Nursery 3
99 Richter	1 (Feb-April 2001)	0	0	100	100	100	100
101-14 Mgt		0	0	85	100	100	90
99 Richter	2 (Nov-Dec 2001)	0	0	0	63	78	80
101-14 Mgt		- [y]	0	0	-	88	90
99 Richter	3 (March-April 2002)	0	0	0	63	78	83
101-14 Mgt		-	0	0	-	83	90

[x] Percentage based on the number of samples that tested positive out of the total number of samples tested.

[y] Rootstock not available.

Table 8. A quantitative assessment of *Phytophthora* and *Pythium* in rhizosphere soils of various rootstocks of nursery grapevines collected from three nurseries during three surveys

Rootstock	Survey (season)	*Phytophthora* (%) [x]			*Pythium* (%) [x]		
		Nursery 1	Nursery 2	Nursery 3	Nursery 1	Nursery 2	Nursery 3
99 Richter	1 (Feb-April 2001)	0	0	1160	240	864	1072
101-14 Mgt		0	0	42	1500	1040	540
99 Richter	2 (Nov-Dec 2001)	0	0	0	1001	1105	957
101-14 Mgt		- [y]	0	0	-	872	870
99 Richter	3 (March-April 2002)	0	0	0	610	852	408
101-14 Mgt		-	0	0	-	1016	447

[x] Number of Colony Forming Units (CFUs) per gram of soil.

[y] Rootstock not available.

Figure 1. Diseased patch in a grapevine nursery

Figure 2. Diseased roots of a nursery grapevine

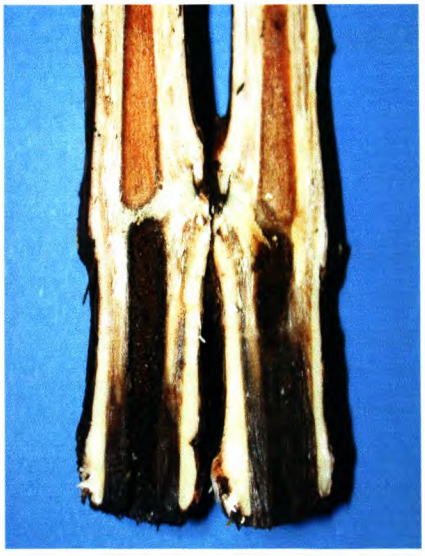


Figure 3. Diseased crown of a nursery grapevine

3. CHARACTERISATION AND PATHOGENICITY OF *CYLINDROCLADIELLA* SPP. ASSOCIATED WITH ROOT AND CUTTING ROT SYMPTOMS OF GRAPEVINES IN NURSERIES

ABSTRACT

Species of *Cylindrocladiella* occur on a variety of hosts, where they are known to act as pathogens or saprobes. Seven species from this genus are currently recognised, of which five have been reported previously in South Africa, namely *C. camelliae*, *C. elegans*, *C. lageniformis*, *C. parva*, and *C. peruviana*. In a recent survey of soilborne diseases of nursery grapevines in the Western Cape province of South Africa, various isolates of *Cylindrocladiella* were obtained from a newly established 99 Richter mother block exhibiting decline symptoms. The DNA phylogeny of these and additional isolates, also obtained from declining grapevines, was determined by sequencing the ITS (ITS-1, ITS-2 and 5.8S) as well as ß-tubulin gene regions. Results from this study revealed that four species of *Cylindrocladiella* occur on grapevines in South Africa, namely *C. lageniformis*, *C. parva*, *C. peruviana*, as well as a new species described here as *C. viticola*, which forms part of the *C. infestans* species complex. Pathogenicity trials, using stem inoculations on green and 1-year-old 99 Richter cuttings were conducted to determine the pathogenicity of selected isolates of these species, but these results were inconclusive.

INTRODUCTION

Boesewinkel (1982) proposed a new hypocrealean genus, *Cylindrocladiella* Boesew., to accommodate small-spored species of *Cylindrocladium* Morgan. This decision was based on the fact that species of *Cylindrocladiella* had different conidiophore branching patterns, conidial shapes, dimensions, cultural characteristics and teleomorphs than *Cylindrocladium*. Although a number of researchers continued to consider *Cylindrocladiella* and *Cylindrocladium* as a single genus (Mandal & Dasgupta, 1983; Peerally, 1991; Sharma & Mohanan, 1991), Crous, Wingfield & Lennox (1994) confirmed the two genera to be distinct, with *Cylindrocladium* having *Calonectria* teleomorphs (Rossman, 1979; Crous, 2002), and *Cylindrocladiella* having *Nectricladiella* Crous & C.L. Schoch teleomorphs (Schoch *et al.*, 2000).

Victor *et al.* (1998) recognised seven species in the genus *Cylindrocladiella* that could be distinguished on Restriction Fragment Length Polymorphisms and AT-DNA data (A+T-rich), as well as morphology. Schoch *et al.* (2000) compared phylogenies derived from the ITS regions flanking the 5.8S ribosomal RNA gene, as well as the 5' end of the ß-tubulin gene of various *Cylindrocladiella* isolates, and distinguished the same seven species recognised by Victor *et al.* (1998), as well as a further species, *C. microcylindrica* Crous & D. Victor. The latter taxon was hitherto incorrectly treated as *C. infestans* Boesew. As was further noted by Schoch *et al.* (2000), and Crous (2002), significant DNA sequence variation was observed within isolates identified as *C. infestans*, and these strains were thus best treated as a species complex until their status could be resolved.

In South Africa, five species of *Cylindrocladiella* have been reported as pathogens or saprobes on various hosts. *Cylindrocladiella camelliae* (Venkataram. & C.S.V. Ram) Boesew. has been associated with cutting rot of *Eucalyptus* spp., *C. elegans* Crous & M.J. Wingf. with roots of *Arachis hypogaea* L. and *Eucalyptus* leaf litter, *C. lageniformis* Crous, M.J. Wingf. & Alfenas with soil and roots of *Vitis vinifera* L., *C. parva* (P.J. Anderson) Boesew. with soil, *Eucalyptus* cuttings and roots of *Fragaria* sp., *Persea americana* Mill., *Pinus* spp., *Prunus* sp. and *V. vinifera*, and *C. peruviana* (Bat., J.L. Bezerra & M.M.P. Herrera) Boesew. with root and cutting rot of *Acacia mearnsii* De Wild., *Eucalyptus* spp., *Protea* sp. and *V. vinifera* (Crous & Wingfield, 1993; Victor *et al.*, 1998; Crous, 2002). Although isolates of *C. lageniformis*, *C. parva* and *C. peruviana* have over the past ten years been isolated from roots and rootstocks of mature grapevines, cuttings and the graft union of grafted young grapevine material (S.C. Lamprecht and P.W. Crous, unpubl. data), Koch's postulates have never been proven, and their role as pathogens on *Vitis* spp. still needs to be elucidated.

In a recent survey of soilborne pathogens of grapevines in nurseries, isolates of several *Cylindrocladiella* spp. were obtained from a newly established 99 Richter (*Vitis berlandieri* PI x *Vitis rupestris* Sch. var. du Lot) mother block, showing symptoms of decline, e.g. chlorotic leaves and stunted growth, while another isolate of a *Cylindrocladiella* sp. was obtained from a declining grafted nursery grapevine (*Vitis vinifera* x 101-14 Mgt [*Vitis riparia* M x *Vitis rupestris* Sch.]) (Chapter 2). The aims of this study were to characterise the species of *Cylindrocladiella* associated with grapevines in South Africa and to determine their pathogenicity on grapevines.

MATERIALS AND METHODS

Cultures. Strains used in previous studies (Crous & Wingfield, 1993; Victor *et al.*, 1998; Schoch *et al.*, 2000; Crous, 2002) and deposited at the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U) were included. Freshly obtained isolates from diseased grapevines encountered during surveys at a grapevine nursery in the Western Cape province of South Africa (chapter 2) were also included. Representative strains were deposited at the Centraalbureau voor Schimmelcultures (CBS) at Utrecht, the Netherlands (Table 1).

DNA extraction, sequencing and phylogeny. Genomic DNA was isolated from fungal mycelium following the protocol of Lee and Taylor (1990), after which the ITS and ß-tubulin regions were amplified (Kang, Crous & Schoch, 2001). The primers ITS1 and ITS4 (White *et al.*, 1990) were used to amplify the ITS1 region, 5.8S rRNA gene and the ITS2 region of the nuclear-encoded ribosomal RNA gene. Part of the ß-tubulin gene was amplified with the primers T1 (O'Donnell & Cigelnik, 1997) and Bt-2b (Glass & Donaldson, 1995). The amplification products were visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining. The amplified fragments were purified using a commercial kit (NucleoSpin Extract 2 in 1 Purification Kit, Macherey-Nagel GmbH, Germany) and the PCR primers were used to sequence both strands of the purified products using an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready reaction Kit (PE Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut).

The sequences generated, together with BLASTn retrievals from GenBank, were assembled and aligned using Sequence Alignment Editor v2.0a11 (Rambaut, 2002). Sequences of *Fusarium subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun & W.F.O Marasas were used as outgroup for both the ITS and ß-tubulin alignment. The phylogenetic analysis of the sequence alignment was done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analyses were performed for all data sets using the heuristic search option with 100 random taxon additions and

tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The resulting trees were evaluated with 1000 bootstrap replications to test the clade stability (Hillis & Bull, 1993). Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC). The resulting trees were printed with TreeView Version 1.6.6 (Page, 1996). A partition homogeneity test (Farris *et al.*, 1994) was conducted in PAUP (Swofford, 2000) to examine the possibility of a joint analysis of the two data sets.

Morphological and cultural comparisons. Strains were cultured on 2% malt extract agar (MEA) (Biolab Diagnostics, Midrand, South Africa), plated onto carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous, Phillips & Wingfield, 1992), incubated at 25°C under near-ultraviolet light, and examined after 7 days. Only material growing on carnation leaves was examined. Mounts were prepared in lactophenol, examined using Nomarski interference phase contrast and bright-field phase contrast microscopy, and measurements made at x 1000 magnification. The 95% confidence intervals were determined from at least 30 observations and the minimum and maximum ranges given in parentheses. Temperature requirements for growth and cultural characteristics were determined after 7 days on MEA in the dark at 25°C, using procedures described by Crous and Wingfield (1994). Colony colours were determined using the colour charts of Rayner (1970).

Stem inoculations. Results from the inoculation of grapevine roots with the different *Cylindrocladiella* spp. were inconclusive and are not shown here. The response of grapevine rootstocks to isolates of the different *Cylindrocladiella* spp. was determined with stem inoculations in two different trials, which were both replicated twice. In the first experiment, the isolates were inoculated onto green shoots (7–15 mm diam., 30 cm long), and in the second experiment, 1-year-old shoots (10–20 mm diam., 30 cm long) were inoculated. Eighteen isolates of *Cylindrocladiella*, obtained from declining grapevines, were used in this study, namely six of *C. lageniformis*, one of *C. parva*, nine isolates of *C. peruviana*, and two of an unknown *Cylindrocladiella* species. Green shoots (7–15 mm diam., 30 cm long) were pruned from a visually healthy 99 Richter mother-block. The shoots were placed in 500 mL flasks and 50 mL municipal tap water was applied to each flask, in order to establish sap flow in the shoots. One day later, the grapevines were subjected to two different inoculation treatments, namely wounded and unwounded inoculation. For the wounded inoculation, shoots were wounded 5 cm from the

apical end with a 4-mm-diam. cork borer to remove the cortex tissue (one wound per shoot). Colonised mycelial plugs from two-week-old cultures of the different *Cylindrocladiella* cultures, grown on 2% potato dextrose agar (PDA; Biolab Diagnostics, Midrand, South Africa) were inserted into the wounds and sealed with Parafilm. Two control treatments were used, namely uncolonised PDA plugs, and a non-pathogen control, which consisted of mycelial plugs colonised with *Clonostachys rosea* (Link : Fr.) Schroers, Samuels, Seifert & W. Gams. For the unwounded treatment, the mycelial plugs were placed on the outside of the shoot, 5 cm from the apical end of the shoot, and sealed with Parafilm. All treatments were replicated three times, with one inoculation per shoot. The shoots were placed in a laboratory in a dew chamber in a completely randomised design. The laboratory temperature was maintained at 22°C, with a 12-h fluorescent white light/dark regime. After 14 days, lesion lengths were determined, and re-isolations were made from lesion margins onto PDA (Biolab Diagnostics, Midrand, South Africa) to confirm Koch's postulates. Lesion lengths were calculated by subtracting the diameter of the cork-borer from the lesion formed. The same method was used for inoculation on 1-year-old shoots in dew chambers, with 12 shoots/dew chamber.

Statistical analysis. Data obtained from the experiments were subjected to analysis of variance using SAS statistical software version 8.02 (SAS Institute Inc., Cary, NC, USA). Fischer's protected least significant difference (Snedecor & Cochran, 1989) was calculated at the 5% level of probability to establish the significance of differences between lesion lengths caused by the different taxa.

RESULTS

DNA phylogeny. DNA amplification products of approximately 550 bp were obtained for both ITS and ß-tubulin. The manually adjusted alignment of the ITS and ß-tubulin nucleotide sequences contained 39 taxa and 970 characters including alignment gaps. Of the aligned nucleotide sites, 148 characters were parsimony-informative, 167 variable characters were parsimony-uninformative and 655 were constant. The result of the partition homogeneity test ($P = 0.345$; where $P \geq 0.05$ was taken as significantly incongruent) showed that the two data sets were combinable. Fifty equally most parsimonious trees (TL = 457 steps, CI = 0.867, RI = 0.963, RC = 0.834) were obtained from maximum parsimony analysis of the combined sequence

data, one of which is shown in Fig. 1. New sequences were deposited in GenBank (Table 1), and the alignments in TreeBASE.

The *Cylindrocladiella* isolates grouped in two main clades with 93% and 90% bootstrap support, respectively. The first main clade contained an isolate of *C. microcylindrica* and two *Cylindrocladiella* species in a basal polytomy. Also in this clade were strains of *C. camelliae* (93% bootstrap support), and *C. peruviana* (95% bootstrap support). The second main clade contained three sub-clades, each with a high bootstrap support. The first subclade (97% bootstrap support) contained a basal polytomy consisting of a *Cylindrocladiella* species and two isolates of *Nectricladiella infestans*. Also in this sub-clade were two groups of *N. infestans* isolates (82% and 96% bootstrap support, respectively), that clustered together with a bootstrap support value of 61%. The second sub-clade (100% bootstrap support) grouped with the first sub-clade with a bootstrap support value of 100%, and contained isolates of *C. lageniformis*. The third sub-clade contained isolates of *C. parva* and had a bootstrap support value of 100%.

Morphology and Taxonomy. Four species of *Cylindrocladiella* were found to occur on grapevines in South Africa. The single isolate of *C. parva* obtained (STE-U 5736), proved to have conidia that were 12–20 x 2–3 µm in size, thus similar to that reported for the species (Crous, 2002). Vesicles were subcylindrical to pyriform, or clavate with an acutely rounded apex (Fig. 296, Crous, 2002), thus closely agreeing with the description of the species. Isolates of *C. lageniformis* had ovoid to ellipsoidal, or lageniform to pyriform vesicles, which agreed with the species (Crous, 2002), but were generally narrower (7–9 µm wide), than reported for the ex-type strain. Conidia were 9–16 x 1.5–2 µm, closely matching that of the species (Crous, 2002). Isolates of *C. peruviana* were characterised by their ellipsoidal to lanceolate vesicles, and conidia which were 9–15 x 2–3 µm in size, also closely matching those of the species (Crous, 2002).

In addition to confirming that these three species occur on grapevines in South Africa, a previously undescribed species was also elucidated in the *C. infestans* species complex. Although this complex has been acknowledged as being variable based on sequence data obtained in previous studies (Schoch *et al.*, 2000; Crous, 2002), the inclusion of additional isolates have shown this complex to consist of at least 3 taxa (Fig. 1), namely *C. infestans* (ATCC 44816, ex-type), *Nectricladiella infestans* (STE-U 2319, ex-type), and a third, undescribed species which is treated below.

Cylindrocladiella viticola Crous & G.J. van Coller, **sp. nov.** Fig. 2

Characteribus culturae et morphologia C. infestanti similis sed distincta propter conidia minoria. Conidia hyalina 1-septata, cylindracea apicibus obtusis, (8–)9–14(–15) × 2–2.5(–3) µm.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, or dimorphic, penicillate and subverticillate, mononematous, hyaline; penicillate conidiophores comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; subverticillate conidiophores comprising a stipe, and one or two series of phialides; stipe septate, hyaline, smooth; stipe extensions aseptate, straight, 80–145 µm long, thick-walled, with one basal septum, terminating in thin-walled, irregularly ellipsoid to clavate vesicles, 4–8 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate or 1-septate, 13–25 × 3–4 µm; secondary branches aseptate, 9–13 × 2.5–3 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–15 × 2.5–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* in moderate numbers, phialides cymbiform to subcylindrical, 10–25 × 2.5–3 µm. *Conidia* cylindrical, rounded at both ends, straight, (8–)9–14(–15) × 2–2.5(–3) µm (mean = 13 × 2.5 µm), (0–)1-septate, frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Cultural characteristics: Colonies raised, cottony, with smooth margins, white with straw (21'd) tint in patches (surface), umber (13'i) (reverse); chlamydospores extensive throughout medium, occurring in chains; reaching 29 mm after 6 days on MEA at 25°C in the dark.

Type: SOUTH AFRICA. Western Cape province, Wellington, *Vitis vinifera*, March 2001, *G.J. van Coller*, herb. CBS 7951 (holotype), ex-type culture STE-U 5606 = CBS 112897.

Symptoms: Cutting rot.

Additional culture: SOUTH AFRICA. Western Cape province, Wellington, *Vitis vinifera*, August 2002, *F. Halleen*, STE-U 5620.

Notes: *Cylindrocladiella viticola* is presently only known from grapevines in South Africa. It has smaller conidia (8–)9–14(–15) × 2–2.5(–3) µm than *C. infestans* and the morphologically similar anamorph of *Nectricladiella infestans*, namely (10–)14–16(–20) × 2(–3) µm. Furthermore, *C. viticola* has irregularly ellipsoidal to clavate vesicles, in contrast to the cylindrical vesicles of *C. infestans*, and the cylindrical to lanceolate vesicles of *N. infestans*.

Stem inoculations.

Green shoots. Analysis of variance were conducted to determine the effects of inoculation of green 99 Richter shoots with different isolates of *Cylindrocladiella* spp. by means of wounded and unwounded treatments, on lesion length (Table 2).

A significant treatment x species interaction (P = 0.0004) occurred when inoculating on green shoots (Table 2). With the unwounded treatment, none of the species resulted in lesions with a significantly (P = 0.05) higher length than either the non-pathogen control (*C. rosea*), or the agar-control (Table 3). None of the species resulted in a significant longer lesion than the two control treatments, or between each other (Table 3). *Cylindrocladiella parva* and *C. peruviana* was the only taxa that caused lesions on unwounded shoots, although the length of these lesions was not significantly longer than the control treatments (Table 3).

Furthermore, a significant treatment x isolate interaction (P = 0.0004) occurred when inoculating on green shoots (Table 2). With the unwounded treatment, no isolates of any species resulted in lesions with a significantly (P = 0.05) higher length than either the non-pathogen control (*C. rosea*), or the agar-control (Table 3). However, with the wounded treatment, inoculation with the *C. lageniformis* isolate STE-U 5611 gave a significantly higher lesion length than the two control treatments. The lesion length from this isolate did not differ significantly from the lesion length obtained by isolate STE-U 5606 of *C. viticola* (Table 3), but differed significantly from the lesion length obtained after inoculation with all isolates of *C. peruviana*, and the isolate of *C. parva* (STE-U 5735) (Table 3). In general, it appears as if isolates of *C. lageniformis* resulted in the highest lesion length from all four species, although these results were not statistically significant (P = 0.05) (Table 3).

One-year-old-shoots. Analysis of variance were conducted to determine the effects of inoculation of wounded and unwounded shoots of 1-year-old 99 Richter with different isolates of *Cylindrocladiella* spp. on lesion length (Table 4).

No significant interactions were observed after inoculation on one-year-old shoots, and therefore main effects were compared. Inoculation with any species of *Cylindrocladiella* did also not result in a significant (P = 0.05) higher lesion length than obtained on 1-year-old shoots after inoculation with either the non-pathogen control, or agar-control. Inoculation with any isolate of any species of *Cylindrocladiella* did not result in a significantly (P = 0.05) higher lesion than

obtained on one-year-old shoots after inoculation with either the non-pathogen control, or agar-control (Table 5).

Results obtained by comparing wounded and unwounded treatments indicated that the wounded treatment resulted in significantly higher lesion lengths than the unwounded treatments (Table 6).

DISCUSSION

Four species of *Cylindrocladiella* were found in this study to be associated with grapevines in South Africa, namely *C. lageniformis*, *C. parva*, *C. peruviana*, as well as a new species within the *C. infestans* complex, which is described here as *C. viticola*.

Although the present study has confirmed earlier records that *C. lageniformis*, *C. parva* and *C. peruviana* occur on grapevines in South Africa, it has also added valuable information to our understanding of the *C. infestans* species complex, and distinguished a new species within this complex, described here as *C. viticola*. Schoch *et al.* (2000) provided ITS and ß-tubulin sequence data to corroborate the RFLP data of Victor *et al.* (1998), proving that *Nectricladiella camelliae* was not the teleomorph of *C. infestans*. Furthermore, a newly collected isolate from Madagascar proved to be morphologically and phylogenetically similar to *C. infestans*, and produced a teleomorph in culture which was subsequently described as *N. infestans*. With the inclusion of additional sequence data and isolates in the present study, however, it appears that the connection proposed by Schoch *et al.* (2000) is probably incorrect, and that *C. infestans* could be a cryptic species closely related to *N. infestans*. Although vesicle morphology is a reliable feature aiding in the identification of *Cylindrocladium* spp. (Crous, 2002), it seems considerably less reliable in *Cylindrocladiella*. The latter is possibly due to the fact that the stipe extensions are aseptate, which in turn results in vesicles that are more prone to variation depending on the osmotic potential of the medium used (Crous *et al.*, 1992). Furthermore, DNA sequence data from additional isolates lead me to conclude that the taxonomy of *Cylindrocladiella* is much more complicated than currently assumed, and that many more species await formal description. Their potential role as plant pathogens, however, remains to be resolved.

Although the *Cylindrocladiella* isolates used for inoculations in this study were all obtained from diseased grapevines exhibiting symptoms of dieback, chlorotic leaves and a general unthrifty appearance, it was not possible to obtain clear stem lesions with any of the

species in the inoculation experiments conducted here. As was mentioned earlier, five species of *Cylindrocladiella* have been reported from South Africa on various hosts, three of which were also recorded on declining *V. vinifera* (Crous & Wingfield, 1993; Victor *et al.*, 1998; Crous, 2002). Although these reports clearly confirm the pathogenic ability of *Cylindrocladiella* to various hosts, we were not able to prove Koch's postulates on grapevines. Further studies would therefore have to be conducted testing different plant parts e.g. roots and canes, under different environmental conditions, e.g. high moisture regimes, and also using different methods of inoculation, to resolve the pathogenic status of *Cylindrocladiella* spp. to grapevines.

LITERATURE

Boesewinkel, H.J. 1982. *Cylindrocladiella*, a new genus to accommodate *Cylindrocladium parvum* and other small-spored species of *Cylindrocladium*. *Canadian Journal of Botany* 60: 2288–2294.

Crous, P.W. 2002. *Taxonomy and pathology of* Cylindrocladium *(*Calonectria*) and allied genera*. APS Press: Minnesota, USA. 294 pp.

Crous, P.W. & Phillips, A.J.L. & Wingfield, M.J. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* 84: 497–504.

Crous, P.W. & Wingfield, M.J. 1993. A re-evaluation of *Cylindrocladiella*, and a comparison with morphological similar genera. *Mycological Research* 97: 433–448.

Crous, P.W., Wingfield, M.J. & Lennox, C.L. 1994. A comparison of generic concepts in *Calonectria* and *Nectria* with anamorphs in *Cylindrocladium* and *Cylindrocladiella*. *South African Journal of Science* 90: 485–488.

Farris, J.S., Kallersjo, M., Kluge, A.G. & Bult, C. 1994. Testing significance of incongruence. *Cladistics* 10: 315–320.

Fisher, N.L., Burgess, L.W., Toussoun, T.A. & Nelson, P.E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

Glass, N.L. & Donaldson, G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330.

Hillis, D.M. & Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.

Kang, J.C., Crous, P.W. & Schoch, C.L. 2001. Species concepts in the *Cylindrocladium floridanum* and *Cy. spathiphylli* complexes (*Hypocreaceae*) based on multi-allelic sequence data, sexual compatibility and morphology. *Systematic and Applied Microbiology* 24: 206–217.

Lee, S.B. & Taylor, J.W. 1990. Isolation of DNA from fungal mycelia and single spores. In *PCR Protocols: a guide to methods and applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White) pp. 282–287. Academic Press: New York, USA.

Mandal, N.C. & Dasgupta, M.K. 1983. Postharvest diseases of perishables in West Bengal 1: new host records and a new fungus from India. *Indian Journal of Mycology and Plant Pathology* 13: 73–77.

O'Donnell, K. & Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7: 103–116.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357–358.

Peerally, A. 1991. The classification and phytopathology of *Cylindrocladium* species. *Mycotaxon* 40: 323–366.

Rambaut, A. 2002. Sequence Alignment Editor. Version 2.0. Department of Zoology, University of Oxford, Oxford.

Rayner, R.W. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew, Surrey, 175h, 34P, UK.

Rossman, A.Y. 1979. A preliminary account of the taxa described in *Calonectria*. *Mycotaxon* 8: 485–558.

Schoch, C.L., Crous, P.W., Wingfield, M.J. & Wingfield, B.D. 2000. Phylogeny of *Calonectria* and selected hypocrealean genera with cylindrical macroconidia. In *Molecules, morphology and classification: towards monophyletic genera in the Ascomycetes* (eds. K.A. Seifert, W. Gams, P.W. Crous, G.J. Samuels) *Studies in Mycology* 45: 45–62.

Sharma, J.K. & Mohanan, C. 1991. *In vitro* evaluation of fungicides against *Cylindrocladium* spp. causing diseases of *Eucalyptus* in Kerala, India. *European Journal of Forest Pathology* 21: 17–26.

Snedecor, G.W. & Cochran, W.G. 1989. *Statistical methods*. Eighth edn. 503 pp. Iowa State University Press: Iowa, USA.

Swofford, D.L. 2000. *PAUP. Phylogenetic analysis using parsimony (and other methods) version* 4. Sunderland, Massachusetts, USA: Sinauer Associates.

Victor, D., Crous, P.W., Janse, B.J.H., Van Zyl, W.H., Wingfield, M.J. & Alfenas, A.C. 1998. Systematic appraisal of species complexes within *Cylindrocladiella*. *Mycological Research* 102: 273–279.

White, T.J., Bruns, T.D., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a guide to methods and applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White) pp. 315–322. Academic Press: New York, USA.

Table 1. *Cylindrocladiella* isolates included in this study for sequence analysis and/or morphological comparison

Anamorph	Teleomorph	Accession no.[w]	Substrate	Country	Collector	Areas sequenced	
						ITS	Beta-tubulin
Cylindrocladiella camelliae	Unknown	STE-U 234	*Eucalyptus grandis*	Northern Province, South Africa	P.W. Crous	AF220952	
Cylindrocladiella camelliae	Unknown	STE-U 277	*Eucalyptus grandis*	Northern Province, South Africa	P.W. Crous	AF220953	
Cylindrocladiella elegans	Unknown	STE-U 518	Litter	Western Cape, South Africa	P.W. Crous	AF220954	
Cylindrocladiella elegans[x]	Unknown	CBS 338.92	Leaf litter	KwaZulu-Natal, South Africa	I. Rong		
Cylindrocladiella infestans[x]		ATCC44816	*Pinus pinea*	New Zealand	H.J. Boesewinkel	AF220955	AF320190
Cylindrocladiella infestans		IMI299376	*Arenga pinnata*	Indonesia	K.B. Boedijn & J. Reitsma	AF220956	
Cylindrocladiella lageniformis	Unknown	STE-U 5607	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella lageniformis	Unknown	STE-U 5608	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella lageniformis	Unknown	STE-U 5609	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella lageniformis	Unknown	STE-U 5610	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella lageniformis	Unknown	STE-U 5611	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella lageniformis	Unknown	STE-U 5736	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		

Table 1. Continue

Anamorph	Teleomorph	Accession no.[w]	Substrate	Country	Collector	Areas sequenced	
						ITS	Beta-tubulin
Cylindrocladiella lageniformis [x]	Unknown	UFV115 CBS 338.92	*Eucalyptus* sp.	Brazil	A.C. Alfenas	AF220959	
Cylindrocladiella microcylindrica [x]	*Nectricladiella camelliae*	ATCC 38571	*Pinus pinea*	Australia	W.A. Shipton	AF220960	AF320191
Cylindrocladiella novaezelandiae [x]	Unknown	ATCC 44815	*Rhododendron indicum*	New Zealand	H.J. Boesewinkel	AF220963	
Cylindrocladiella parva [x]	Unknown	ATCC 28272	*Telopea speciosissima*	New Zealand	H.J. Boesewinkel	AF220964	
Cylindrocladiella parva	Unknown	STE-U 373	*Pinus radiata*	Western Cape, South Africa	P.W. Crous	AF220965	
Cylindrocladiella parva	Unknown	STE-U 5735	*Vitis vinifera* cv. 101-14 Mgt [z]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella peruviana	Unknown	STE-U 395	*Acacia mearnsii*	KwaZulu-Natal, South Africa	P.W. Crous	AF220967	
Cylindrocladiella peruviana	Unknown	STE-U 683	Soil	Thailand	M.J. Wingfield	AF220961	
Cylindrocladiella peruviana	Unknown	STE-U 4210	*Vitis vinifera*	California, U.S.A.			
Cylindrocladiella peruviana	Unknown	STE-U 5612	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5613	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5614	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		

Table 1. Continue

Anamorph	Teleomorph	Accession no.[w]	Substrate	Country	Collector	Areas sequenced	
						ITS	Beta-tubulin
Cylindrocladiella peruviana	Unknown	STE-U 5615	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5616	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5617	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5618	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana	Unknown	STE-U 5619	*Vitis vinifera*	Western Cape, South Africa	S.C. Lamprecht		
Cylindrocladiella peruviana[x]	Unknown	IMUR 1843	Ants	Peru	M.P. Herrera	AF220966	
Cylindrocladiella sp.	Unknown	STE-U 10451	*Echeveria elegans*	Indonesia	C.F. Hill		
Cylindrocladiella sp.	Unknown	STE-U 10452	*Agalonema commutatum*	U.S.A.	C.F. Hill		
Cylindrocladiella sp.	Unknown	STE-U 10490	*Archontophoenix purpurea*	Australia			
Cylindrocladium sp.	Unknown	IMI 384951	*Oryza sativa* ex rice field	India			
Cylindrocladiella viticola[x]	Unknown	STE-U 5606 CBS 112897	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	G.J. van Coller		
Cylindrocladiella viticola	Unknown	STE-U 5620	*Vitis vinifera* cv. 99 Richter[y]	Western Cape, South Africa	F. Halleen		

Table 1. Continue

Anamorph	Teleomorph	Accession no.[w]	Substrate	Country	Collector	Areas sequenced	
						ITS	Beta-tubulin
Cylindrocladiella sp.	*Nectricladiella infestans*	STE-U 708	Soil	Hong Kong	M.J. Wingfield	AF220958	
Cylindrocladiella sp.	*Nectricladiella infestans* [x]	STE-U 2319	Soil	Madagascar	J.E. Taylor	AF220957	

[w] ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham lane, U.K.; IMUR: Institute of Mycology, University of Recife, Brazil; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; UFV: (A.C. Alfenas), Department of Plant Pathology, University of Viçosa, Viçosa, Minas Gerais, Brazil.

[x] Ex-type cultures.

[y] Richter 99 = *Vitis berlandieri* PI x *Vitis rupestris* Sch. var. du Lot.

[z] 101-14 Mgt = *Vitis riparia* M x *Vitis rupestris* Sch.

Table 2. Analysis of variance for effects of inoculation of green 99 Richter shoots with different isolates (I) of different *Cylindrocladiella* spp. (S) by means of wounded and unwounded treatments (T) on lesion length

Source of variation	**DF**	**SS**	**MS**	**Pr > F**
Model	79	1051.0000	13.3038	< 0.0001
Experiment	1	19.9580	19.9580	0.0019
Sp	5	14.4522	2.8904	0.3474
I	19	50.6228	2.6644	0.1492
T	1	787.6466	787.6466	< 0.0001
T x Sp	5	59.2653	11.8531	0.0010
T x I	19	122.3674	6.4404	0.0004
Error	128	326.0000	2.5469	
Corrected total	207	1377.0000		

Table 3. Mean lesion lengths after inoculation of wounded and unwounded green shoots of 99 Richter with different isolates of *Cylindrocladiella*

Species	Isolate no.	Lesion length (mm) [z]	
		Unwounded	Wounded
C. lageniformis	STE-U 5607	0.00 k	4.67 bc
	STE-U 5608	0.00 k	5.83 ab
	STE-U 5609	0.00 k	5.00 a-c
	STE-U 5610	0.00 k	4.50 b-d
	STE-U 5611	0.00 k	6.60 a
	STE-U 5736	0.00 k	4.83 bc
C. lageniformis (Mean)		0.00 k	5.21 a-c
C. parva	STE-U 5735	0.50 jk	4.75 bc
C. parva (Mean)		0.50 jk	4.75 bc
C. peruviana	STE-U 4210	0.00 k	4.17 b-f
	STE-U 5612	0.80 i-k	4.00 c-f
	STE-U 5613	1.60 h-k	2.60 e-h
	STE-U 5614	1.25 h-k	2.50 f-i
	STE-U 5615	1.33 h-k	2.17 g-j
	STE-U 5616	0.00 k	4.40 b-d
	STE-U 5617	1.20 h-k	3.60 c-g
	STE-U 5618	0.00 k	4.60 b-c
	STE-U 5619	0.00 k	2.80 d-h
C. peruviana (Mean)		0.66 jk	3.45 c-g
C. viticola	STE-U 5606	0.00 k	5.80 ab
	STE-U 5620	0.00 k	2.60 e-h
C. viticola (Mean)		0.00 k	4.20 b-e
Clonostachys rosea	Control	0.00 k	4.25 b-e
Clonostachys rosea (Mean)	Control	0.00 k	4.25 b-e
Control	Control	0.00 k	4.50 b-d
Control (Mean)	Control	0.00 k	4.50 b-d

[z] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 4. Analyses of variance for effects of inoculation of 1-year-old 99 Richter shoots with different isolates (I) of *Cylindrocladiella* spp. by means of wounded and unwounded treatments (T) on lesion length

Source of variation	DF	SS	MS	Pr > F
Model	79	555.6600	7.0337	< 0.0001
Experiment	1	6.3550	6.3550	0.1685
S	5	21.4643	4.2929	0.1754
T	1	325.0003	325.0003	< 0.0001
I	19	50.5315	2.6596	0.6679
T x S	5	21.7433	4.3487	0.1697
T x I	19	47.8565	2.5188	0.7146
Error	145	272.5000	1.8793	
Corrected total	224	828.1600		

Table 5. Mean lesion length after inoculation of 1-year-old 99 Richter shoots with different *Cylindrocladiella* isolates

Species	Isolate no.	Mean lesion length [z]
C. lageniformis	STE-U 5607	1.30 a
	STE-U 5608	1.55 a
	STE-U 5609	1.09 a
	STE-U 5610	0.67 a
	STE-U 5611	1.00 a
	STE-U 5736	1.58 a
C. lageniformis (Mean)		1.19 a
C. parva	STE-U 5735	2.17 a
C. parva (Mean)		2.17 a
C. peruviana	STE-U 4210	1.00 a
	STE-U 5612	0.58 a
	STE-U 5613	0.92 a
	STE-U 5614	0.92 a
	STE-U 5615	1.27 a
	STE-U 5616	2.30 a
	STE-U 5617	1.64 a
	STE-U 5618	1.27 a
	STE-U 5619	0.80 a
C. peruviana (Mean)		1.17 a
C. viticola	STE-U 5606	1.00 a
	STE-U 5620	1.80 a
C. viticola (Mean)		1.38 a
Clonostachys rosea	Control	0.58 a
Clonostachys rosea (Mean)		0.58 a
Control	Control	0.58 a
Control (Mean)		0.58 a

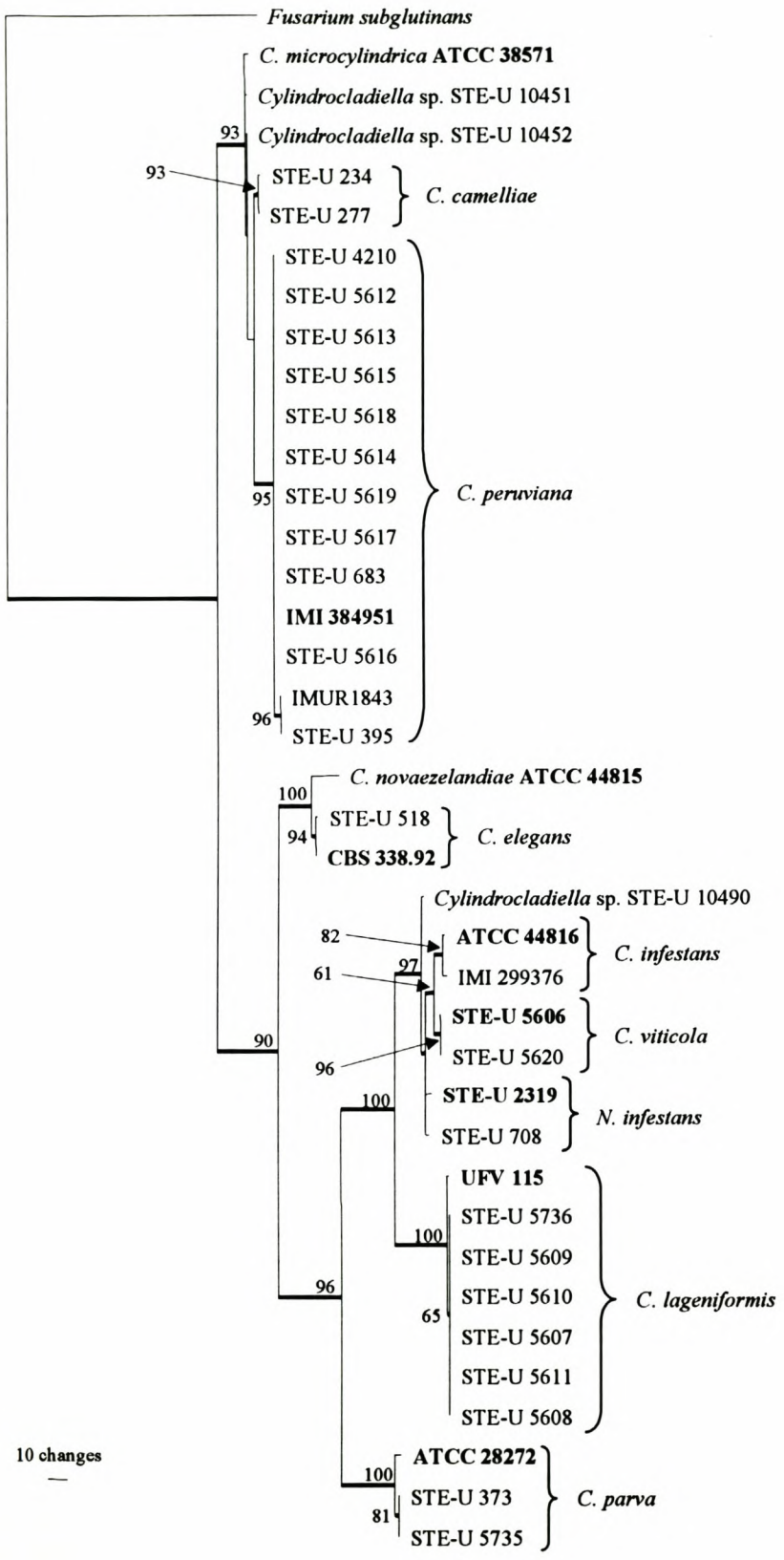
[z] Isolate means within a column followed by the same letter do not differ significantly; species means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 6. Mean lesion length observed after wounding and unwounded inoculations on 1-year-old 99 Richter shoots

Treatment	**Mean lesion length (mm)** [z]
Wounded	2.41 a
Unwounded	0.00 b

[z] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Figure 1. One of fifty most parsimonious trees obtained from the combined ITS and β-tubulin sequence data (TL = 457 steps, CI = 0.867, RI = 0.963, RC = 0.834). The scale bar indicates 10 changes and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The GenBank sequences of *Fusarium subglutinans* (U34559 and U34417, respectively for ITS and β-tubulin) were included as outgroup.



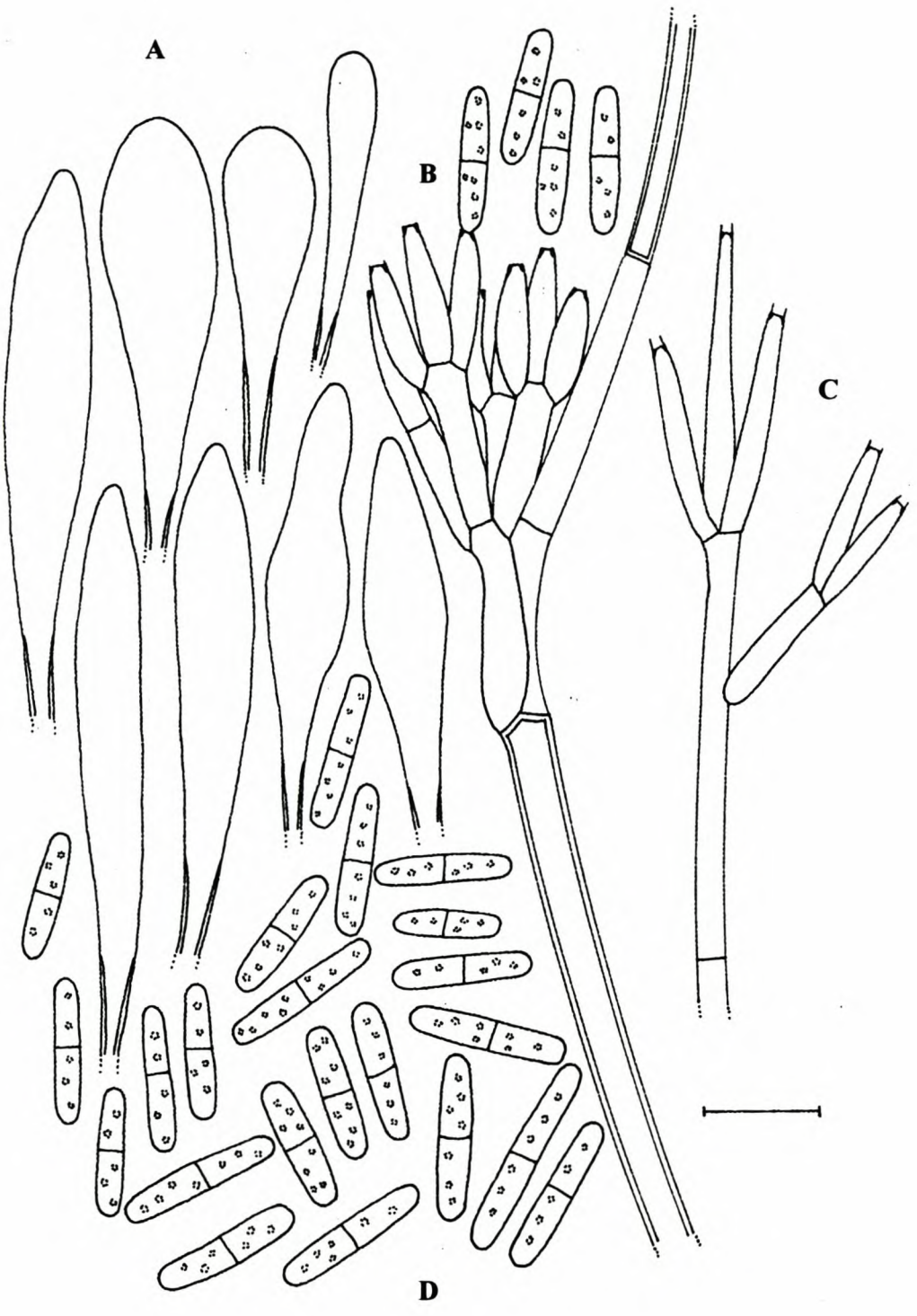


Fig. 2A–D. *Cylindrocladiella viticola*. A. Ellipsoidal to clavate vesicles. B. Penicillate conidiophore. C. Subverticillate conidiophore. D. One-septate conidia. Bar = 10 µm.

4. IDENTIFICATION AND PATHOGENICITY OF *FUSARIUM* SPECIES ASSOCIATED WITH NURSERY GRAPEVINES IN THE WESTERN CAPE PROVINCE

ABSTRACT

Fusarium spp. are important soilborne pathogens of grapevines in some countries, causing root necrosis and decline. Surveys conducted in a previous study to determine the status of soilborne diseases of grapevines in three nurseries in the Western Cape province, showed that *Fusarium* spp. were frequently associated with diseased grapevine crowns and roots. Subsequent species identification revealed that *F. oxysporum*, *F. proliferatum* and *F. solani* were predominantly isolated, while low frequencies of *F. acuminatum*, *F. anthophilum*, *F. chlamydosporum*, *F. equiseti*, *F. nygamai*, *F. scirpi* and *F. semitectum* were recorded. Pathogenicity was evaluated in glasshouse studies to determine the pathogenic status of *F. oxysporum*, *F. proliferatum* and *F. solani*. A significant ($P = 0.05$) reduction of root volume, root dry mass, length of new shoot growth, number of leaves but an increase in percentage of chlorotic leaves, as well as significantly higher root rot severity indices were caused by *F. oxysporum* and *F. solani*. Inoculation with *F. proliferatum* caused a significant reduction of dry mass of new shoot growth, number of leaves, as well as higher root rot than the controls. Inoculation of the rootstock 101-14 Mgt with *F. proliferatum* resulted in a significant reduction of dry root mass and length of new shoot growth, but a significant increase in dry root mass and length of new shoot growth was recorded for the rootstock cultivar 99 Richter after inoculation with *F. proliferatum*. The root rot severity index was significantly negatively correlated with all other parameters except the percentage of chlorotic leaves. Although the three species do not differ significantly with regard to their pathogenicity, it appears as if *F. solani* may be more virulent than *F. oxysporum*, followed by *F. proliferatum*. This is the first report of the pathogenicity of *F. oxysporum* and *F. solani* as pathogens of grapevines in South Africa. It is also the first report of *F. proliferatum* as a pathogen of grapevines in the world.

INTRODUCTION

Fusarium spp. are commonly associated with grapevines, and have been isolated from this host in Australia (Highet & Nair, 1995), Brazil (Andrade, 1993), Chile (Mujica, 1944), Egypt (Mahrous, 2001), France (Foëx & Ayoutantis, 1924, 1926; Rives, 1927; Branas & Bernon, 1933),

India (Sinha, Jawanda & Chohan, 1969; Lele *et al.*, 1978), Italy (Petri, 1932), Russia (Mil'ko, 1959; Shterenburg, 1959), Sardinia (Petri, 1932), Sicily (Grasso, 1984), South Africa (Du Plessis, 1936; Marais, 1979; Ferreira, Matthee & Thomas, 1989), Switzerland (Osterwalder, 1943; Benzoni, 1954), and the USA (Sprague, 1953; Gugino, Travis & Stewart, 2001). *Fusarium oxysporum* Schltdl. em. W.C. Snyder & H.N. Hansen and *Fusarium solani* (Mart.) Appel & Wollenw. are the two *Fusarium* spp. most commonly associated with grapevine decline. *Fusarium oxysporum* causes both root rot and wilt of grapevines, while *F. solani* has been associated with root rot only. Fusarium root rot is often restricted to feeder roots and contributes to the decline syndrome, but is seldom fatal to the plant. On the other hand, most plants diseased with vascular wilt do not recover and eventually die. Consequently Fusarium wilt of grapevines is considered a very important disease with great economic implications (Lele *et al.* 1978; Grasso, 1984; Andrade, 1993; Highet & Nair, 1995; Gugino *et al.*, 2001).

In Brazil, Andrade (1993) reported a *Fusarium* sp., cited as "*F. oxysporum* f.sp. *herbemontis*" as the most important pathogen of grapevines in some regions in Santa Catarina. *Fusarium oxysporum* together with *Cylindrocarpon destructans* (Zinssm.) Scholten were also consistently isolated from grapevines in Sicily. These grapevines were characterised by leaf yellowing, wilting, and cane blight with necrosis of cortical tissues, discolouration and tylosis of the xylem (Grasso, 1984). A number of grapevine diseases have also been attributed to non-wilt causing forms of *F. oxysporum*. In South Africa, a *Fusarium* sp., cited as "*F. oxysporum* var. *auranticum*" was associated with wastage of export grapes (Du Plessis, 1936) and *F. oxysporum* with declined grapevines (Ferreira *et al.*, 1989). *Fusarium oxysporum* was also associated with decline of grapevines in Pennsylvania (Gugino *et al.*, 2001). In California, Granett *et al.* (1998) reported wounds on roots caused by phylloxera (*Daktulosphaira vitifolia* Fitch) to become infested by various fungi, including *Fusarium* spp. cited as "*Fusarium roseum* (Schlecht)" and *F. oxysporum*, as well as *Pythium ultimum* Tröw.

Fusarium solani was the predominant species of *Fusarium* isolated from grapevines with a high mortality rate in Brazil (Andrade, 1993). In India, severe crown and root galls on 2-year-old grapevines were reported that were caused by combined infections with fungi, a nematode sp. and bacterium, cited as "*Rotylenchulus reniformis*, *Agrobacterium tumefaciens* and *F. solani*" (Lele *et al.*, 1978). In California, Omer *et al.* (1995) reported *F. solani* causing further damage to grapevines that were already damaged by phylloxera. In Egypt, Mahrous (2001) identified *Fusarium* spp., cited as "*F. solani* and *Fusarium moniliforme*", and other fungi, cited as

"*Rhizoctonia solani* and *Botryodiplodia theobromae*" as the predominant pathogens involved in decline observed on nursery grapevines. It was recently decided that the name "*Fusarium verticillioides*" be used instead of "*Fusarium moniliforme*", since the name *F. moniliforme* represents an unacceptable broad species concept and *F. verticillioides* is indisputably the older name for the species now commonly referred to as *F. moniliforme* (Hawksworth, 2003). Since the name *F. moniliforme* was used loosely, no equivalency should be made between *F. moniliforme* and *F. verticillioides*, especially when dealing with older literature (Hawksworth, 2003). *Fusarium solani* was also associated with decline of grapevines in Pennsylvania (Gugino *et al.*, 2001).

Other *Fusarium* spp. associated with diseased grapevines were cited as "*Fusarium viticola*" (Foëx & Ayoutantis, 1924; Rives, 1927), "*Fusarium merismoides*" (Morquer, 1940), "*Fusarium acuminatum*" (Sprague, 1953), "*Fusarium avenaceum*" (Morquer, 1940; Benzoni, 1954), "*Fusarium equiseti*" (Andrade, 1993; Gugino *et al.*, 2001), and "*Fusarium moniliforme* var. *anthophilum*" and "*Fusarium moniliforme* var. *subglutinans*" (Andrade, 1993). *Fusarium acuminatum* was isolated from grape berries in Washington, USA but no indication of pathogenicity was given (Sprague, 1953). Similarly, *F. avenaceum* was found in old excised branches or emerging from the cortex of Bonarda grapevines in sheltered sites on calcareous soil, but pathogenicity was not confirmed. According to Morquer (1940), in Toulouse, France, *F. avenaceum* and *F. merismoides* grew and reproduced in the viscous exudates of grapevines in spring. Tests using artificial inoculation demonstrated that these fungi resided as saprobes in the vine sap (Morquer, 1940). According to Morquer (1940), *F. avenaceum* and *F. merismoides* could propagate and reproduce in sap of grapevines in spring. Artificial inoculation tests confirmed that these fungi reside as saprobes in the vine sap (Morquer, 1940). In Brazil, in an experimental area with high grapevine mortality, *Fusarium equiseti*, *F. moniliforme* var. *anthophilum* and *F. moniliforme* var. *subglutinans* were isolated from roots (Andrade, 1993). *Fusarium* spp., cited as "*Fusarium equiseti*" and "*Fusarium sporotrichioides*" together with "*F. oxysporum*", "*F. solani*", "*C. destructans*" and a *Diplodina* sp. were associated with decline of grapevines in Pennsylvania. Foëx and Ayoutantis (1924) and Rives (1927) reported *F. viticola* to be associated with diseased grapevines in France. Rives (1927), however, stated that insufficient information was available to confirm whether *F. viticola* was responsible for this condition.

In the USA, Falk *et al.* (1996) reported on the use of *Fusarium proliferatum* (T. Matsushima) Nirenberg as a biological control agent against grape downy mildew and

maintained that the formulation tested was not pathogenic to grapes and no fumonisin mycotoxins occurred in grape juice from treated plants. Later on, Bakshi, Sztejnberg and Yarden (2001) isolated and characterised a cold-tolerant strain of *F. proliferatum* as a biological control agent against grape downy mildew.

Fusarium spp. associated with declining and wilting grapevines are often not identified to species level. Unidentified *Fusarium* spp. associated with wilting of vines were reported in Italy and Sardinia by Petri (1932), as well as by Branas and Bernon (1933) in France on soil previously cultivated with lucerne. Unidentified *Fusarium* spp. associated with decline of grapevines was reported by Agnihotrudu (1968) in Andhra Pradesh, India, by Shterenburg (1959) in Russia, by Bumbieris (1972) in Australia, and by Marais (1979) and Halleen, Crous and Petrini (2003) in South Africa, although no indication of the pathogenic status of these *Fusarium* isolates was given.

In view of the recent increase in the number of vineyards in South Africa and the demand for high quality disease free planting stock the need to reassess the status of root diseases on nursery grapevines became clear. With the evident lack of information regarding *Fusarium* species and their effect on grapevines in South Africa, a study was initiated to investigate this area. The aims of this study were to determine which *Fusarium* spp. are associated with nursery grapevines grafted onto popular commercial grapevine rootstocks in the Western Cape province and origin of material (different grapevine nurseries). Furthermore, the effect of isolation procedures, e.g. disinfestation and non-disinfestation of grapevine material prior to isolation, on the incidence of different *Fusarium* spp. needs to be elucidated. Pathogenicity and relative virulence of the most predominant species were also assessed on commonly planted rootstocks, using different parameters for the evaluation.

MATERIALS AND METHODS

Surveys. *Fusarium* species associated with diseased grapevines in nurseries were recorded as part of a survey programme (Chapter 2) to determine the incidence of pathogenic fungi on nursery grapevines. The surveys were conducted in three commercial nurseries in the Western Cape province over three seasons. The first survey was conducted from February to April 2001 when the grapevines were 5-7 months old, the second survey from November to December 2001 when the grapevines were 1-2 months old, and the third survey from March to

April 2002, when the grapevines were 6-7 months old. Declining grafted nursery grapevines were sampled and isolations made from root and crown tissue onto *Phytophthora* selective medium (PH medium) (Solel & Pinkas, 1982); *Pythium* selective medium (P medium) (PH medium without hymexazol); potato dextrose agar (PDA) (Biolab Diagnostics; Midrand, South Africa); water agar (WA) (Agar Bacteriological, Biolab Diagnostics, Midrand, South Africa) containing 0.04g/L streptomycin sulphate, unamended WA, and Komada's medium (*Fusarium oxysporum* selective medium) (Komada, 1975). For surveys two and three, the same culturing media were used, but the WA amended with streptomycin sulphate was left out, and Komada's medium was replaced with selective *Fusarium* agar (Tio *et al.*, 1977) to isolate a wider range of *Fusarium* spp. All fungal colonies that developed were transferred to divided Petri dishes containing 2% potato dextrose agar (PDA; Biolab Diagnostics, Midrand, South Africa) in one side, and carnation leaf agar (Fisher *et al.*, 1982) in the other side. *Fusarium* species were identified to species level according to Nelson *et al.* (1983).

Pathogenicity trials. Two pathogenicity trials were conducted in separate glasshouses. In the first glasshouse, grafted grapevines were inoculated with *F. oxysporum* and *F. solani*, and in the second glasshouse, grapevines were inoculated with *F. proliferatum*. In each glasshouse a complete set of control plants were present. The trials were conducted separately in order to avoid contamination of *F. oxysporum* and *F. solani* treatments with the *F. proliferatum* treatment, because *F. proliferatum* can easily be dispersed. The materials and methods for both trials were the same.

Isolates and preparation of inoculum. Three isolates each of *F. oxysporum* (STE-U 5725, STE-U 5726, STE-U 5727), *F. proliferatum* (STE-U 5715, STE-U 5716, STE-U 5719), and *F. solani* (STE-U 5728, STE-U 5729, STE-U 5730) were plated onto 90 mm diam. Petri dishes containing PDA amended with 0.04 g/L streptomycin sulphate. The developing cultures were incubated for 14 days at 24°C under near ultraviolet and cool white lights with a 12 h photoperiod.

The millet seed inoculation method developed by Strauss and Labuschagne (1995) was used in this study. Two inoculum levels were prepared, namely 0.5% and 1% m/v inoculum. The millet seed was soaked in distilled water for 12 h (250 g seed/125 mL in 1 L Schott bottles), and then autoclaved for 20 min at 120°C on two consecutive days. The bottles were shaken

before autoclaving, as well as each time after autoclaving. Mycelial discs of the different *Fusarium* isolates were made with a sterile cork borer (5 mm diam.) and 13 mycelial plugs were transferred to each respective bottle. The bottles were incubated in the dark at 25°C for 10 days and shaken every third day to ensure thorough seed colonisation.

Preparation of plant material. Dormant 1-year-old grafted nursery grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon on 99 Richter (*Vitis berlandieri* PI x *Vitis rupestris* Sch. var. du Lot), and *V. vinifera* cv. Cabernet Sauvignon on 101-14 Mgt (*Vitis riparia* M x *Vitis rupestris* Sch.) rootstocks were subjected to hot water treatment (50°C for 30 min) to rid them of pathogens that may be present (Von Broembsen & Marais, 1978; Crous, Swart & Coertze, 2001). Following this treatment, the grapevines were hydrated in cold water for 24 h in order to break the heat shock (Crous *et al.*, 2001). The potting mixture consisted of fine sand and composted pine bark (1 : 1) (Nucellar nurseries, P.O. Box 73, Simondium), with a pH value of 6.2, and was amended with either 0.5% or 1% m/v inoculum. The grapevines were transplanted into 5 L plastic pots (one plant per pot) containing the amended potting mixture. The plants were irrigated daily with municipal tap water until field capacity was reached and fertilised weekly with a balanced, water-soluble fertiliser (Chemicult, Chemicult Producers (Pty) Ltd. P.O. Box 133, Camps Bay, 8040). A cloth dipped in penconazole (Topaz EW, Syngenta), was placed in front of the air-conditioners of the glasshouse, and replaced weekly, to prevent the development of powdery mildew (*Uncinula necator* (Schwein.) Burrill). No pest control was necessary.

Experimental design. The two experiments were conducted in separate glasshouses. Both were factorial experiments in randomised block designs. The factors were two rootstocks (101-14 Mgt, 99 Richter), three species in experiment 1 (control, *F. oxysporum*, *F. solani*), two species in experiment 2 (control, *F. proliferatum*), and two inoculum densities (0.5% m/v, 1% m/v) replicated randomly in five blocks. An experimental unit was one plant in a pot.

Disease assessments. Disease symptoms on the above-ground and below-ground parts of the plants were assessed 3 months after inoculation. The following below-ground parameters were used (i) a visual root rot rating on a scale of 0–4 (0 = no root rotting, 1 = >1–25% root rotting, 2 = >25–50% root rotting, 3 = >50–75% root rotting, 4 = >75–100% root rotting), (ii) root volume (cm^3) and (iii) dry root mass (g). To determine the root volume, the washed roots were excised and placed into a 2 L measuring cylinder containing 1 L of water. Root volume was

determined by measuring the water displacement (cm^3). Above-ground parameters assessed for every plant were (i) the length of new shoot growth (mm), (ii) dry mass of new shoot growth (g), (iii) number of leaves, and (iv) percentage of chlorotic leaves. The length of new shoot growth was determined by cutting off all new shoot growth (growth that occurred after the grapevines were planted), and measuring the sum total of new growth (mm).

Re-isolation of Fusarium spp. Re-isolation of the different *Fusarium* spp. was carried out by plating two pieces of root and crown tissue per plant onto both PDA and selective *Fusarium* agar.

Statistical analysis. Data obtained from the surveys and from the two pathogenicity experiments were subjected to analysis of variance using SAS statistical software version 8.02 (SAS Institute Inc., Cary, NC, USA). Fischer's protected least significant difference (Snedecor & Cochran, 1989) was calculated at the 5% level of probability to compare treatment means. The test of Shapiro and Wilk (1965) were carried out to test for non-normality. In order to compare the relative virulence of the three different *Fusarium* spp. tested in the two separate glasshouses, an adjusted analysis of variance were performed with residual results obtained by subtracting the mean value of the control treatments from the mean values of the inoculated treatments (Snedecor & Cochran, 1989). The analysis of variance was also performed with SAS statistical software version 8.02. The method described by Shapiro and Wilk (1965) was carried out to test for non-normality. Fischer's protected least significant difference (Snedecor & Cochran, 1989) was calculated at the 5% level of probability to compare treatment means. Pearson's product moment correlation test was performed to determine the correlation between parameters used for evaluating pathogenicity (Ott, 1998).

RESULTS

***Fusarium* species.** Ten *Fusarium* species, viz. *F. acuminatum* Ell. & Ev. Sensu Gordon, *F. anthophilum* (A. Braun) Wollenw., *F. chlamydosporum* Wollenw. & Reinking, *F. equiseti* (Corda) Sacc., *F. nygamai* Burgess & Trimboli, *F. oxysporum* Schlecht. Emend. Snyd. & Hans., *F. proliferatum* (Matsushima) Nirenberg, *F. scirpi* Lambotte & Fautr., *F. semitectum* Berk. & Rav., and *F. solani* (Mart.) Appel & Wollenw. Emend. Snyd. & Hans., were isolated from root and crown tissue of nursery grapevines.

Crown tissue. Results of the analyses of variance for the incidence of the different *Fusarium* spp. obtained from crown tissue of nursery grapevines are given in Table 1. In crown tissue, *Fusarium oxysporum* was isolated significantly ($P = 0.05$) more than any other *Fusarium* spp., whereas *F. proliferatum* and *F. solani* were significantly ($P = 0.05$) more frequently isolated than the remaining *Fusarium* spp. (Table 2).

Rootstocks and origin of material (i.e. nurseries) also affected the incidence of *Fusarium* spp. obtained from grapevine crown tissue. A significant rootstock x nursery interaction ($P = 0.0387$) was observed for the incidence of *Fusarium* spp. obtained from crown tissue of grapevines (Table 3). Crown tissue of the rootstock 101-14 Mgt at nursery 1 yielded a significantly ($P = 0.05$) higher incidence of *Fusarium* spp. than 99 Richter. However, the incidence of *Fusarium* spp. in crown tissue of these rootstocks at the other nurseries were not statistically significant (Table 3).

Surface disinfestation did not significantly ($P = 0.05$) affect the incidence of *Fusarium* spp. obtained from crown tissue (Table 2).

Nurseries differed with regard to the incidence of *Fusarium* spp. obtained from crown tissues during the different surveys and a significant survey x nursery interaction ($P = 0.0030$) was observed (Table 5). Grapevines collected at Nursery 1 yielded a significantly higher incidence of *Fusarium* spp. during the first survey than the second and third surveys. There were no significant differences in the incidences of *Fusarium* spp. in crown tissue of grapevines collected at the other two nurseries during all three seasons (Table 5).

Roots. Results from the incidence of different *Fusarium* spp. obtained from root tissue of nursery grapevines were subjected to analyses of variance (Table 6). A significant interaction ($P = 0.0002$) was observed between the incidence of *Fusarium* species obtained from root tissue of different grapevine rootstocks subjected to surface disinfestation and non-surface disinfestation (Table 7). Roots of 99 Richter yielded a significantly ($P = 0.05$) higher level of *F. oxysporum* than 101-14 Mgt when not surface disinfested, whereas 101-14 Mgt roots yielded significantly more *F. oxysporum* than 99 Richter when surface disinfested (Table 7). Roots of 99 Richter yielded significantly more *F. solani* whereas 101-14 Mgt roots did not yield significantly more *F. solani* when not surface disinfested (Table 7).

Data on the effect of nurseries and different surveys on the incidence of *Fusarium* spp. on grapevine roots are given in Table 8. A significant (P = 0.0005) survey x nursery interaction was recorded for the incidence (%) of *Fusarium* species obtained from root tissue of grapevines. The highest incidence of *F. oxysporum* was recorded on grapevine roots collected at nursery 2 during the first survey (Table 8). *Fusarium proliferatum* was isolated more frequently during the second and third surveys and *F. solani* more frequently during the first survey (Table 8).

Pathogenicity of *F. oxysporum* and *F. solani*.

Below-ground parameters. Significant rootstock x species ($P = 0.0144$) and rootstock x isolate ($P = 0.0473$) interactions occurred for the root rot parameter, after inoculation of plants with *F. oxysporum* and *F. solani* (Table 9). Significantly ($P = 0.05$) higher root rot indices were recorded for plants from the two rootstocks inoculated with *F. oxysporum* and *F. solani* compared to their controls. Root rot indices recorded for 99 Richter plants inoculated with the *F. oxysporum* isolates and isolate STE-U 5729 of *F. solani* were significantly higher than for 101-14 Mgt (Table 9).

There were also significant differences in the root rot indices recorded after inoculation of plants with different inoculum levels of *F. oxysporum* and *F. solani* (Table 10). Significantly more root rot was recorded at the 0.5% and 1% inoculum levels than the 0% (control) level, but the root rot indices at the 0.5% and 1% inoculum levels did not differ significantly (Table 10). Root volumes of plants inoculated with *F. oxysporum* and *F. solani* at the 1% inoculum level were significantly lower than that at the 0% inoculum level, but did not differ significantly from the root volumes of plants inoculated at the 0.5% inoculum level (Table 10).

Root volumes of plants inoculated with *F. oxysporum* (STE-U 5725) and *F. solani* (STE-U 5728 and STE-U 5730) were significantly lower than those of the control plants (Table 11). Isolates did not differ significantly with regard to their effect on root volumes. Root volumes for 101-14 Mgt plants inoculated with *F. oxysporum* and *F. solani* were also significantly higher than 99 Richter (Table 12).

Root dry mass followed a similar pattern, except for *F. solani* (STE-U 5729), which also caused a significant reduction in root dry mass compared to the controls (Table 11). Root dry mass for 101-14 Mgt plants inoculated with *F. oxysporum* and *F. solani* was also significantly higher than those of 99 Richter inoculated with these fungi (Table 12). Root dry masses of plants

inoculated with *F. oxysporum* and *F. solani* at the 10% inoculum level were significantly lower than that at the 0% inoculum level, but did not differ significantly from the root dry masses of roots of plants inoculated at the 0.5% inoculum level (Table 10).

Above-ground parameters. Both *F. oxysporum* and *F. solani* caused a significant (P = 0.05) reduction in new shoot growth of inoculated plants (Table 11). All *F. oxysporum* and *F. solani* isolates except for *F. solani* (STE-U 5729) caused a significant reduction in new shoot growth (Table 11). 99 Richter also had significantly less new shoot growth compared to 101-14 Mgt (Table 12). New shoot growth of plants inoculated with a 1% inoculum level of *F. oxysporum* and *F. solani* was significantly lower than the controls (0% inoculum level) but did not differ significantly from those inoculated at the 0.5% inoculum level (Table 10).

Both *F. oxysporum* and *F. solani* caused a significant reduction in mass of new shoot growth of inoculated plants (Table 11). All *F. oxysporum* and *F. solani* isolates except for *F. solani* (STE-U 5729) caused a significant reduction in mass of new shoot growth (Table 11). 99 Richter also had a significantly lower mass of new shoot growth compared to 101-14 Mgt (Table 12). Shoot mass of plants inoculated at the 1% inoculum level also differed significantly from the controls and plants inoculated at the 0.5% level (Table 10).

The number of leaves on plants inoculated with *F. oxysporum* and *F. solani* was significantly less than the number of leaves recorded on control plants, except for plants inoculated with *F. solani* (STE-U 5729) (Table 11). The number of leaves of plants inoculated at the 1% inoculum level of *F. oxysporum* and *F. solani* was significantly lower than that of the controls but did not differ significantly from the number of leaves on plants inoculated at the 0.5% inoculum level (Table 10).

No significant differences were observed in the percentage of chlorotic leaves on plants inoculated with *F. oxysporum* and *F. solani* compared to the controls (Table 11). The number of leaves and percentage chlorotic leaves were significantly higher for 101-14 Mgt than for 99 Richter plants inoculated with these fungi (Table 12). After inoculation with *F. oxysporum* and *F. solani*, the total number of leaves decreased significantly at the 1% inoculum level compared to the 0% level, whereas the percentage chlorotic leaves at the different levels did not differ significantly (Table 10).

Pathogenicity of *F. proliferatum*.

Below-ground parameters. Plants inoculated with *F. proliferatum* had significantly higher root rot indices compared with the controls (Table 13). Isolates of *F. proliferatum* did not differ from each other with regard to root rot indices (Table 13). 99 Richter plants had significantly more root rot than 101-14 Mgt after inoculation with *F. proliferatum* (Table 14). Significantly more root rot was recorded at the 0.5 and 1 % inoculum levels than the 0% (control) level, but the root rot indices at the 0.5 and 1% inoculum levels did not differ significantly (Table 15).

A significant rootstock x species interaction ($P = 0.0268$) was recorded for root volumes of plants inoculated with *F. proliferatum*. Root volumes of 99 Richter and 101-14 Mgt plants inoculated with *F. proliferatum* did not differ significantly from those of the control plants (Table 16). A significant rootstock x inoculum level interaction ($P = 0.0227$) was recorded for root volumes of plants inoculated with *F. proliferatum* (Table 17). Root volumes of 99 Richter and 101-14 Mgt plants did not differ significantly at the 0.5 and 1% inoculum levels (Table 17).

Significant rootstock x species ($P = 0.0031$) and rootstock x inoculum level ($P = 0.0074$) interactions were recorded for root dry mass of plants inoculated with *F. proliferatum* (Table 16 and 17). Dry masses of roots of 101-14 Mgt plants inoculated with *F. proliferatum* were significantly lower than that of the control plants, but dry masses of roots of 99 Richter plants inoculated with *F. proliferatum* were not significantly lower than the controls (Table 16). Root dry masses of both 99 Richter and 101-14 Mgt plants did not differ significantly at the 0, 0.5 and 1% inoculum levels (Table 17), except for 101-14 Mgt plants, which had significantly lower dry root masses when inoculated at the 1% inoculum level than at the 0 and 0.5% levels (Table 16).

Above-ground parameters. Data on new shoot growth after inoculation with *F. proliferatum* are given in Tables 16 and 17. Significant rootstocks x species ($P = 0.0263$) and rootstock x isolate ($P = 0.0398$) interactions occurred for new shoot growth of plants inoculated with *F. proliferatum* (Table 16). All *F. proliferatum* isolates caused a significant reduction in growth of 101-14 Mgt, but not of 99 Richter (Table 16).

A significant ($P = 0.0038$) rootstock x inoculum level interaction occurred for new shoot growth of plants inoculated with *F. proliferatum* (Table 17). Higher levels of *F. proliferatum* inoculum decreased new shoot growth of 101-14 Mgt, but not of 99 Richter (Table 17).

A significant (P = 0.0268) rootstock x inoculum level interaction was recorded for mass of new shoot growth of plants inoculated with *F. proliferatum* (Table 17). Higher levels of *F. proliferatum* inoculum significantly decreased mass of new shoot growth of 101-14 Mgt, but not of 99 Richter (Table 17).

Number of leaves on plants inoculated with the *F. proliferatum* isolate STE-U 5716 was significantly lower than those for plants inoculated with the other *F. proliferatum* isolates and control plants (Table 13). Percentage of chlorotic leaves of plants inoculated with *F. proliferatum* and control plants did not differ significantly (Table 13). The number of leaves at different inoculum levels did not differ for plants inoculated with *F. proliferatum* (Table 15). A significant (P = 0.0594) rootstock x inoculum level interaction was recorded for the percentage chlorotic leaves on plants inoculated with *F. proliferatum* (Table 17). An increase in inoculum levels of *F. proliferatum* lead to a higher percentage of chlorotic leaves on Richter 99, but not on 101-14 Mgt. (Table 17).

Comparison between *F. oxysporum*, *F. proliferatum* and *F. solani*.

Below-ground parameters. There were significant rootstock x species interactions for root rot severity (P = 0.0086), root volume (P = 0.0031) and dry mass of roots (P = 0.0006) when the three *Fusarium* spp. were compared (Table 18). Inoculation of 101-14 Mgt with *F. solani* resulted in significantly more root rot than inoculation of this cultivar with *F. oxysporum* and *F. proliferatum*, while there were no differences in root rot severity after inoculation of 99 Richter with these *Fusarium* spp. (Table 20). Root volume of 99 Richter, inoculated with *F. oxysporum*, was significantly lower than after inoculation with *F. proliferatum* and *F. solani* (Table 18). There were no significant differences in root volumes after inoculation of 101-14 Mgt with the three *Fusarium* spp. (Table 18). Inoculation of 99 Richter with *F. proliferatum* resulted in a significantly higher dry root mass than *F. oxysporum* and *F. solani* (Table 18). There were no differences with regard to root dry mass after inoculation of 101-14 Mgt with the three *Fusarium* spp. (Table 18).

There were no significant differences in root rot severity at the 0.5 and 1% inoculum levels when comparing *F. oxysporum*, *F. proliferatum* and *F. solani*, but inoculum levels of 1% caused a significant decrease in root volume and root dry mass when compared with the 0.5% inoculum level (Table 19).

Above-ground parameters. The three *Fusarium* species did not differ significantly with regard to new shoot growth, mass of new shoot growth, number of leaves, and percentage chlorotic leaves on inoculated plants (Table 19). The 1% inoculum level caused a significant reduction in new shoot growth, mass of new shoot growth, and number of leaves after inoculation with the three *Fusarium* spp. (Table 19).

The 1% inoculum level caused a significant reduction in the number of leaves after the inoculation with the three *Fusarium* spp. There were no differences between 99 Richter and 101-14 Mgt with regard to new shoot length, new growth mass and stem diameter, while 101-14 Mgt had significantly more leaves than 99 Richter (Table 20). There was, however, a significant (P = 0.0390) rootstock x inoculum level interaction for percentage chlorotic leaves after inoculation with the three *Fusarium* spp. (Table 21). Significantly more chlorotic leaves were recorded on 99 Richter at the 1% inoculum level compared to the 0.5%, but there was no significant difference in the percentage chlorotic leaves on 101-14 Mgt at the 0.5 and 1% inoculum levels (Table 21).

Comparison between pathogenicity parameters. Correlations between the different parameters used for pathogenicity were determined. Root rot rating, which is most often used as a parameter in pathogenicity tests with root rot pathogens, was significantly ($P < 0.0001$) negatively correlated with root volume (r = - 0.5982), root dry mass (r = - 0.4472), length of new shoot growth (r = - 0.6602), dry mass of new growth (r = - 0.4942), and number of leaves (r = - 0.5510). There was no significant correlation between root rot rating and number of chlorotic leaves.

Re-isolation of Fusarium *spp.* The respective *Fusarium* spp. were re-isolated from inoculated plant material, but not from the control plants.

DISCUSSION

Ten *Fusarium* spp. were isolated from crowns and roots of nursery grapevines. It is the first report of *F. acuminatum*, *F. anthophilum*, *F. chlamydosporum*, *F. equiseti*, *F. nygamai*, *F. proliferatum*, *F. scirpi*, and *F. semitectum* from grapevines in South Africa. *Fusarium oxysporum* and *F. solani* were the predominant *Fusarium* species associated with crowns and roots of diseased grapevines in nurseries in the Western Cape province, concurring with findings

of other researchers in Australia (Highet & Nair, 1995) and the USA (Gugino *et al.*, 1999). *Fusarium proliferatum* was also frequently isolated from crowns of diseased grapevines, but was less frequently obtained from roots. The role of the different *Fusarium* spp. associated with grapevines in vineyards of various ages in South Africa needs to be assessed.

The results obtained from the identification of the *Fusarium* spp. suggest that the number of *Fusarium* spp. associated with declining grapevines have increased over the last 20-30 years. Marais (1979) indicated three different groups of *Fusarium* that were infrequently obtained from declining grapevines in the Western Cape province. These groups were, however, not identified to species level. One possible explanation for this increase may be the use of rotation crops such as legumes in grapevine nurseries (J. Wiese, pers. comm.). These crops may introduce *Fusarium* spp. to grapevine nursery soil. This aspect needs to be further investigated.

In the pathogenicity trial, all three *Fusarium* species, *viz. F. oxysporum*, *F. proliferatum*, and *F. solani* caused root rot, and reduced growth of the rootstock cultivars 99 Richter and 101-14 Mgt. Although the three species do not differ significantly with each other, it appears as if *F. solani* may be slightly more virulent than *F. oxysporum*, and *F. proliferatum* although still pathogenic appears to be the least virulent of the three species tested. *Fusarium oxysporum* and *F. solani* have been reported as pathogens of grapevines in Australia (Highet & Nair, 1995), Brazil (Andrade, 1993), India (Lele *et al.*, 1978), and the USA (California and Pennsylvania) (Omer *et al.*, 1999; Gugino *et al.*, 2001). Ferreira *et al.* (1989) also reported *F. oxysporum* to be associated with declining grapevines in South Africa. However, this is the first report on the pathogenicity of *F. oxysporum* and *F. solani* as pathogens of grapevines in South Africa. It is also the first report of *F. proliferatum* as a pathogen of grapevines in the world.

It is well known that *F. proliferatum* produces fumonisins (Rheeder, Marasas & Vismer, 2002). This fungus has been evaluated for biocontrol of downy mildew on grapevines (Falk *et al.*, 1996). Falk *et al.* (1996) reported that the isolate used for biocontrol is not a fumonisin producer. The fact that the *F. proliferatum* isolates evaluated in our study are pathogenic has important implications for the use of *F. proliferatum* for biocontrol. Significant differences in the effect of different *F. proliferatum* isolates on grapevine roots were, however, observed. Whether these differences in pathogenicity are linked to fumonisin production is uncertain.

Root rot rating seems to be a reliable parameter to measure pathogenicity of *Fusarium* spp. on grapevines. All the above and below-ground parameters used were significantly

negatively correlated with root rot rating. The number of parameters for pathogenicity can therefore be reduced considerably.

It is still unclear whether the two rootstocks used in this study differ with regard to susceptibility to the *Fusarium* spp. tested. In earlier studies it was reported that 99 Richter was more susceptible to the soilborne pathogen, *P. cinnamomi* (Von Broembsen & Marais, 1978). The reason for this higher susceptibility of 99 Richter to soilborne pathogens is still unclear and needs to be determined. In the case of infection of the rootstock 99 Richter by *P. cinnamomi*, Marais and Hattingh (1985) showed that the higher levels of arginine, aspartic acid and glutamic acid in the root exudates of 99 Richter resulted in a higher chemotactic index for zoospores of *P. cinnamomi*. This may therefore be partially responsible for the higher susceptibility of 99 Richter to *P. cinnamomi*. Omer *et al.* (1999) determined the pathogenicity of *F. oxysporum* to various rootstocks. The rootstock Carignane was most susceptible, followed by the *V. vinifera*-hybrid rootstock AXR#1, and lastly the non-*V. vinifera* rootstocks 110R and St. George. They also mentioned that the cultivars that are highly susceptible to phylloxera appear to be susceptible to *F. oxysporum*. The use of resistant rootstocks will be a useful means of disease management therefore screening available rootstocks might yield valuable information for disease management.

LITERATURE

Agnihotrudu, V. 1968. A root rot of grapes in Andhra Pradesh. *Current Science* 37: 292–294.

Andrade, E.R. de. 1993. Ocorrência de espécies do gênero *Fusarium* em unaccompanied cultivado com videira (*Vitis* spp.) em Santa Catarina. [Occurrence of *Fusarium* species in grapevine (*Vitis* spp.) in Santa Catarina.] *Fitopatologia Brasileira* 18: 502–505 (*Abstract in Review of Plant Pathology* 74: 657).

Bakshi, S., Sztejnberg, A. & Yarden, O. 2001. Isolation and characterisation of *Fusarium proliferatum*, a biocontrol agent of grape downy mildew. *Phytopathology* 91: 1062–1068.

Benzoni, C. 1954. Appunti su diversi mutamenti dei Vigneti del Cantone Ticino meridionale e la comparsa di alcune specie di Pionnotes (Fries). [Notes on various changes in the vineyards in the south of the canton of Ticino and the appearance of some species of *Pionnotes* (Fries).] *Schweizerische Zeitschrift für Pilzkunde* 32: 155–158 (*Abstract in Review of Plant Pathology* 33: 275).

Branas, M. & Bernon, G. 1933. Note sur deux cas de dépérissement et leur relation avec la presence de certains champignons dans le vieux bois de la Vigne. [A note on two cases of wilt and their relation to the presence of certain fungi in old wood of the Vine.] *Annales de l'Ecole Nationale d'Agriculture de Montpellier* 22: 331–334 (*Abstract in Review of Plant Pathology* 13: 353).

Bumbieris, M. 1972. Observations on some pythiaceous fungi associated with grapevine decline in South Australia. *Australian Journal for Agricultural Research* 23: 651–657.

Crous, P.W., Swart, L. & Coertze, S. 2001. The effect of hot water treatment on fungi occurring in apparently healthy grapevine cuttings. *Phytopathologia Mediterranea* 40, Supplement: S464–S466.

Du Plessis, S.J. 1936. Studies on the wastage of export grapes with special reference to that caused by *Botrytis cinerea*, Pers. *Scientific Bulletin of the Department of Agriculture of South Africa* 151, 156 pp. (*Abstract in Review of Plant Pathology* 15, 701).

Falk, S.P., Pearson, R.C., Gadoury, D.M., Seem, R.C. & Sztejnberg, A. 1996. *Fusarium proliferatum* as a biocontrol agent against grape downy mildew. *Phytopathology* 86: 1010–1017.

Ferreira, J.H.S., Matthee, F.N. & Thomas, A.C. 1989. Fungi associated with dieback and pruning wounds of grapevines in South Africa. *South African Journal of Enology and Viticulture* 10: 62–66.

Fisher, N.L., Burgess, L.W., Toussoun, T.A. & Nelson, P.E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

Foëx, E. & Ayoutantis, A. 1924. Sur une production rouge qui revêt parfois la Vigne au printemps. [A red substance which sometimes covers the Vine in spring.] *Comptes Rendus de l'Academie d'Agriculture de France* 10: 1071–1074 (*Abstract in Review of Plant Pathology* 4: 255).

Foëx, E. & Ayoutantis, A. 1926. D'une production rouge à consistence gélatineuse que revêt parfois la Vigne au printemps. [On a red gelatinous substance which sometimes covers the Vine in spring]. *Bulletin Société Mycologique de France* 40: 318–331 (*Abstract in Review of Plant Pathology* 5: 593).

Granett, J., Omer, A.D., Pessereau, P. & Walker, M.A. 1998. Fungal infections of grapevine roots in phylloxera-infested vineyards. *Vitis* 37: 39–42.

Grasso, S. 1984. Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. [Infections of *Fusarium oxysporum* and *Cylindrocarpon destructans* associated with a decline of young grapevine plants in Sicily.] *Informatore Fitopatologico* 34: 59–63.

Gugino, B.K., Travis, J.W. & Stewart, E.L. 2001. Pathogenicity of *Fusarium* spp., *Cylindrocarpon* sp. and a *Diplodina* sp. on grape roots. *Phytopathology* 91, Supplement: S34.

Halleen, F., Crous, P.W. & Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australasian Plant Pathology* 32: 47–52.

Hawksworth, D.L. 2003. The name *Fusarium moniliforme* should no longer be used. *Mycological Research* 107: 643–644.

Highet, A.S. & Nair, N.G. 1995. *Fusarium oxysporum* associated with grapevine decline in the Hunter Valley, NSW, Australia. *Australian Journal of Grape and Wine Research* 1: 48–50.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8: 114–125.

Lele, V.C., Durgapal, J.C., Agarwal, D.K. & Sethi, C.L. 1978. Crown and root gall of grape (*Vitis vinifera* L.) in Andhra Pradesh. *Current Science* 47: 280 (*Abstract in Review of Plant Pathology* 58: 28).

Mahrous, H.A.H. 2001. Reaction of different grapevine cultivars to infection with root-rot fungi and its control. *Phytopathologia Mediterranea* 40, Supplement: S484.

Marais, P.G. 1979. Fungi associated with root rot in vineyards in the western cape. *Phytophylactica* 11: 65–68.

Marais, P.G. 1988. Grapevine roots and soilborne fungi. In *The Grapevine Root and its Environment*. (eds. J.A. van Zyl), pp. 106–137. South Africa Department of Agriculture and Water Supply Technical Bulletin 215.

Mil'ko, A.A. 1959. On the specific composition of the fungi causing rot of Vine roots damaged by phylloxera.] *Izvestiya Moldavskogo Filiala Akademii Nauk SSR.* 8: 55–66 (*Abstract in Review of Plant Pathology* 39: 264).

Morquer, R. 1940. Sur quelques *Fusarium* dans l'écoulement muqueux de la Vigne au printemps. [On some *Fusarium* species found in the mucous exudate of the Vine in spring.] *Bulletin de Societe d'Histoire Naturelle de Toulouse* 75: 193–208 (*Abstract in Review of Plant Pathology* 25: 43).

Mujica, F. 1944. El género *Fusarium* en Chile. Aporté preliminary a su conocimiento. [The genus *Fusarium* in Chile. Preliminary contribution to its recognition.] *Agricultura Tecnica (Chile)* 4: 224–229 (*Abstract in Review of Plant Pathology* 23: 341).

Nelson, P.E., Toussoun, T.A. & Marasas, W.F.O. 1983. Fusarium *species. An Illustrated Manual for Identification*. 193 pp. The Pennsylvania University Press: Pennsylvania, USA.

Omer, A.D., Granett, J., De Benedictus, J.A. & Walker, M.A. 1995. Effects of fungal root infections on the vigor of grapevines infested by root-feeding grape phylloxera. *Vitis* 34: 165–170.

Omer, A.D., Granett, J. & Wakeman, R.J. 1999. Pathogenicity of *Fusarium oxysporum* on different *Vitis* rootstocks. *Journal of Phytopathology* 47: 433–436.

Osterwalder, A. 1943. Von teilweisen Lahmstieler-Trauben. [On grapes with partial pedicel lameness.] *Schweizerische Zeitschrift fuer Obst-und Weinbau* 52: 635-638 (*Abstract in Review of Plant Pathology* 22: 205–206).

Ott, R.L. 1998. *An introduction to statistical methods and data analyses*. Belmont, California, Duxbury Press. 105 pp.

Petri, L. 1932. Rassegna dei casi fitopatologici osservati nel 1932. [Review of phytopathological records observed in 1932.] *Bolletino della stazione di patologia vegetale di Roma* 13: 1–73. (*Abstract in Review of Plant Pathology* 11: 745).

Rheeder, J.P., Marasas, W.F.O. & Vismer, H.F. 2002. Production of fumonisin analogs by *Fusarium* species. *Applied and Environmental Microbiology* 68: 2101–2105.

Rives, L. 1927. Sur la Fusariose (?) du Seibel 4986. [On fusariosis (?) of Seibel 4986.] *Progrés Agricola et Viticola* 88: 8–9. (*Abstract in Review of Plant Pathology* 6: 74).

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591–611.

Shterenberg, P.N. 1959. [Epiphytic fungus flora of Vine.] *Agrobiology, Moscow* 1: 49–53. (*Review of Plant Pathology* 37: 347).

Sinha, M.K., Jawanda, J.S. & Chohan, J.S. 1969. Occurrence of foot-rot in grapes and its control measures. *Punjab Horticultural Journal* 9: 127–129. (*Review of Plant Pathology* 51: abstract 1725).

Snedecor, G.W. & Cochran, W.G. 1989. *Statistical methods*. Eighth edn. Iowa State University Press: Iowa, USA. 503 pp.

Solel, Z. & Pinkas, Y. 1982. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. *Phytopathology* 74: 506–508.

Sprague, R. 1953. Field rots of grapes in north-central Washington. *Northwest Science* 27: 1–16. (*Abstract in Review of Plant Pathology* 32: 537).

Strauss, J. & Labuschagne, N. 1995. Pathogenicity of *Fusarium solani* isolates on citrus roots and evaluation of different inoculum types. *Toegepaste Plantwetenskap* 9: 48–52.

Tio, M., Burgess, L.W., Nelson, P.E. & Toussoun, T.A. 1977. Techniques for the isolation, culture and preservation of the fusaria. *Australian Plant Pathological Society Newsletter* 6: 11–13.

Von Broembsen, S. & Marais, P.G. 1978. Eradication of *Phytophthora cinnamomi* by hot water treatment. *Phytophylactica* 10: 25–27.

Table 1. Analysis of variance for the incidence of different *Fusarium* spp. isolated from crown tissue of nursery grapevines

Source of variation	DF	SS	MS	Pr > F
Model	279	21798.9227	78.1323	< 0.0001
Nursery (N)	2	130.0908	65.0454	0.3005
Survey (S)	2	33.1769	16.5884	0.7358
N x S	3	748.2119	249.4040	0.0032
Rootstock (R)	1	16.8338	16.8338	0.5769
N x R	2	517.4170	258.7085	0.0084
R x S	2	10.2461	5.1231	0.9096
N x R x S	1	37.5156	37.5156	0.4049
Treatment (T)	1	3.1569	3.1569	0.8091
N x T	2	72.8722	36.4361	0.5098
S x T	2	29.6658	14.8329	0.7601
N x S x T	3	83.3176	27.7725	0.6729
R x T	1	80.5445	80.5445	0.2224
N x R x T	2	183.6535	91.8268	0.1832
R x S x T	2	70.2044	35.1022	0.5226
N x R x S x T	1	0.7656	0.7656	0.9053
Species (Sp)	9	11235.4505	1248.3834	< 0.0001
N x Sp	18	408.3911	22.6884	0.9844
S x Sp	18	875.9645	48.6647	0.5786
N x S x Sp	27	1255.7620	46.5097	0.6725
R x Sp	9	149.8075	16.6453	0.9726
N x R x Sp	18	923.7326	51.3185	0.5176
R x S x Sp	18	102.7096	5.7061	1.000
N x R x S x Sp	9	425.1406	47.2379	0.5482
T x Sp	9	107.5225	11.9470	0.9916
N x Sp x T	18	494.3417	27.4634	0.9561
S x Sp x T	18	632.8511	35.1584	0.8617
N x S x Sp x T	27	852.7861	31.5847	0.9567
R x Sp x T	9	455.4097	50.6011	0.4929
N x R x Sp x T	18	1329.7074	73.8726	0.1377
R x S x Sp x T	18	459.1576	25.5088	0.9702
N x R x S x Sp x T	9	72.5156	8.0573	0.9982
Error	2680	144910.8333	54.0712	
Corrected total	2959	166709.7560		

Table 2. Mean incidence of different *Fusarium* spp. isolated from crown tissue of nursery grapevines

***Fusarium* species**	**Incidence [xz]**
F. acuminatum	0.41 c
F. anthophilum	0.00 c
F. chlamydosporum	0.29 c
F. equiseti	0.24 c
F. nygamai	0.08 c
F. oxysporum	6.55 a
F. proliferatum	1.99 b
F. scirpi	0.34 c
F. semitectum	0.00 c
F. solani	2.39 b

[x] Means followed by the same letter do not differ significantly ($P = 0.05$).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 3. Effect of rootstocks and nursery on the incidence of *Fusarium* spp. on crown tissue of grapevines

Rootstock	**Incidence (%) [xy]**		
	Nursery 1	**Nursery 2**	**Nursery 3**
99 Richter	0.85 b	1.16 b	1.16 b
101-14 Mgt	3.33 a	1.18 b	0.92 b

[x] Means followed by the same letter do not differ significantly ($P = 0.05$).

[y] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 4. Mean incidence of *Fusarium* spp. isolated from crown tissue of nursery grapevines subjected to different treatments

Treatment [x]	Incidence [yz]
D	1.20 a
ND	1.26 a

[x] D = plant material disinfected; ND = plant material not disinfected.

[y] Means followed by the same letter do not differ significantly ($P = 0.05$).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 5. Effect of surveys and nurseries on the incidence of *Fusarium* spp. on crowns of grapevines in nurseries

Survey	Incidence (%) [xy]		
	Nursery 1	Nursery 2	Nursery 3
1 (Feb-April 2001)	2.50 a	- [z]	0.54 b
2 (Nov-Dec 2001)	0.83 b	1.10 b	1.30 b
3 (March-April 2002)	0.90 b	1.25 b	1.44 ab

[x] Means followed by the same letter do not differ significantly ($P = 0.05$).

[y] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

[z] *Fusarium* spp. not isolated.

Table 6. Analysis of variance for the incidence of different *Fusarium* spp. isolated from root tissue of nursery grapevines

Source of variation	DF	SS	MS	Pr > F
Model	319	66470.6830	208.3720	< 0.0001
Nursery (N)	2	1419.9173	709.9587	< 0.0001
Survey (S)	2	374.2281	187.1140	0.0302
N x S	4	1131.9774	282.9943	0.0003
Rootstock (R)	1	57.2617	57.2617	0.3005
N x R	2	47.9985	23.9992	0.6381
R x S	2	55.8755	27.9377	0.5927
N x R x S	2	68.0955	34.0478	0.5287
Treatment (T)	1	709.1474	709.1474	0.0003
N x T	2	53.7574	26.8787	0.6046
S x T	2	13.5761	6.7881	0.8806
N x S x T	4	365.1882	91.2971	0.1450
R x T	1	414.7502	414.7502	0.0054
N x R x T	2	138.6870	39.3435	0.2731
R x S x T	2	18.3674	9.1837	0.8420
N x R x S x T	2	66.7280	33.3640	0.5355
Species (Sp)	9	39841.9438	4426.8827	< 0.0001
N x Sp	18	2747.4644	152.6369	< 0.0001
S x Sp	18	2140.5289	118.9183	0.0021
N x S x Sp	36	3876.9376	107.6927	0.0003
R x Sp	9	362.4772	40.2752	0.6593
N x R x Sp	18	810.0228	45.0013	0.6503
R x S x Sp	18	160.1899	8.8994	1.000
N x R x S x Sp	18	851.3162	47.2953	0.5967
T x Sp	9	3080.2970	342.2552	< 0.0001
N x Sp x T	18	863.7533	47.9863	0.5805
S x Sp x T	18	1168.6762	64.9265	0.2382
N x S x Sp x T	36	2486.5878	69.0719	0.1136
R x Sp x T	9	1832.4649	203.6072	< 0.0001
N x R x Sp x T	18	503.3385	27.9633	0.9488
R x S x Sp x T	18	485.3506	26.9639	0.9574
N x R x S x Sp x T	18	323.77816	17.9877	0.9959
Error	3060	163425.3935	53.4070	
Corrected total	3379	229896.0765		

Table 7. Effect of rootstock and surface disinfestation of plant material on the incidence of *Fusarium* spp. on roots of grapevines in nurseries

Rootstock	Treatment [w]	Incidence (%) [xyz]									
		F. acu	***F. ant***	***F. chl***	***F. equ***	***F. nyg***	***F. oxy***	***F. pro***	***F. sci***	***F. sem***	***F. sol***
99 Richter	D	0.00 h	0.00 h	0.43 h	1.51 gh	0.13 h	7.61 cd	0.89 gh	0.51 h	0.00 h	2.96 fg
	ND	0.00 h	0.00 h	0.54 h	1.73 f-h	0.00 h	16.27 a	0.79 gh	1.08 gh	0.10 h	9.62 bc
101-14 Mgt	D	0.74 h	0.00 h	0.51 h	2.02 f-h	0.00 h	11.15 b	1.58 e-h	0.74 gh	0.00 h	3.84 ef
	ND	0.00 h	0.68 h	0.14 h	0.66 h	0.34 h	9.26 bc	1.15 gh	0.84 gh	0.00 h	6.08 de

[w] D = plant material surface disinfested; ND = plant material not surface disinfested.

[x] *Fusarium* spp: *F. acu* = *F. acuminatum*, *F. ant* = *F. anthophilum*, *F. chl* = *F. chlamydosporum*, *F. equ* = *F. equiseti*, *F. nyg* = *F. nygamai*, *F. oxy* = *F. oxysporum*, *F. pro* = *F. proliferatum*, *F. sci* = *F. scirpi*, *F. sem* = *F. semitectum*, *F. sol* = *F. solani*.

[y] Means followed by the same letter do not differ significantly ($P = 0.05$).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 8. Effect of survey and nursery on the incidence of *Fusarium* spp. on roots of grapevines in nurseries

Survey	Nursery	Incidence (%) [xyz]									
		F. acu	***F. ant***	***F. chl***	***F. equ***	***F. nyg***	***F. oxy***	***F. pro***	***F. sci***	***F. sem***	***F. sol***
1 (Feb-Apr 2001)	1	1.67 h-k	0.00 k	0.00 k	0.00 k	0.00 k	5.00 e-h	0.00 k	0.00 k	0.00 k	1.67 h-k
	2	0.00 k	1.04 i-k	0.00 k	4.17 f-i	0.00 k	19.79 a	1.04 i-k	4.17 f-i	0.00 k	10.42 cd
	3	0.00 k	0.00 k	0.00 k	0.22 k	0.00 k	7.87 de	0.00 k	0.67 jk	0.00 k	9.67 d
2 (Nov-Dec 2001)	1	0.00 k	0.00 k	0.00 k	1.25 i-k	0.00 k	9.17 d	0.00 k	0.00 k	0.00 k	3.75 f-j
	2	0.00 k	0.00 k	1.88 g-k	1.50 i-k	0.00 k	9.69 d	0.56 jk	0.56 jk	0.25 k	5.63 ef
	3	0.00 k	0.00 k	0.50 jk	0.00 k	0.00 k	8.00 de	2.00 g-k	0.00 k	0.00 k	3.75 f-j
3 (Mar-Apr 2002)	1	0.00 k	0.00 k	1.00 i-k	3.50 f-k	0.00 k	16.00 b	1.50 i-k	0.00 k	0.00 k	5.50 ef
	2	0.00 k	0.00 k	0.31 jk	2.81 f-k	0.00 k	13.75 bc	3.13 f-k	0.00 k	0.00 k	5.31 e-g
	3	0.00 k	0.00 k	0.31 jk	0.31 jk	0.94 i-k	10.94 cd	1.25 i-k	0.31 jk	0.00 k	2.50 f-k

[x] *Fusarium* spp: *F. acu* = *F. acuminatum*, *F. ant* = *F. anthophilum*, *F. chl* = *F. chlamydosporum*, *F. equ* = *F. equiseti*, *F. nyg* = *F. nygamai*, *F. oxy* = *F. oxysporum*, *F. pro* = *F. proliferatum*, *F. sci* = *F. scirpi*, *F. sem* = *F. semitectum*, *F. sol* = *F. solani*.

[y] Means followed by the same letter do not differ significantly ($P = 0.05$).

[z] Mean value calculated as follows: Number of tissue pieces yielding fungus / total number of tissue pieces x 100.

Table 9. Effect of *F. oxysporum* and *F. solani* on root rot of different grapevine rootstocks after inoculation of plants in a glasshouse

Rootstock	Species	Isolate no.	Root rot index [xy]
99 Richter	*F. oxysporum*	STE-U 5725	2.50 a
		STE-U5726	2.25 ab
		STE-U 5727	2.75 a
Mean (*F. oxysporum*)			2.50 a
	F. solani	STE-U 5728	2.30 ab
		STE-U 5729	2.35 a
		STE-U 5730	2.15 ab
Mean (*F. solani*)			2.27 ab
	Control	Control	1.37 c
Mean (control)			1.37 c
101-14 Mgt	*F. oxysporum*	STE-U 5725	1.45 cd
		STE-U5726	1.50 cd
		STE-U 5727	1.00 de
Mean (*F. oxysporum*)			1.32 c
	F. solani	STE-U 5728	2.15 ab
		STE-U 5729	1.75 bc
		STE-U 5730	1.80 bc
Mean (*F. solani*)			1.90 b
	Control	Control	0.73 d
Mean (control)			0.73 d

[x] Isolate means within a column followed by the same letter do not differ significantly; species means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Disease severity rating (0 = no root rot, 1= >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

Table 10. Effect of different inoculum levels of *F. oxysporum* and *F. solani* on grapevines after inoculation of roots in a glasshouse

Inoculum level	Below-ground [x]			Above-ground [x]			
	Root rot index [y]	Root volume (cm^3)	Dry root mass	New shoot length (mm)	New shoot mass (g)	Number of leaves	Chlorotic leaves (%) [z]
0	1.15 b	48.20 a	12.09 a	698.40 a	6.93 a	30.70 a	9.25 a
0.5	1.74 a	41.40 ab	10.10 ab	586.11 ab	5.83 a	25.39 ab	11.56 a
1	1.97 a	32.11 b	7.96 b	420.30 b	3.94 b	18.20 b	9.80 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Disease severity rating (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

[z] Percentage chlorotic leaves / total number of leaves x 100.

Table 11. Effect of different isolates of *F. oxysporum* and *F. solani* on grapevines after inoculation of roots in a glasshouse

Species	Isolate	Below-ground [x]		Above-ground [x]			
		Root volume (cm^3)	Dry root mass (g)	New shoot length (mm)	New growth mass (g)	Number of leaves	Chlorotic leaves (%) [y]
F. oxysporum	STE-U 5725	33.55 b	7.62 b	456.80 b	3.94 b	19.40 b	9.08 a
	STE-U5726	36.45 ab	9.15 ab	458.40 b	4.65 b	18.65 b	9.90 a
	STE-U 5727	40.35 ab	10.16 ab	472.20 b	4.72 b	20.40 b	9.67 a
Mean (*F. oxysporum*)		36.78 b	8.98 b	462.43 b	4.44 b	19.48 b	9.56 a
F. solani	STE-U 5728	33.10 b	8.08 b	389.10 b	3.95 b	19.05 b	11.81 a
	STE-U 5729	37.55 ab	8.32 b	525.40 ab	5.38 ab	23.75 ab	11.20 a
	STE-U 5730	30.40 b	8.24 b	497.00 b	4.60 b	20.90 b	12.94 a
Mean (*F. solani*)		33.68 b	8.21 b	470.48 b	4.64 b	21.23 b	11.94 a
Control	Control	46.67 a	11.79 a	715.30 a	6.95 a	30.50 a	10.27 a
Mean (control)		46.67 a	11.79 a	715.27 a	6.94 a	30.50 a	10.27 a

[x] Isolate means within a column followed by the same letter do not differ significantly; species means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Percentage chlorotic leaves / total number of leaves x 100.

Table 12. Effect of *Fusarium oxysporum* and *F. solani* on grapevine rootstocks after inoculation of roots in a glasshouse

Rootstock	Below-ground [x]		Above-ground [x]			
	Root volume (cm^3)	Dry root mass (g)	New shoot length (mm)	New growth mass (g)	Number of leaves	Chlorotic leaves (%) [y]
99 Richter	29.52 b	7.39 b	393.57 b	3.78 b	18.78 b	8.34 b
101-14 Mgt	45.52 a	11.07 a	638.87 a	6.25 a	26.00 a	12.45 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Percentage chlorotic leaves / total number of leaves x 100.

Table 13. Effect of *F. proliferatum* on grapevines after inoculation of roots in a glasshouse

Species	Isolate no.	Below-ground [x]	Above-ground [x]		
		Root rot index [y]	New growth mass (g)	Number of leaves	Chlorotic leaves (%) [z]
F. proliferatum	STE-U 5715	1.73 a	8.30 a	32.30 a	12.28 a
	STE-U 5716	1.83 a	6.10 b	25.15 b	13.85 a
	STE-U 5719	1.63 a	7.22 ab	31.60 ab	12.18 a
Mean (*F. proliferatum*)		1.73 a	7.21 b	29.68 b	12.69 a
Control	Control	0.78 b	8.89 a	35.87 a	13.39 a
Mean (Control)		0.78 b	8.89 a	35.87 a	13.39 a

[x] Isolate means within a column followed by the same letter do not differ significantly; species means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Disease severity rating (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).Percentage chlorotic leaves / total number of leaves x 100.

[z] Percentage chlorotic leaves / total number of leaves x 100.

Table 14. Effect of *F. proliferatum* on grapevine rootstocks after inoculation of roots in a glasshouse

Rootstock	Below-ground [x]	Above-ground [x]
	Root rot index [y]	Number of leaves
99 Richter	1.79 a	29.00 a
101-14 Mgt	1.19 b	33.42 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Disease severity rating (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

Table 15. Effect of different inoculum levels of *F.proliferatum* on grapevines after inoculation of roots in a glasshouse

Inoculum level (%m/v)	Below-ground [x]	Above-ground [x]
	Root rot index [y]	Number of leaves
0	0.67 b	35.22 a
0.5	1.50 a	32.29 a
1	1.63 a	29.50 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Disease severity rating (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

Table 16. Effect of different isolates of *F. proliferatum* on grapevine rootstocks after inoculation of roots in a glasshouse

Rootstock	Species	Isolate no.	Root volume (cm^3) [x]	Dry root mass (g) [x]	New shoot length (mm) [x]
99 Richter	*F. proliferatum*	STE-U 5715	60.00 ab	15.71 b	902.80 b
		STE-U 5716	37.00 c	9.64 d	444.50 g
		STE-U 5719	47.60 bc	11.93 cd	730.80 d
Mean (*F. proliferatum*)			48.20 bc	12.43 bc	692.70 b
	Control	Control	38.88 c	9.60 d	684.88 f
Mean (control)			38.88 c	9.60 d	684.88 f
101-14 Mgt	*F. proliferatum*	STE-U 5715	64.00 a	15.85 b	838.00 c
		STE-U 5716	56.60 a	13.06 bd	687.50 f
		STE-U 5719	57.30 a	13.66 bc	717.40 e
Mean (*F. proliferatum*)			59.30 ab	14.19 b	747.63 b
	Control	Control	67.80 a	18.02 a	1094.80 a
Mean (control)			67.80 a	18.02 a	1094.80 a

[x] Species means within a column followed by the same letter do not differ significantly ($P = 0.05$); Isolate means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 17. Effect of different inoculum levels of *F. proliferatum* on rootstocks

Rootstock	Inoculum level (%m/v)	Below-ground [x]		Above-ground [x]		
		Root volume (cm^3)	Dry root mass (g)	New shoot length (mm)	New growth mass (g)	Chlorotic leaves (%) [y]
99 Richter	0	45.00 b	11.79 b	662.25 b	7.08 a	8.16 b
	0.5	43.50 b	10.97 b	612.83 b	5.87 a	9.81 ab
	1	49.63 b	12.81 b	786.25 a	7.67 a	16.19 ab
101-14 Mgt	0	66.20 a	18.51 a	1016.60 a	10.35 a	15.46 ab
	0.5	68.85 a	17.28 a	1004 65 a	9.46 a	17.44 a
	1	54.40 ab	12.90 b	683.75 b	6.97 a	8.23 b

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Percentage chlorotic leaves / total number of leaves x 100.

Table 18. Adjusted means for the effect of rootstock and *Fusarium* spp. after inoculation of grapevine roots in a glasshouse

Rootstock	Species	Root rot index [xy]	Root volume (cm^3) [x]	Dry root mass (g) [x]
99 Richter	*F. oxysporum*	1.15 bc	-13.10 c	-2.82 b
	F. proliferatum	0.75 ac	12.92 a	5.27 a
	F. solani	0.92 a-c	-0.33 b	-1.99 b
101-14 Mgt	*F. oxysporum*	0.67 a	-5.13 bc	-2.49 b
	F. proliferatum	0.80 ac	-9.30 bc	-3.59 b
	F. solani	1.25 b	-14.77 c	-4.85 b

[x] Adjusted means within a column followed by the same letter do not differ significantly (P = 0.05).

[y] Adjusted disease severity rating (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

Table 19. Adjusted means of the relative effect of different inoculum levels of *F. oxysporum*, *F. proliferatum*, and *F. solani* on grapevines in a glasshouse with respect to different disease assessment parameters

Inoculum level (%m/v)	Below-ground [x]			Above-ground [x]		
	Root rot index [y]	Root volume (cm^3)	Dry root mass (g)	New shoot growth (mm)	New growth mass (g)	Number of leaves
0.5	1.01 a	-4.13 a	-0.94 a	-162.82 a	-1.24 a	-5.67 a
1	0.88 a	-13.55 b	-4.23 b	-386.74 b	-3.30 b	-12.59 b

[x] Adjusted means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Adjusted disease severity (0 = no root rot, 1 = >0-25%, 2 = >25-50%, 3 = >50-75%, 4 = >75-100%).

Table 20. Adjusted means of the effect of *F. oxysporum*, *F. proliferatum* and *F. solani* on grapevine rootstocks after inoculation of roots in a glasshouse

Rootstock	Above-ground [x]		
	New shoot length (mm)	New growth mass (g)	Number of leaves
99 Richter	-217.68 a	-1.86 a	-11.83 b
101-14 Mgt	-313.00 a	-2.53 a	-6.73 a

[x] Adjusted means within a column followed by the same letter do not differ significantly (P = 0.05).

Table 21. Adjusted means of the relative effect of rootstocks and inoculum levels on the percentage chlorotic leaves after inoculation with *F. oxysporum*, *F. proliferatum*, and *F. solani*

Rootstock	Inoculum level (% m/v)	Chlorotic leaves (%) [xy]
99 Richter	5	0.46 a
	10	7.08 b
101-14 Mgt	5	-1.87 a
	10	-5.93 a

[x] Adjusted means within a column followed by the same letter do not differ significantly (P = 0.05).Percentage chlorotic leaves / total number of leaves x 100.

[y] Percentage chlorotic leaves / total number of leaves x 100.

5. THE EFFECT OF CHEMICALS REGISTERED AGAINST DOWNY MILDEW OF GRAPEVINES ON THE INFECTION OF NURSERY GRAPEVINES BY *PHYTOPHTHORA CINNAMOMI*

ABSTRACT

Phytophthora cinnamomi was previously (1970s-1980s) reported to be the most important soilborne pathogen associated with grapevines in nurseries in the Western Cape province. In a recent survey conducted in three nurseries in this province, *P. cinnamomi* was isolated at low frequencies from susceptible declined grapevines, although the pathogen was present in the rhizosphere soil. A glasshouse trial was conducted to assess the effect of chemicals used to combat downy mildew on Phytophthora root rot of nursery grapevines. Fosetyl-Al, metalaxyl, and phosphorous acid were applied to the plants as foliar sprays every fortnight for 31 weeks. A very low percentage of *P. cinnamomi* isolates was obtained from both treated and untreated inoculated vines, indicating that the inoculation technique was insufficient for this trial. There was very little discernable effect of the chemicals tested relative to the control plants for the parameters measured. Consequently it can be concluded that the experimental technique needs to be further developed to ensure conclusive results. However, although not statistically significant, some trends were evident. Plants treated with phosphorous acid tended to be taller and had more leaves, greater stem diameter and root volume than the controls or plants treated with the other chemicals.

INTRODUCTION

Pythiaceous fungi, in particular species from the genera *Phytophthora* and *Pythium*, have been identified as, among others, the cause of rapid decline and death of grapevines in many countries such as Australia (McGechan, 1966; Bumbieris, 1970, 1972), British Colombia (Utkhede & Vielvoye, 1984), California, USA (Chiarappa, 1959), Chile (Latorre, Wilcox & Banados, 1997) India (Agnihothrudu, 1968), South Africa (Van der Merwe, Joubert & Matthee, 1972; Marais, 1979, 1980), and Spain (Mansilla, Pintos & Salinero, 1993). In South Africa, Marais (1980) indicated that *Phytophthora* spp. were, after *Pythium* spp., the most common fungal pathogens isolated from the roots and rhizosphere soils of dead and dying nursery

grapevines. The *Phytophthora* spp. associated with diseased grapevines were *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cinnamomi* Rands, *P. cryptogea* Pethybr. & Laff., *P. megasperma* Dreschler, and *P. nicotianae* Breda de Haan, with *P. cinnamomi* the predominantly isolated species and the most aggressive root pathogen (Marais, 1980).

Effective control of soilborne pathogens in nurseries, and in particular *P. cinnamomi*, is crucial for the production and cultivation of high quality grapevines (Stephens, Davoren & Wicks, 1999). Marais and Hattingh (1986) maintained that in established vineyards the most effective way of preventing infection of grapevines by *P. cinnamomi* is by using fungicides. Marais and Hattingh (1986) tested a variety of chemicals as preventative and curative treatments on 20 cm cuttings of rootstock cultivar 99 Richter (*Vitis berlandieri* PI x *Vitis rupestris* Sch. var. du Lot) in glasshouse and field trials. They concluded that grapevine nursery soil should be treated with a drench of metalaxyl (2 g a.i./m^2) before planting, and that established grapevines should receive drench applications of metalaxyl every five months, or monthly foliar applications of fosetyl-Aluminium (3.2 g a.i./L), beginning in spring when shoots are 10 cm long.

Results obtained from recent surveys conducted in three selected grapevine nurseries near the town of Wellington in the Western Cape province indicated that *P. cinnamomi* was present in 40% of the soils, but in only 1.9% of the diseased plants tested (chapter 2). Metalaxyl (chemical class phenylamides) and the phosphonate fungicides, fosetyl-Al and phosphorous acid, are commonly used against downy mildew in these nurseries. Since these chemicals were found to be effective against *Phytophthora* (Marais & Hattingh, 1986) the question arose whether the chemicals applied against downy mildew in grapevine nurseries could be preventing *P. cinnamomi* from infecting nursery grapevines. The phosphonates are fully systemic chemicals registered against Oomycetous plant pathogens, and are translocated both upward and downward through the plant (Guest & Grant, 1991; Schwinn & Staub, 1996) and therefore foliar applications could well have enormous value in controlling root pathogens. Metalaxyl is also a systemic chemical and has very good curative activity, although pathogen resistance to metalaxyl is well known (Schwinn & Staub, 1996). Metalaxyl has very little downward translocation (Schwinn & Staub, 1996), and therefore has to be applied as a soil drench or as granules worked into the soil to be effective against Phytophthora root rot (Marais & Hattingh, 1986). It could therefore be anticipated that foliar applications of metalaxyl would have little to no effect on *Phytophthora* root infections.

The aim of this study therefore was to determine whether the chemicals fosetyl-Al, metalaxyl, and phosphorous acid, registered against downy mildew on grapevines in South Africa and applied as a foliar spray (Nel *et al.*, 1999), could protect the roots of nursery grapevines from infection by *P. cinnamomi*.

MATERIALS AND METHODS

Plant material. One-year-old dormant grafted nursery grapevines (*Vitis vinifera* L cultivar Cabernet Sauvignon on 99 Richter and *V. vinifera* cultivar Cabernet Sauvignon on 101-14 Mgt) were subjected to hot water treatment at 50°C for 30 min in order to rid the plants of *P. cinnamomi* propagules (Von Broembsen & Marais, 1978), as well as other pathogens (Crous, Swart & Coertze, 2001). Following hot water treatment, the grapevines were hydrated in cold water for 24 h in order to break the heat shock (Crous *et al.*, 2001). The grapevines were transplanted into 5 L plastic bags (one plant per bag) in a pasteurised fine sand, composted bark mixture (Nucellar nurseries, P.O. Box 73, Simondium, Paarl, South Africa) and Hygrotech mixture ® (P.O. Box 379, Strand, South Africa) planting medium (1:1:0.5). The Hygrotech-mixture was added since it has excellent water-holding capacity that is essential for *P. cinnamomi*. The plants were irrigated daily with municipal tap water until field capacity was reached, and fertilised weekly with a balanced, water-soluble, liquid-fertiliser (Hydropon®, Solute, P.O. Box 3172, Matieland). A cloth, dipped in penconazole (Topaz EW, Syngenta), was placed in front of the air-conditioners of the glasshouses, and changed weekly, in order to prevent the development of powdery mildew (*Uncinula necator* (Schwein.) Burrill) (W. Schwabe, pers. comm.). No pest control was necessary. The grapevines were pruned back to 2 nodes three weeks after planting, and were inoculated with *P. cinnamomi* after two months.

Determination of mating type of *P. cinnamomi* isolates. Isolates of *P. cinnamomi* were obtained from surveys conducted at three commercial grapevine nurseries near Wellington in the Western Cape (Chapter 2). The mating type of the isolates was determined by placing a 4 mm diam agar plug of mycelium from each isolate, made with a sterile cork-borer, onto potato carrot agar opposite a 4 mm mycelial plug of strains of *P. cinnamomi* of known mating types (A1 or A2) (Ribeiro, 1978). Plates were incubated for 6 weeks in the dark, and checked for the presence of oospores (Ribeiro, 1978). Two isolates of mating type A1 (accession numbers STE-U 5731, STE-U 5732) and two of mating type A2 (accession numbers STE-U 5733, STE-U 5734) isolated

from 99 Richter at Wellington in 2001 were selected. All cultures are preserved in the culture collection of the Department of Plant Pathology at the University of Stellenbosch.

Inoculum and inoculation method. The inoculation method of Swart and Denman (2000) was used. Young shoots were cut from *Leucospermum cordifolium* (Salisb. ex Knight) Fourc.) bushes, defoliated and dried in an oven at 60°C for 2 days. The shoots were then cut into 2.5 cm fragments, autoclaved at 120°C for 15 min, dried overnight in a laminar flow cabinet and irradiated with gamma rays in order to completely sterilise the shoot pieces. The sterile *Leucospermum* shoot pieces were colonised with *P. cinnamomi* by placing a 4 mm diam circular mycelial plug of a *P. cinnamomi* isolate on a 90 mm diam Petri dish containing Corn Meal Agar (CMA, Biolab Diagnostics, Midrand, South Africa) and positioning five *Leucospermum* shoot pieces around the plug. In the negative controls, sterile shoot pieces were exposed to uncolonised CMA plugs only. The dishes were incubated for three weeks under cool white lights with a 12 h photoperiod by which time the shoot pieces were thoroughly colonised with the pathogen. The plants were inoculated by pushing three inoculated shoot pieces equally spaced 10 cm around each plant into the planting mixture to a depth of about 5 cm. Sterile shoot pieces that were incubated on CMA plates for 3 weeks were used as controls.

Chemicals. The chemicals used in this trial were fosetyl-Al (800 g a.i./kg Aliette WG, Aventis) applied at 3.25 g/L of water, metalaxyl (640 g a.i./kg Ridomil Gold WP, Syngenta) at 2.75 g/L of water, and phosphorous acid (400 g a.i./L Fosguard 400SL, Ocean Agriculture) at 4.00 ml/L of water. Application of all chemicals commenced two weeks after inoculation with *P. cinnamomi* and all chemicals were applied as a foliar spray every two weeks throughout the duration of the trial. Chemicals were applied to plants until runoff. Some of the chemicals dripped onto the potting mixture following foliage runoff, but this was minimal. A "no chemical" treatment in which water only was applied to the plants was also included. The trial was terminated 31 weeks after inoculation with *P. cinnamomi*, which was two weeks after the last application of chemicals.

Experimental design. The experimental design was a 2 x 4 x 5 factorial (2 rootstocks x 4 fungicides x 5 isolates) design. The experiment was laid out as a randomised block design with four blocks.

Disease assessments. Disease symptoms on the above-ground and below-ground parts of the plants were assessed at termination of the experiment. The following above-ground parameters were recorded: (i) Shoot height (mm), (ii) the number of leaves, (iii) the percentage (%) chlorotic leaves, and (iv) the stem diameter (mm). Below-ground parameters assessed for every plant were (i) the root volume (cm^3) and (ii) the root length (mm). To determine the root volume, the washed roots of vines were submerged up to the crown area of the vine in a 2 L measuring cylinder that was filled with 1 L of water. Root volume was determined by measuring the water displacement (cm^3).

Re-isolation of *P. cinnamomi*. Re-isolation of *P. cinnamomi* was carried out by excising and plating three pieces each of feeder roots, medium-sized roots, large roots and one piece of crown tissue of every plant onto Phytophthora selective medium (PH) (Solel & Pinkas, 1982). The plates were incubated for 3 weeks after which the number of *P. cinnamomi* colonies was recorded.

Soil studies. Plants were removed from the pots and soil adhering to the roots was removed with free running municipal tap water. Soil from each of the four replicate plants per treatment was collected for soil studies. The presence of *P. cinnamomi* in the collected soil samples was confirmed by using the avocado leaf disc baiting bioassay described by Pegg (1977). Ten gram of soil were weighed from each soil sample and placed in a 90 mm diam Petri-dish, and covered with carbon-filtered water until a film of water approximately 1 mm deep appeared on the soil. Four leaf discs of sterile avocado leaves were made with a sterile cork borer (4 mm diam), and floated adaxial side up onto the water film of each soil sample. The dishes were incubated at room temperature (20–23°C) for 72 h, after which the leaf discs were lifted off the soil suspension, rinsed in sterile water and placed onto freshly prepared PH-medium. The plates were incubated for a further 72 h and the development of *P. cinnamomi* recorded.

Statistical analysis. Analysis of variance (ANOVA) was performed on the data using SAS statistical software version 8.02 (SAS Institute Inc., Cary, NC, United States of America). Fisher's protected least significant difference (Snedecor & Cochran, 1989) was calculated at the 5% level of probability to compare treatment means.

RESULTS

There were no significant interactions ($P > 0.05$), therefore main effects were compared (Table 1-6). These data are presented below.

Re-isolation of *P. cinnamomi* from plants. Overall there was a very low level of back-isolation of the pathogen from inoculated plant material (Fig. 1) and *P. cinnamomi* was not re-isolated from the uninoculated (negative) controls, indicating that the method of inoculation was not suitable for this trial. Although levels of re-isolation were too low to carry out statistical tests for significance, a trend in the data was evident. Lower numbers of isolates were consistently obtained from inoculated 101-14 Mgt rootstocks than from inoculated 99 Richter rootstocks, except for plants treated with phosphorous acid, where almost no difference was visible between the number of *P. cinnamomi* isolates obtained from the 2 rootstocks (Fig 1).

Isolates. There were no differences in aggressiveness between isolates for most parameters measured. However, isolate STE-U 5734 of the A2 mating type significantly ($P = 0.05$) reduced root length relative to the other isolates, except the A1 isolate STE-U 5732 (Table 7). None of the isolates significantly affected the above-ground parameters (height, number of leaves, percentage yellowed leaves, stem diameter) compared to the control (uninoculated). Root volume was also not significantly affected by any of the isolates compared to the uninoculated control.

Treatments (chemical effect). At the 95% confidence limit, there was very little discernable effect of the chemicals tested relative to the control plants for the parameters measured. However, a distinctive trend in the data was evident. Plants treated with phosphorous acid tended to be taller and have more leaves, greater stem diameter and root volume than the controls or plants treated with the other fungicides (Table 8). The phosphorous acid treatment resulted in a significantly ($P = 0.05$) higher number of leaves per plant than the controls (Table 8).

Rootstocks. A significantly lower number of leaves and root volume were recorded for the 99 Richter rootstock compared to the 101-14 Mgt rootstock. There were, however, no other significant differences in the other parameters between these two rootstocks (Table 9).

Soil studies. At the end of the experiment *P. cinnamomi* was baited from the inoculated soils but not from the uninoculated (negative control) soil (Fig 2). In general a higher number of baits from 99 Richter soils yielded the pathogen compared with soils in which 101-14 Mgt rootstocks were planted.

DISCUSSION

Although these tests confirmed that *P. cinnamomi* could infect grapevines, the lack of statistically significant differences between the treatments make the results difficult to interpret. In future it is recommended that a higher level of inoculum and periodic flooding or water-logged conditions be applied. By challenging the plant and the treatments with extreme conditions suitable for the pathogen differences are likely to be more clearly high-lighted. Furthermore if a longer test period was used where grapevines were allowed to go through dormancy differences in the treatment, effects might be emphasised in the spring flush, which is dependent upon the root system. Removal of the grapevines from the plastic bags resulted in a massive loss of diseased feeder roots, despite careful work to minimise it. Therefore, if the grapevines are left in the plastic bags through dormancy, the onset of the new growing season may result in a large amount of inoculum in the root system, which may result in more observable results.

This study did show, however, that none of the chemicals tested were able to eradicate *P. cinnamomi* from plant tissues or soil. Although this is well documented (Marais & Hattingh, 1986) it has serious implications for the management of this pathogen. Although not statistically significant, a trend that was visible, was that fungicide treatments improve aboveground health relative to the untreated ones in terms of plant height, number of leaves and root volume. This trend suggests that the different chemicals may help the plants to overcome damage caused by *P. cinnamomi*. Results obtained suggest that phosphorous acid is probably the most promising compound tested, but more severe testing conditions are required to confirm this.

In this study, the two different mating types of *P. cinnamomi* (A1 and A2), did not differ from each other with respect to relative virulence, since no significant differences were obtained with the different isolates and the control with regards to any growth parameter. The only exception was observed with root length, where the A2 isolate STE-U 5734 resulted in a significant lower root length than the A2 isolate STE-U 5733, and the A1 isolate STE-U 5731. Little information is available on the relative virulence of the two mating types of *P. cinnamomi*,

although Zentmyer (1980) reported that on *Persea* (avocado), the A2 mating type is considered to be more virulent to *P. americana* Mill., *P. indica* (L.) K. Spreng., and *P. borbonia* (L.) K. Spreng. than the A1 mating type. No difference in virulence was observed, however, between the two mating types on *P. pachypoda* Nees. It is therefore important that the relative virulence of the A1 and A2 mating types on grapevines be determined in a separate experiment using more isolates of each mating type, since both mating types occur on South African grapevines (chapter 2; Zentmyer, 1976).

In this study, 99 Richter had a significantly lower number of leaves and root volume than 101-14 Mgt. Marais (1988) showed that 99 Richter was more susceptible to *P. cinnamomi* than 101-14 Mgt. Since 99 Richter rootstocks have a lower root volume than 101-14 Mgt, any further loss of roots caused by pathogens is likely to enhance its decline and susceptibility to infections. This factor may contribute to its greater susceptibility than 101-14 Mgt.

The data obtained in this study are not conclusive with regard to the effect of the chemicals registered against downy mildew on grapevines on the infection of nursery grapevines with *P. cinnamomi*. More glasshouse as well as field trials will therefore need to be conducted, which will enable a better understanding of the possible effect of these chemicals on the microbial shift observed on grapevines in nurseries (Chapter 2).

LITERATURE

Agnihothrudu, V. 1968. A root rot of grapes in Andhra Pradesh. *Current Science* 37: 292–294.

Bumbieris, M. 1970. Effect of DBCP on pythiaceous fungi. *Plant Disease Reporter* 54: 622–624.

Bumbieris, M. 1972. Observations on some pythiaceous fungi associated with grapevine decline in South Australia. *Australian Journal of Agricultural Research* 23: 652–657.

Chiarappa, L. 1959. The root rot complex of *Vitis vinifera* in California. *Phytopathology* 49: 670–674.

Crous, P.W., Swart, L. & Coertze, S. 2001. The effect of hot water treatment on fungi occurring in apparently healthy grapevine cuttings. *Phytopathologia. Mediterrania* 40: Supplement S464–S466.

Guest, D. & Grant, B. 1991. The complex action of phosphonates as antifungal agents. *Biological Reviews* 66: 159–187.

Latorre, B.A., Wilcox, W.F. & Banados, M.P. 1997. Crown and root rots of table grapes caused by *Phytophthora* spp. in Chile. *Vitis* 36: 195–197.

Mansilla, J.P., Pintos, C. & Salinero, M.C. 1993. Isolation and identification of *Phytophthora cinnamomi* Rands. as a pathogen of grape in the province of Pontevedra. *Boletin de Sanidad Vegetal, Plagas* 19: 541–549.

Marais, P.G. 1979. Fungi associated with root rot in vineyards in the Western Cape. *Phytophylactica* 11: 65–68.

Marais, P.G. 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. *Phytophylactica* 12: 9–13.

Marais, P.G. & Hattingh, M.J. 1986. Reduction of root rot caused by *Phytophthora cinnamomi* in grapevines by chemical treatments. *Plant Disease* 70: 109–111.

Marais, P.G. 1988. Grapevine roots and soilborne fungi. In *The Grapevine Root and its Environment*. (eds. J.A. van Zyl), pp. 106–137. South Africa Department of Agriculture and Water Supply Technical Bulletin 215.

McGechan, J.K. 1966. *Phytophthora cinnamomi* responsible for a root rot of grapevines. *Australian Journal of Science* 28: 354.

Nel, A., Krause, M., Ramautar, N. & Van Zyl, K. 1999. *A guide for the control of plant diseases*. National Department of Agriculture, Technical Advice (Act No. 36/1947), Directorate: Agricultural Production Inputs, Republic of South Africa, 122 pp.

Pegg, K.G. 1977. Soil application of elemental sulphur as a control of *Phytophthora cinnamomi* root and heart rot of pineapple. *Australian Journal of Experimental Agriculture and Animal Husbandry* 17: 859–865.

Ribeiro, O.K. 1978. *A sourcebook on the genus* Phytophthora. J. Cramer: Lehre, Germany. 420 pp.

Schwinn, F. & Staub, T. 1995. Oomycetes fungicides. In *Modern Selective Fungicides. Properties, Applications, Mechanisms of Action* (ed. H. Lyr), pp. 323–354. Gustav Fischer Verlag, Jena, Stuttgart, New York.

Snedecor, G.W. & Cochran, W.G. 1989. *Statistical methods*. Eighth edn. Iowa State University Press: Iowa, USA. 503 pp.

Solel, Z. & Pinkas, Y. 1982. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. *Phytopathology* 74: 506–508.

Stephens, P.M., Davoren, C.W. & Wicks, T. 1999. Effect of methyl bromide, metham sodium and the biofumigants Indian mustard and canola on the incidence of soilborne fungal pathogens and growth of grapevine nursery stock. *Australasian Plant Pathology* 28: 187–196.

Swart, L. & Denman, S. 2000. Chemical control of *Phytophthora cinnamomi* in potted *Leucospermum* plants. *Australasian Plant Pathology* 29: 230–239.

Utkhede, R.S. & Vielvoye, J. 1984. Cause and control of decline of grapevines in British Colombia. *Plant Disease* 68: 820–822.

Von Broembsen, S.L. & Marais, P.G. 1978. Eradication of *Phytophthora cinnamomi* from grapevine by hot water treatment. *Phytophylactica* 10: 25–27.

Van der Merwe, J.J.H., Joubert, D.J. & Matthee, F.N. 1972. *Phytophthora cinnamomi* root rot of grapevines in the Western Cape. *Phytophylactica* 4: 133–136.

Zentmyer, G.A. 1976. Distribution of the A1 mating type of *Phytophthora cinnamomi*. *Phytopathology* 66: 701–703.

Zentmyer, G.A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monograph 10. The American Phytopathological Society, St. Paul, Minnesota. 96 pp.

Table 1. Analysis of variance for the number of leaves of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	15942.6354	265.7106	0.2571
Rep	3	5650.7010	1883.5670	< 0.0001
Rootstock (R)	1	683.7745	683.7745	0.0287
Treatment (T)	3	1537.2150	512.4050	0.0198
R x T	3	688.7823	229.5941	0.1662
Error	39	8498.5247	217.9109	
Corrected total	99	24441.1600		

Table 2. Analysis of variance for the percentage of chlorotic leaves (%) of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	3.4574	0.05762	0.0767
Rep	3	0.7028	0.2343	0.0034
Rootstock (R)	1	0.2064	0.2064	0.0270
Treatment (T)	3	0.0795	0.0265	0.5392
R x T	3	0.1226	0.0409	0.3566
Error	39	1.4600	0.0374	
Corrected total	99	4.9174		

Table 3. Analysis of variance for root length (mm) of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	1340449.8260	22340.8300	0.2626
Rep	3	274675.1885	91558.3962	0.0008
Rootstock (R)	1	379.2549	379.2549	0.8505
Treatment (T)	3	7920.1728	2640.0576	0.8572
R x T	3	136422.2083	45474.0694	0.0575
Error	39	718165.1740	18414.4920	
Corrected total	99	2058615.0000		

Table 4. Analysis of variance for root volume (cm^3) of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	44063.3806	734.3897	0.1143
Rep	3	5386.7930	1795.5977	0.0614
Rootstock (R)	1	10414.9583	10414.9583	0.0006
Treatment (T)	3	1122.2361	374.0787	0.6164
R x T	3	601.7084	200.5695	0.8053
Error	39	19912.6194	510.5800	
Corrected total	99	63976.0000		

Table 5. Analysis of variance for shoot height (mm) of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	3.9394	0.0657	0.8727
Rep	3	0.4442	0.1481	0.0730
Rootstock (R)	1	0.0137	0.0137	0.6208
Treatment (T)	3	0.1876	0.0625	0.3522
R x T	3	0.0757	0.0252	0.7081
Error	39	3.5403	0.0908	
Corrected total	99	7.4796		

Table 6. Analysis of variance for stem diameter (mm) of different grapevine rootstocks (R) inoculated with different mating types of *P. cinnamomi*, and subjected to treatment (T) with different chemicals in a glasshouse

Source of variation	DF	SS	MS	Pr > F
Model	60	139.6515	2.3275	0.7786
Rep	3	8.3689	2.7896	0.4013
Rootstock (R)	1	1.3035	1.3035	0.4959
Treatment (T)	3	3.0744	1.0248	0.7688
R x T	3	8.3158	2.7719	0.4041
Error	39	112.7636	2.8914	
Corrected total	99	252.4151		

Table 7. Effect of the inoculation of grapevines with *P. cinnamomi* in a glasshouse on above- and below-ground parameters

Isolate number	Mating type	Above-ground [x]				Below-ground [x]	
		Height (mm) [y]	Number of leaves [y]	Chlorotic leaves (%) [yz]	Stem diameter (mm) [y]	Root volume (cm^3) [y]	Root length (mm) [y]
STE-U 5731	A1	757.23 a	36.59 a	59.79 a	10.58 a	76.14 a	770.41 a
STE-U 5732	A1	724.43 a	41.05 a	64.12 a	10.60 a	76.19 a	733.29 ab
STE-U 5733	A2	747.56 a	35.73 a	68.00 a	9.87 a	73.41 a	780.77 a
STE-U 5734	A2	671.99 a	38.00 a	61.42 a	9.85 a	66.32 a	653.16 b
Control		672.92 a	40.44 a	58.34 a	9.88 a	73.13 a	754.69 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Parameters measured.

[z] Number of chlorotic leaves/ total number of leaves x 100.

Table 8. Effect of foliar application of chemicals on above- and below-ground parameters measured on grapevines in a glasshouse

Fungicide treatment	Above-ground [x]				Below-ground [x]	
	Height (mm) [y]	Number of leaves [y]	Chlorotic leaves (%) [yz]	Stem diameter (mm) [y]	Root volume (cm^3) [y]	Root length (mm) [y]
Fosetyl-Al	729.68 a	39.59 ab	63.57 a	10.08 a	73.33 a	755.74 a
Metalaxyl	716.17 a	36.52 ab	57.68 a	10.01 a	69.29 a	726.67 a
Phosphorous acid	752.69 a	42.76 a	62.23 a	10.48 a	77.20 a	729.56 a
Control	676.36 a	33.96 b	65.74 a	10.12 a	72.41 a	744.67 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Parameters measured.

[z] Number of chlorotic leaves/ total number of leaves x 100.

Table 9. Differences between rootstocks in a glasshouse with regard to above- and belowground parameters

	Above-ground [x]				Below-ground [x]	
Rootstock	**Height (mm) [y]**	**Number of leaves [y]**	**Chlorotic leaves (%) [yz]**	**Stem diameter (mm) [y]**	**Root volume (cm^3) [y]**	**Root length (mm) [y]**
99 Richter	728.99 a	36.07 b	65.65 a	10.31 a	65.27 b	745.75 a
101-14 Mgt	703.44 a	40.96 a	58.69 a	10.01 a	83.30 a	732.91 a

[x] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

[y] Parameters measured.

[z] Number of chlorotic leaves/ total number of leaves x 100.

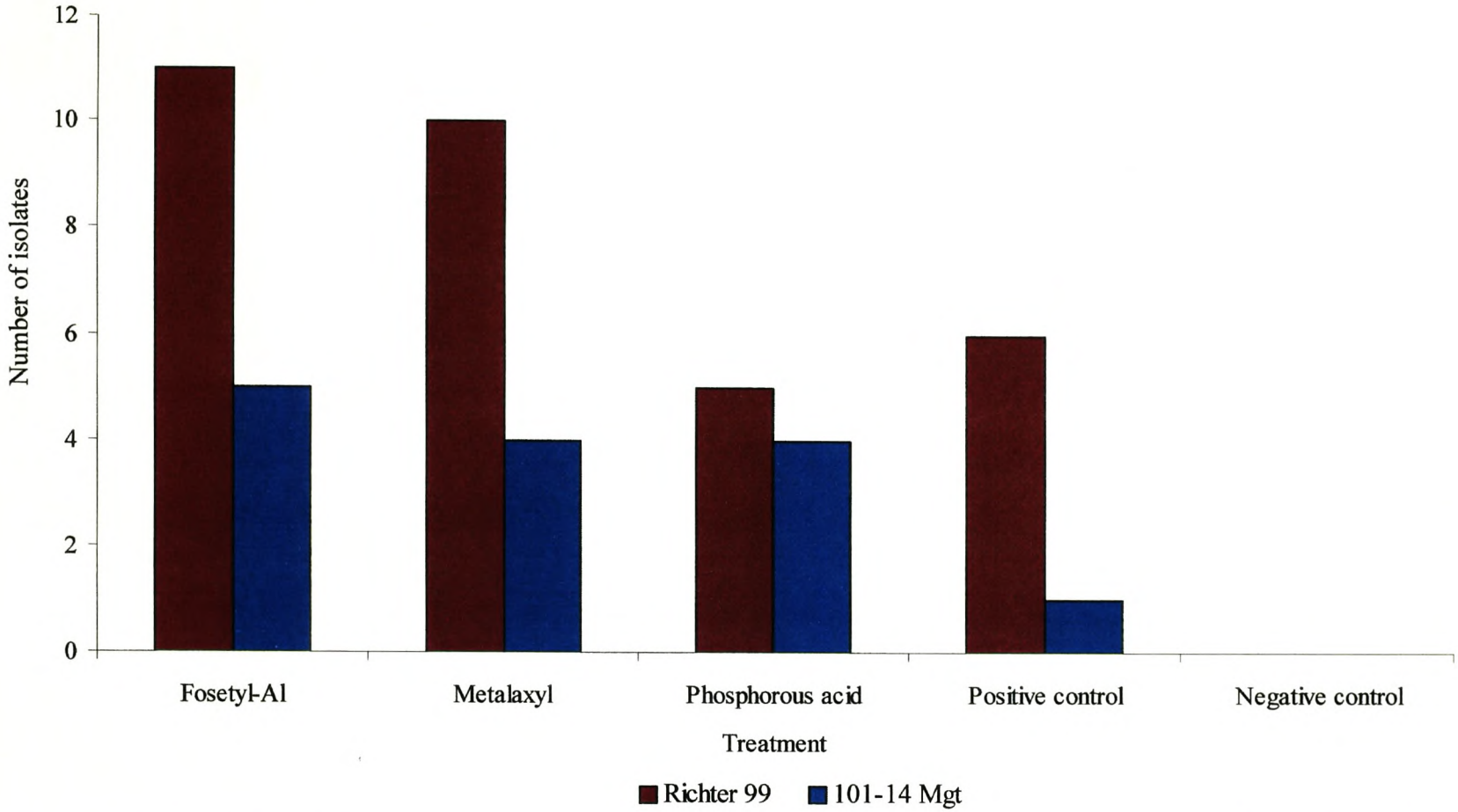


Figure 1. Detection of *P. cinnamomi* from excised tissue of inoculated grapevine rootstocks that were treated with various fungicides in glasshouse trials; positive control = plants inoculated with *P. cinnamomi*, and receiving no fungicide treatments negative control = plants not inoculated with *P. cinnamomi*.

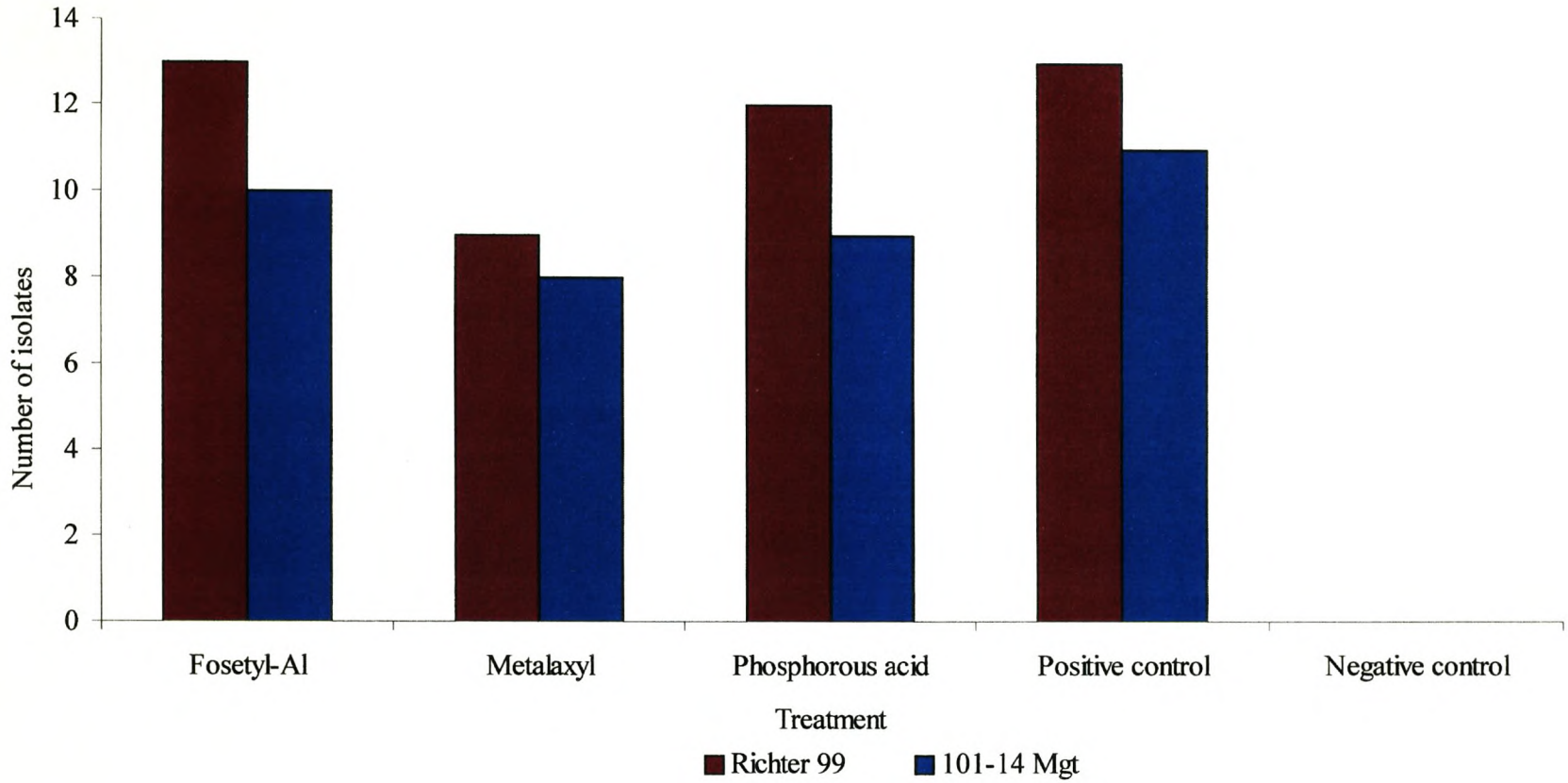


Figure 2. Number of artificially inoculated soil samples that tested positive for *P. cinnamomi* after different fungicide treatments were applied to the plants growing in the soils; positive control = plants inoculated with *P. cinnamomi*, and receiving no fungicide treatments, negative control = plants not inoculated with *P. cinnamomi*.