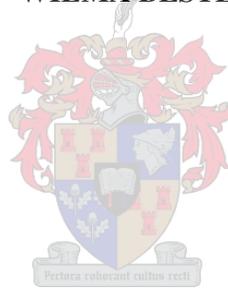


**CHARACTERISATION AND MANAGEMENT OF TRUNK DISEASE-CAUSING
PATHOGENS ON TABLE GRAPEVINES**

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Science in Agriculture at the University of Stellenbosch**

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CHARACTERISATION AND MANAGEMENT OF TRUNK DISEASE-CAUSING PATHOGENS ON TABLE GRAPEVINES

SUMMARY

Phaeoconiella chlamydospora, *Eutypa lata*, *Phomopsis*, *Phaeoacremonium*, and *Botryosphaeria* spp. are important trunk disease pathogens that cause premature decline and dieback of grapevine. Previous research has focused primarily on wine grapes and the incidence and symptomatology of these pathogens on table grapes were largely unknown. A survey was therefore conducted to determine the status and distribution of these pathogens and associated symptoms in climatically diverse table grape growing regions. Fifteen farms were identified in the winter rainfall (De Doorns, Paarl and Trawal) and summer rainfall (Upington and Groblersdal) areas. Samples were taken in July and August 2004 from Dan-ben-Hannah vineyards that were 8 years and older. Distal ends of arms were removed from 20 randomly selected plants in each vineyard. These sections were dissected and isolations were made from each of the various symptom types observed: brown or black vascular streaking, brown internal necrosis, wedge-shaped necrosis, watery necrosis, esca-like brown and yellow soft wood rot, as well as asymptomatic wood. Fungal isolates were identified using molecular and morphological techniques. *Pa. chlamydospora* was most frequently isolated (46.0%), followed by *Phaeoacremonium aleophilum* (10.0%), *Phomopsis viticola* (3.0%), *Botryosphaeria obtusa* (3.0%), *B. rhodina* (2.2%), *B. parva* (2.0%), *Fusicoccum vitifusiforme* (0.6%), *B. australis*, *B. dothidea* and an undescribed *Diplodia* sp. (0.2% each), while *E. lata* was not found. Most of these pathogens were isolated from a variety of symptom types, indicating that disease diagnosis can not be based on symptomatology alone. *Pa. chlamydospora* was isolated from all areas sampled, although most frequently from the winter rainfall region. *Pm. aleophilum* was found predominantly in Paarl, while *P. viticola* only occurred in this area. Although *B. obtusa* was not isolated from samples taken in De Doorns and Groblersdal, it was the most commonly isolated *Botryosphaeria* sp., being isolated from Upington, Paarl and Trawal. *B. rhodina* occurred only in Groblersdal and *B. parva* in Paarl, Trawal and Groblersdal, while *B. australis* was isolated from Paarl only. The rest of the isolates (33%) consisted of sterile cultures, *Exochalara*, *Cephalosporium*, *Wangiella*, *Scytalidium*, *Penicillium* spp. and two unidentified basidiomycetes, which were isolated from five samples with yellow esca-like symptoms from the Paarl area.

These findings clearly illustrate that grapevine trunk diseases are caused by a complex of fungal pathogens, which has serious implications for disease diagnosis and management.

Protection of wounds against infection by any of these trunk disease pathogens is the most efficient and cost-effective means to prevent grapevine trunk diseases. However, previous research on the effectiveness of chemical pruning wound protectants has mostly focused on the control of *Eutypa dieback* only. Fungicide sensitivity studies have been conducted for *Pa. chlamydospora*, *P. viticola* and *Eutypa lata*, but no such studies have been conducted for the pathogenic *Botryosphaeria* species from grapevine in South Africa. Ten fungicides were therefore tested *in vitro* for their efficacy on mycelial inhibition of the four most common and/or pathogenic *Botryosphaeria* species in South Africa, *B. australis*, *B. obtusa*, *B. parva* and *B. rhodina*. Iprodione, pyrimethanil, copper ammonium acetate, kresoxim-methyl and boscalid were ineffective in inhibiting the mycelial growth at the highest concentration tested (5 µg/ml; 20 µg/ml for copper ammonium acetate). Benomyl, tebuconazole, prochloraz manganese chloride and flusilazole were the most effective fungicides with EC₅₀ values for the different species ranging from 0.36-0.55, 0.07-0.17, 0.07-1.15 and 0.04-0.36 µg/ml, respectively. These fungicides, except prochloraz manganese chloride, are registered on grapes in South Africa and were also reported to be effective against *Pa. chlamydospora*, *P. viticola* and *E. lata*. Results from bioassays on 1-year-old Chenin Blanc grapevine shoots indicated that benomyl, tebuconazole and prochloraz manganese chloride were most effective in limiting lesion length in pruning wounds that were inoculated with the *Botryosphaeria* spp after fungicide treatment. The bioassay findings were, however, inconclusive due to low and varied re-isolation data of the inoculated lesions. Benomyl, tebuconazole, prochloraz manganese chloride and flusilazole can nonetheless be identified as fungicides to be evaluated as pruning wound protectants in additional bioassays and vineyard trials against *Botryosphaeria* spp. as well as the other grapevine trunk disease pathogens.

KARAKTERISERING EN BESTUUR VAN STAMSIEKTE PATOGENE OP TAFELDRUIWE

OPSOMMING

Phaeomoniella chlamydospora, *Eutypa lata*, *Phomopsis*, *Phaeoacremonium*, en *Botryosphaeria* spesies is die mees belangrikste stamsiekte patogene wat agteruitgang en vroeë terugsterwing van wingerd veroorsaak. Voorafgaande navorsing het hoofsaaklik gefokus op wyndruiwe en die voorkoms en simptomatologie van hierdie patogene op tafeldruiwe is dus grootliks onbekend. 'n Opname is gevolglik gedoen in verskillende klimaatareas waar tafeldruiwe verbou word om die voorkoms en verspreiding, asook die simptome geassosieer met hierdie patogene, te bepaal. Vyftien plase is geïdentifiseer in die winter- (De Doorns, Paarl en Trawal) en somer-reënval (Upington en Groblersdal) streke. Wingerde (8 jaar en ouer) met die kultivar Dan-ben-Hannah is gekies vir opname en monsters is gedurende Julie en Augustus 2004 geneem. Die distale deel van 'n arm is verwyder vanaf 20 lukraak gekose plante in elke wingerd. Hierdie dele is ontleed en isolasies is gemaak vanuit elke simptoontipe wat beskryf is, naamlik bruin en swart vaskulêre verkleuring, bruin interne nekrose, wig-vormige nekrose, waterige nekrose, esca-geassosieerde bruin en geel sagte houtverrotting en asimptomatiese hout. Identifikasie van die swamagtige isolate is gedoen op grond van morfologiese eienskappe en molekulêre tegnieke. *Pa. chlamydospora* is die meeste geïsoleer (46.0%), gevolg deur *Phaeoacremonium aleophilum* (10.0%), *Phomopsis viticola* (3.0%), *Botryosphaeria obtusa* (3.0%), *B. rhodina* (2.2%), *B. parva* (2.0%), *Fusicoccum vitifusiforme* (0.6%), *B. australis*, *B. dothidea* en 'n onbeskryfde *Diplodia* sp. (0.2% elk), terwyl *E. lata* nie geïsoleer is nie. Hierdie patogene is elk geïsoleer vanuit 'n verskeidenheid simptoontipes, wat daarop dui dat siekediagnose nie alleenlik op simptomatologie gebaseer kan word nie. *Pa. chlamydospora* is geïsoleer vanuit al die gebiede, alhoewel die patogeen opmerklik meer voorgekom het in die winter-reënval area. *Pm. aleophilum* het hoofsaaklik voorgekom in Paarl, terwyl *P. viticola* slegs in hierdie area voorgekom het. Alhoewel *B. obtusa* nie voorgekom het in die De Doorns en Groblersdal areas nie, was dit die mees algemeen geïsoleerde *Botryosphaeria* sp. en het in Upington, Paarl en Trawal voorgekom. *B. rhodina* het slegs in Groblersdal voorgekom, *B. parva* in Paarl, Groblersdal en Trawal en *B. australis* het slegs in Paarl voorgekom. Die res van die

isolate (33%) het bestaan uit steriele kulture, *Exochalara*, *Cephalosporium*, *Wangiella*, *Scytalidium*, en *Penicillium* spesies asook twee onbekende basidiomycete isolate, geïsoleer vanuit vyf monsters met geel eska-geassocieerde simptome vanuit die Paarl area. Hierdie resultate illustreer dus die feit dat wingerdstamsiektes deur 'n kompleks van swampatogene veroorsaak word, wat belangrike implikasies het vir die bestuur en diagnose van hierdie siektes.

Wondbeskerming teen infeksie van enige van hierdie stamsiekte patogene is die mees doeltreffende en koste-effektiewe manier om wingerdstamsiektes te voorkom. Vorige navorsing aangaande die effektiwiteit van chemiese wondbeskermingsmiddels het egter slegs gefokus op die beheer van *Eutypa* terugsterwing. *In vitro* swamdoder sensitiviteitstoetse is gedoen vir *Pa. chlamydospora*, *P. viticola* en *Eutypa lata*, maar geen studies is al gedoen ten opsigte van die patogeniese *Botryosphaeria* spesies op wingerd in Suid-Afrika nie. Tien swamdoders is dus getoets vir inhibisie van *in vitro* miseliumgroei van die vier mees algemene en/of patogeniese *Botryosphaeria* spesies wat in Suid-Afrika voorkom, naamlik *B. australis*, *B. obtusa*, *B. parva* en *B. rhodina*. Iprodione, pyrimethanil, koper ammonium asetaat, kresoxim-metiel en boscalid was oneffektief by die hoogste konsentrasies getoets (5 µg/ml; 20 µg/ml vir koper ammonium asetaat). Benomyl, tebuconasool, prochloraz mangaan chloried en flusilasool was die mees effektiewe swamdoders met EC₅₀ waardes tussen 0.36-0.55, 0.07-0.17, 0.07-1.15 en 0.04-0.36 µg/ml, onderskeidelik vir die verskillende spesies. Hierdie fungisiedes, behalwe prochloraz mangaan chloried, is geregistreer op druiwe in Suid-Afrika en is ook effektief gevind teenoor *Pa. chlamydospora*, *P. viticola* en *E. lata*. Resultate van biotoetse op 1-jaar-oue Chenin Blanc wingerd lote het getoon dat benomyl, tebuconasool en prochloraz mangaan chloried die effektiefste was om die lengte van letsels in snoeiwonde, geïnokuleer met *Botryosphaeria* spesies na die aanwending van swamdoder behandelings, te verminder. Die bevindinge was egter onbeslis as gevolg van die lae en variërende her-isolerings data. Benomyl, tebuconasool, prochloraz mangaan chloried en flusilasool kan egter geïdentifiseer word as swamdoders wat verder geevalueer kan word as snoeiwond beskermingsmiddels teen *Botryosphaeria* spesies asook ander wingerd stamsiekte patogene in verdere biotoetse en wingerdproewe.

Contents

1. Management of grapevine trunk diseases.....1
2. Trunk disease pathogens of table grapevines: symptomatology and distribution in South Africa.....26

Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* spp.....

1. MANAGEMENT OF GRAPEVINE TRUNK DISEASES

INTRODUCTION

Trunk diseases of grapevines are a constant threat to the grapevine industry and their management is becoming increasingly important in order to sustain long-term grape and wine production. Producers continually report substantial losses in grape yield and quality due to disease, which is often inconspicuous. Symptoms of these diseases include dead spurs, arms, cordons and eventually premature dieback of vines due to wood decay, canker, as well as tylose formation in the vascular tissue. As cankers develop, yield reductions occur due to the loss of productive wood. The impact of grapevine trunk diseases can be of greater severity and significance in older vineyards and subsequent re-establishment costs are astronomical. The pathogens causing trunk diseases infect through unprotected wounds. For that reason it is particularly important to protect these potential infection sites. A complex of pathogens causes grapevine trunk diseases. Global studies show that the microbial population of cankered grapevine tissues frequently include *Phaeoconiella chlamydospora*, *Eutypa lata*, *Botryosphaeria*, *Phaeoacremonium*, *Phomopsis*, *Stereum* and *Fomitiporia* species (Moller and Kasimatis, 1981; Mugnai *et al.*, 1999; Crous and Gams, 2000; Armengol *et al.*, 2001; Larignon *et al.*, 2001; Phillips, 2002; Sosnowski *et al.*, 2003; Edwards and Pascoe, 2004; van Niekerk *et al.*, 2005a).

The most important trunk pathogens of grapevines identified in South Africa are *Phomopsis*, *Botryosphaeria*, *Phaeoacremonium* spp., *Phaeoconiella chlamydospora* and *Eutypa lata* (Ferreira *et al.*, 1989; Swart and De Kock, 1994; Groenewald *et al.*, 2001; Fourie and Halleen, 2004a; van Niekerk *et al.*, 2004; Mostert *et al.*, 2005; van Niekerk *et al.*, 2005b). The prevalence of these pruning wound pathogens in the South African grapevine industry was shown during research completed by the Grapevine Diagnostics Service at ARC Infruitec-Nietvoorbij during 2000 to 2002. The results showed that of the 360 samples of diseased grapevine material, 75% of them were infected by wound pathogens (Fourie and Halleen, 2001).

These pathogens pose an immense threat, especially due to the fact that most of these pathogens can occur in a latent or asymptomatic form in grapevines, causing severe symptoms that only become apparent in later years. It is therefore imperative that integrated management strategies focus on preventative measures. Protection of wound surfaces and removal of potential inoculum sources through sanitation practices are the answer to preventing potential losses by trunk disease pathogens.

EUTYPA DIEBACK

Eutypa dieback, also known as dying arm, eutypiosis or 'Tandpyn' (South Africa), is a potentially lethal disease that affects the woody parts of the grapevine. Originally this disease was confused with Phomopsis shoot blight and has only been recognised as a distinct disease in the past few decades. It kills the woody tissue and can release a toxin (eutypine) into the vascular system, which then results in foliar deformities and inhibition of berry maturation. The toxin is produced by mycelium present in the trunk or arms of the vines where it is transported into the herbaceous parts of the vine. It is furthermore believed that this toxin then causes the characteristic symptoms of dying-arm disease (Deswarte *et al.*, 1994). Eutypa dieback therefore reduces vineyard productivity and longevity. The disease is particularly problematic in established vineyards (Sosnowski *et al.*, 2003), where disease incidence increases with the ageing of grapevines. It was found that the incidence ranged from 6% affected vines in a 7-year-old vineyard, to 78% in a 14-year-old vineyard, to 81% in a 15-year-old vineyard in California (Moller *et al.*, 1974). Van Niekerk *et al.* (2003) calculated yield losses of 367 ton during the 2000/2001 season in the Stellenbosch area, costing producers more than R1, 7 million in lost income. Impact studies done in Australia indicated yield losses of 1500 kg/ha, costing over \$2 800/ha in a Shiraz vineyard where 47% of the vines were infected with *E. lata* (Wicks and Davies, 1999). Economic losses due to dieback can, however, be minor under good grape growing conditions where the diseased arms are removed and replaced by new growth. The most damaging effects can be on older vines with large pruning wounds caused by drastic cutting during retraining.

Symptoms

The name Eutypa dieback was proposed for the syndrome characterised by pruning wound cankers, dwarfed spring foliage, and subsequent dead arms which appear on grapevines in the field (Moller *et al.*, 1974; Moller and Kasimatis, 1978). The symptoms of Eutypa dieback are best observed during the first two months of the annual growth cycle when shoots are 25-50 cm long (Ferreira, 1994). Affected shoots, distributed randomly throughout the vineyard, show deformation (cup-shaped) as well as discolouration and are the most obvious sign of infection (Carter, 1988). The shoots are stunted and have shortened internodes. Leaves on the stunted shoots are usually yellow, tattered, speckled and often dead around the margins. Many of the flowers fall off and most berries that do establish on these affected shoots do not reach maturity (Moller and Kasimatis, 1981). On mildly affected shoots the tattered leaves appear on the first nodes only, and subsequent growth is normal. The disease appears first in one or two spurs and spreads in following seasons to adjacent spurs, eventually killing the arm.

An important diagnostic symptom of the disease is the formation of pruning wound cankers. Three to four years after infection, these cankers can form near an old infection site, such as a pruning scar. The removal of the loose bark shows the extent of the canker. A cross section of the affected area on the cordon or trunk exposes a wedge-shaped zone of necrotic

sapwood extending from the point of origin of the canker. The dead wood is hard, brown and brittle, and transport of water and nutrients to upper parts of the vine are greatly reduced (Sosnowski *et al.*, 2003). Cankers will continue to expand around the cordon or trunk of an infected vine, the wedge of dead tissue increasing in the process, thus gradually killing it.

After infection, the disease progresses very slowly: growth of the fungus proceeds slowly down the host tissue at approximately 20 mm per year (Ferreira, 1994). Therefore, symptoms may only become apparent a few seasons after the original infection when the pathogen colonises the xylem, cambium, and then the phloem tissue. The pathogen is primarily a vascular pathogen and as this tissue is only exposed where the vine is injured, plants that are frequently pruned are more prone to this disease.

Etiology

The causal organism of Eutypa dieback is the ascomycete fungus *Eutypa lata* (Pers.: Fr.) Tul. & C. Tul. (= *E. armeniacae* Hansf. & M.V. Carter). It was found in Northern Californian vineyards that a second species, *E. leptoplaca*, acted as a wound pathogen on young spurs of grapevine and although this species was not isolated from typical wedge-shaped necrotic wood, stromata were found on the wood of diseased vines (Trouillase and Gubler, 2004). This species has recently been found on a grapevine sample in South Africa, but further studies are ongoing to confirm results (F. Halleen, pers. comm.).

Epidemiology

The disease is introduced into vineyards during wet weather when ascospores are splashed or blown into wounds. These types of wounds are caused by pruning or re-working operations. Infected debris in apricot and cherry orchards and grape vineyards are the most likely inoculum source, but *E. lata* has also been reported on a number of other cultivated and native woody species (Munkvold and Marois, 1995).

Ascospores are produced on wood previously killed by the fungus, inside a stromatal layer, which contains perithecia. Ascospores are released when it rains, or alternatively when overhead irrigation water falls onto the ascomata. Distribution and occurrence of the perithecial stage on apricot trees in California is related to the mean annual rainfall (Ramos *et al.*, 1975). In areas of San Francisco Bay, where the mean annual rainfall exceeds 508 mm, stromata develop readily. An infected vine can show symptoms but may never form stromata. Ascospores of *E. lata* enter the xylem vessels through wounds on the grapevine and the fungus then spreads further through the wood (Munkvold and Marois, 1993a; John *et al.*, 2004). The optimum

temperature for germination and growth has been reported to be 22-25°C, and relative humidity of at least 90% is required for germination (Munkvold and Marois, 1995). Stromata remain viable for years and continue to produce ascospores each year, whenever conditions are favourable. Although the disease cycle of *Eutypa dieback* is not complex, it requires a long time to complete. After 2-3 years, foliar symptoms may be seen and only after 5 years the bark weathers away from the canker, exposing the stromata on the dead wood.

Management

Cultural practices. Currently there is no means of eradicating the fungus once it has been established inside a grapevine (Creaser and Wicks, 2004). Therefore management of *Eutypa dieback* presently relies on the timing of pruning, and wound protection with wound sealants (Halleen *et al.*, 2001) along with sanitation practices, which are very important, as the ascomata of *Eutypa* occur on dead wood (Ferreira, 1994).

During the wet season, pruning should be avoided since the risk of ascospores being released from the perithecia is greater. Moller and Kasimatis (1980) found that pruning wounds made on Californian grapevines were highly susceptible to *E. lata* for several days after late winter pruning, where after susceptibility rapidly decreased. Pruning cuts should furthermore be at an angle to encourage water run off and the wound immediately sealed with a protectant.

Munkvold and Marois (1995) researched environmental influences on pruning wound susceptibility. They found that temperature influences the susceptibility of pruning wounds, a decline in susceptibility was found to be highly correlated with an increase in degree-day accumulation, and furthermore concluded that optimum pruning time for disease prevention depended on temperatures after pruning, rather than on pruning date.

Many farmers decide to 'renew' infected vines using remedial surgery, which is the process of removing infected tissue from cordons and trunks. Where both of the cordons are infected or where a canker has formed on the trunk, both cordons are removed using one of two methods (Creaser and Wicks, 2004). The cut and train method involves cutting the trunk 10 cm below any signs of infection and training up a watershoot to replace the lost canopy. For the train and cut method, a healthy shoot is selected from the base of the trunk and trained upwards to form a new canopy. The infected trunk is not removed until the new shoot begins cropping. Remedial surgery can, however, be labour intensive and costly. The infected tissue must be cut out and wounds treated to prevent new infections. For remedial surgery to be effective, the pathogen should be removed completely with the decayed wood (Sosnowski *et al.*, 2005a).

Brotomax, a liquid fertiliser, was tested in South Australia on *Eutypa dieback*-affected grapevines (Sosnowski *et al.*, 2005b). They hypothesised that the fertiliser may stimulate the synthesis of phenolic compounds in fruit and vegetable crops, thus decreasing foliar symptoms

and increasing yield. A 20% yield increase was observed in two of the three sites tested in the third season of application. There was, however, no effect on foliar symptoms and further testing are ongoing.

Chemical. Benomyl and thiabendazole were tested against germination and growth of *E. armeniacae*. These compounds appear to act by inhibiting hyphal growth rather than by preventing spore germination (Carter and Price, 1974). They also found that *Fusarium lateritium*, a potential biological control agent, could sporulate on apricot sapwood after introduction of spore inocula suspended in 300 ppm (parts per million) benomyl or 400 ppm thiabendazole. This biological control agent can therefore tolerate relatively high concentrations of benzimidazole compounds, thus suggesting a promising form of integrated control where mixtures of benzimidazoles and *F. lateritium* spores are applied to wounds. The benzimidazole compounds give initial protection, whereas the *F. lateritium* spores give longer lasting protection. Moller *et al.* (1977) found that spraying apricot tree wounds with chemical protectants did not result in practical control of Eutypa canker, although the use of high doses of benzimidazoles in hand-painted applications to individual wound sites was found to be a practical solution.

Halleen *et al.* (2001) found flusilazole and tebuconazole effective alternative fungicides (EC₅₀ values of 0.005 and 0.01 respectively) to benomyl for grapevine pruning wound protection, as the manufacturing of Benlate was stopped in 2001 (www.colostate.edu/Depts/bspm/vol18/). Work done in Australia showed carbendazim to be effective in preventing infection of pruning wounds by *E. lata*, providing a further alternative to benomyl, since these two fungicides belong to the benzimidazole group (Sosnowski *et al.*, 2004).

Biological. Munkvold and Marois (1993b) found that naturally occurring wound colonisers have a high potential for reducing infection of grapevines by *E. lata*. The efficacy of chemicals tended to decline with time (Munkvold and Marois, 1993a), but it was found that the efficacy of biological control agents increased with time. They tested a number of biological control agents such as *Alternaria alternata*, *Cladosporium herbarum*, *F. lateritium* and *Trichoderma viride*. They found *C. herbarum* and *T. viride* moderately effective in reducing infection of grapevine wounds by *E. lata*. Rapid colonisation of the wounds by the biocontrol agent may be the key to effective control through competition (Munkvold and Marois, 1993b). Microorganisms that arrive at the wound site early have the potential to obstruct colonisation by pathogens. John *et al.* (2004; 2005) found that the volatile metabolites produced by *T. harzianum* inhibited mycelial growth of *E. lata* but it did not, however, prevent mycelial growth. Ferreira *et al.* (1991) found *Bacillus subtilis* to inhibit mycelial growth and ascospore

germination of *E. lata*. They observed mycelial malformation, which was probably due to the antibiotic substances produced by the bacteria, which interfered with normal growth processes.

BOTRYOSPHAERIA CANKER AND DIEBACK

Larignon and Dubos (2001) described black dead arm for the first time in France in 1999. It attacks the wood of the plant, causing decline and eventually death. This disease was easily confused with Esca because of the similarity of the foliar symptoms. Like Esca and Eutypa dieback, it affects grapevines older than eight years and has been observed only in vineyards that have never received sodium arsenite treatments used for Esca control (Larignon and Dubos, 2001). An increase in the disease incidence has furthermore been associated with changes in training systems, such as a shift from freestanding vines to trellised vines (Lehoczky, 1988). Yield losses of 25-30% due to black dead arm have been reported (Lehoczky, 1988). The term black dead arm was introduced to distinguish the disease from the dead arm disease caused by *Phomopsis viticola* (Larignon *et al.*, 2001).

Symptoms

In general, neither the clusters nor the berries of the grapevine are infected during the growing season. The fungus has, however, been reported to cause severe berry and cluster rot on cultivars White Hanepoot and Red Hanepoot in South Africa (Lehoczky, 1988). The berries are infected near ripening and become dark brown, shriveled, and mummified. Symptoms of infection in Western Australia included stunting of leaves and internodes, reduced vigour, dead or dying arms and vines with limited or no bud burst (Taylor *et al.*, 2005).

Symptoms can evolve quickly (severe form) or show different phases (mild form), leading in each case to the premature falling of leaves (Larignon and Dubos, 2001). During the mild form of this disease red grape varieties show red patches on the margin of the leaves or on the blade. These patches grow and join together to form large zones of deterioration between the veins and the margin of the leaf. The white varieties lose their colour and become yellowish-orange. These patches grow and coalesce to form necrotic zones leaving a green zone along the main veins. The severe forms of the disease causes the leaves to dry out starting at the base, and the leaves may later also drop off. Wood symptoms include a brown-black streak 1-2cm wide, visible when the bark is peeled back (Larignon and Dubos, 2001), and internal wedge-shaped and arch-shaped necrosis in cross-sectioned arms (van Niekerk *et al.*, 2005a). These streaks developed in the xylem of infected spurs, arms and trunks but rarely in canes as young as one year (Lehoczky, 1988).

In a study on Semillon grapevines in the Hunter Valley of New South Wales, Castillo-Pando *et al.* (2001) found the disease to be associated with reduced growth, budburst failure,

death of arms and, although not consistently, bleaching of canes and small leaves. Internal symptoms such as brown wood streaking were commonly associated with *Botryosphaeria* dieback in the field. Occasionally, when streaking reaches the pith of the wood, a wedge-shaped sector is observed. They furthermore found that symptoms could occur on both young and mature grapevines.

Etiology

Shoemaker (1964) described two *Botryosphaeria* species occurring on cultivated grapes, namely *B. obtusa* (Schwein.) Shoemaker [anamorph *Diplodia* sp.] and *B. stevensii* Shoem. [anamorph *Diplodia mutila* Fr. apud Mont.]. These fungi were thought to cause black dead arm on grapevines. In addition, *B. rhodina* and *B. ribis* have also been found to cause dieback symptoms in grapevines (Shoemaker, 1964). Studies done to identify the fungi associated with black dead arm in Bordeaux vineyards in France showed that the fungi most frequently isolated from the brown streaks in diseased trunks were *B. obtusa* and *B. dothidea*, which were isolated from 69.6% and 34.8% of all vines, respectively (Larignon *et al.*, 2001). They furthermore showed that *B. obtusa* produced dark lesions on 1-year-old canes but its frequent association with *P. viticola* and *B. dothidea* suggested that it might be a secondary pathogen. *B. dothidea* is, however, pathogenic on peach trees and invades through wounds and lenticel openings (Pusey, 1989) and is furthermore associated with Macrophoma rot or “Bot rot”, affecting bunch and muscadine grapes (Milholland, 1991).

Van Niekerk *et al.* (2004) identified 11 *Botryosphaeria* and -related species from grapevines, but demonstrated only four *Botryosphaeria* species to be highly virulent on grapevines, namely *B. australis*, *B. parva*, *B. stevensii* and *B. ribis*. In Western Australia, Taylor *et al.* (2005) isolated four species from 62.5% of the 16 vineyards sampled, namely *B. obtusa*, *B. stevensii*, *B. rhodina* and *B. australis*. They found *B. rhodina*, *B. stevensii* and *B. australis* to be pathogenic on young healthy grapevines, while *B. obtusa* did not appear to be a major pathogen on grapevines.

Botryosphaeria species have also been recovered from cankers and were determined to be the main cause of canker diseases in some Californian vineyards (Gubler *et al.*, 2005). For several years, *B. rhodina* has been known to cause wedge-shaped canker symptoms in California, and the disease it caused was referred to as ‘Bot canker’. The fungus is now considered to be an endemic species in many vineyards in areas of California that have hot

climatic conditions. In the cooler areas of California, *B. obtusa* and *B. dothidea* appeared to be the dominant species associated with these symptoms (Urbez *et al.*, 2005).

Epidemiology

Botryosphaeria species are wound pathogens entering the vine through fresh pruning wounds. Large numbers of conidia are exuded from black fruiting bodies (pycnidia) found on diseased plant parts, on the surface of bark, trunks, spurs or on the residual pruning wood left in the vineyards (Castillo-Pando *et al.*, 2001). The formation of numerous fruiting bodies provides an excellent source of spores for further infections in the vineyard. Conidia may be easily distributed over the vineyard by wind, or they may be waterborne in splashed drops from rain or sprinkler irrigation.

Botryosphaeria obtusa is found on a wide variety of woody plants other than *Vitis* spp. The pathogen has furthermore been recognised mostly as a wound pathogen, and associated with both dieback symptoms and cankers on stone and pome fruit crops (Castillo-Pando *et al.* 2001). *Botryosphaeria* spp. have also been isolated from asymptomatic plant material, showing that these fungi can survive as endophytes in apparently healthy wood (Smith *et al.*, 1996; van Niekerk *et al.*, 2002).

The temperature range for conidium production relating to *B. dothidea* are 6°C to 30°C with optimum sporulation at 24°C (Copes and Hendrix, 2004). For *B. obtusa*, optimum sporulation occurs at both 18°C and 24°C and for *B. rhodina* the optimum temperatures are between 12°C and 24°C (Copes and Hendrix, 2004).

Management

Cultural practices. Good sanitation practices are recommended. After pruning, the debris should be removed from the vineyard and preferably burnt, as this material can be a source of inoculum that can cause new infections. This practice is supported by the fact that van Niekerk *et al.* (2004) isolated several *Botryosphaeria* spp. from pruning debris collected from the vineyard floor.

As part of a preventative management strategy it is important to prevent unnecessary wounding of plants as most *Botryosphaeria* spp., which occur on grapevines, are regarded as wound pathogens (Larignon and Dubos, 2001). Fourie and Halleen (2004a) found *Botryosphaeria* spp. were isolated more frequently from pruning wound ends than from the basal ends of 2-year-old rootstock canes. When the disease has not spread throughout the whole plant,

the diseased parts can be removed, which is the same practice used for the control of Eutypa dieback. Whole infected plants should be removed from the vineyard (Sosnowski *et al.*, 2005a).

Chemical. Management of *Botryosphaeria*-related diseases is difficult since information on disease control, especially chemical control, is very limited. Currently there are no fungicides registered for use against *Botryosphaeria* dieback in South Africa. France is one of the very few countries where a fungicide, sodium arsenite, is registered for control of black dead arm (Larignon & Dubos, 2001). However, given the high toxicity of sodium arsenite, it was either banned or its use restricted.

Denman *et al.* (2004) found that tebuconazole, benomyl, prochloraz magnesium chloride, iprodione and fenarimol inhibited *in vitro* mycelial growth of *B. protearum*. They furthermore found during field trials that applying prochloraz mc alternated with mancozeb, reduced the occurrence of cankers on proteas. *In vitro* fungicide sensitivity screenings done in Australia, showed tebuconazole and fluazinam to be the most effective fungicides to reduce the mycelial growth of *B. obtusa* and *B. lutea* (Savocchia *et al.*, 2005). Ma *et al.* (2002), however, reported reduced sensitivity of *B. dothidea* isolates toward tebuconazole and therefore recommended that anti-resistance strategies be implemented.

Biological. Hunt (2004) suggested that the protective effect in the grapevine of Trichoderma products should be seen as “vaccination” of the vine and therefore has considerable potential as a treatment during the nursery propagation stage. They furthermore found that strains of *T. harzianum* have shown evidence of lysis and parasitism on various species of *Botryosphaeria*.

PHOMOPSIS CANE AND LEAF SPOT

For many years, grape growers referred to a disease as “dead arm”, of which the causal organism was considered to be *Phomopsis viticola*. In later years, however, it was demonstrated that dead arm symptoms could be caused by two different pathogens, often occurring simultaneously on grapevines. Phomopsis cane and leaf spot describes the cane and leaf spotting phase, whereas Eutypa dieback describes the canker and shoot dieback phase of what was first known as dead arm. The two diseases are distinct from one another, and their management strategies also differ. Infection by *P. viticola* can seriously damage the vine and decrease grape quality and yield (Hewitt and Pearson, 1988). The disease can result in crop loss due to shoots

breaking off where the lesion occurs, stunted shoots, loss of vigour and smaller bunches (Mostert and Crous, 2000).

Symptoms

Infected young shoots have small, pale green or chlorotic, irregular to circular spots with dark centres (Hewitt and Pearson, 1988). During rapid growth of shoots, these dark, necrotic blotches often crack and become open fissures in the cortex tissue. Dark brown to black necrotic spots may also occur along primary and secondary leaf veins and on petioles. The necrotic spots may drop out of the leaf, causing a “shot-hole” appearance. Infected young shoots, cluster stems, and petioles have chlorotic spots with dark centres.

Etiology

Phomopsis cane and leaf spot is caused by *Phomopsis viticola* (Sacc.) Sacc. (formerly known as *Fusicoccum viticolum*) (Merrin *et al.*, 1995). Various *Phomopsis* species occur on grapevine in South Africa, but it was confirmed (Mostert *et al.*, 2001) that *P. viticola* is the primary causal agent of Phomopsis cane and leaf spot.

In Australia, four *Phomopsis* species have been associated with dead arm (Merrin *et al.*, 1995). Mostert and Crous (2000) found three of these species in South African vineyards, as well as a new South African record, *P. amygdali*, which traditionally causes a dieback disease in peach trees. During a reassessment of *Phomopsis* species occurring on grapevines, van Niekerk *et al.* (2005b) identified 15 *Phomopsis* spp. from grapevine and conducted a pathogenicity trial on selected *Phomopsis* species to find that *P. amygdali* and *P. viticola* caused the most severe lesions on young grapevine shoots. *P. viticola* was furthermore isolated from pruning wounds, indicative of its ability to infect wounds.

Australian researchers reported two distinct taxa of *Phomopsis*, taxon 1 and taxon 2 (Melanson *et al.*, 2002). Taxon 2 was identified as *P. viticola* and taxon 1 as *Diaporthe perijuncta* (Mostert *et al.*, 2001). *Diaporthe perijuncta* (sexual stage of *Phomopsis* taxon 1) was also isolated from grapevine in South Africa (Mostert and Crous, 2000). Van Niekerk *et al.* (2005b) later distinguished three clades within *D. perijuncta* isolates. *D. viticola* are applied to isolates originating from Portugal and Germany while *D. australafricana* are proposed for South African and Australian isolates. In Australia, however, it was found that *D. australafricana* was not pathogenic on grapevine shoots or buds (Rawnsley *et al.*, 2004). Taxon 2 infections caused the same symptoms displayed by *P. viticola* in other parts of the world. However, taxon 1 infection manifests itself only in late winter as pycnidia on bleached canes; no symptoms are displayed on the green shoots or leaves during the growing season (Melanson *et al.*, 2002).

Epidemiology

The fungus overwinters as mycelia and pycnidia in the bark of old canes infected during previous seasons (Hewitt and Pearson, 1988). When these pycnidia are wetted by rain, irrigation or dew, thousands of spores are released. Two kinds of spores, i.e. the alpha and beta spores are released by the dark, eustromatic pycnidia through white to cream cirrhi of spore masses (Sergeeva *et al.*, 2003). Only the alpha spores cause infection of young, susceptible tissue; the function of the beta spores, which are considered sterile, is unknown (Merrin *et al.*, 1995). Sergeeva *et al.* (2003), however, reported germination of beta conidia of *P. viticola* *in vitro* and suggested that beta conidia are potentially capable of symptomless infection. Infection of alpha conidia occurs within a couple of hours in free water or at a relative humidity of 100%. Prolonged periods of rain and cold weather are prime factors in the development of an epidemic (Swart and De Kock, 1994). Spread within the vineyard is localised, remaining in close proximity to the inoculum source, because the fungus spreads mostly within a vine rather than from vine to vine. Long distance spread occurs by transport of infected or contaminated propagation materials such as budwood, cane cuttings and nursery stock (Hewitt and Pearson, 1988).

Management

Disease control depends on disease pressure, which varies from year to year, depending on the remaining inoculum and the climate (Mostert and Crous, 2000). A study done by Mostert *et al.* (2001) confirmed the observation that one *Phomopsis* species can infect more than one host and that host switching may have occurred during speciation. These findings suggest that management of diseases caused by *Phomopsis* spp. are complex since alternative hosts might act as inoculum sources for these diseases.

Cultural practices. Pscheidt and Pearson (1989) studied the effect of training and pruning practices on the occurrence of the disease. They found that vines hedged for 2 years or more showed significantly more diseased material than hand-pruned vines. Hedged vines contained more canes and dead material, hereby also increasing the inoculum potential. Growers who apply mechanical pruning practices should employ follow up pruning practices that remove dead and infected canes (Pscheidt and Pearson, 1989). This disease can effectively be managed through sanitation practices in combination with fungicide application. Shoots showing the typical lesions should be removed during pruning.

Hot water treatment (30 min at 50°C) of dormant grapevine cuttings showed a reduction in the survival of *P. viticola* in grapevine cuttings (Clarke *et al.*, 2004). However, it did not eliminate the pathogen. Hot water treatment can therefore be a non-chemical way of reducing

infection of *P. viticola* in grapevine propagation material. It is recommended to select propagation material from healthy, well-maintained vines with no symptoms of disease.

Chemical. Swart and De Kock (1994) found that four applications of chemicals at the development stages 25% budbreak and 50% budbreak, at 2.5 cm and 12.7 cm shoot length, to be effective in managing the disease. The application of fungicides is focused on protecting young growth during critical periods when infection can occur. An *in vitro* study done by Mostert *et al.* (2000), which screened fungicides for possible control of *P. viticola*, showed that sterol biosynthesis-inhibiting fungicides, flusilazole and penconazole, inhibited mycelial growth at low concentrations of the fungicides. Folpet showed moderate inhibition, with an EC₅₀ value of 4.49. They furthermore recommended the use of strobilurin fungicides as alternative to other fungicides when the disease pressure of *P. viticola* increases.

Castillo-Pando *et al.* (1997) treated dormant grapevine canes with fungicides and found that benomyl, fluazinam, mancozeb and 8-hydroxyquinoline sulphate inhibited mycelial growth and spore germination of pycnidia *in situ*. Inhibition of pycnidial viability will reduce the spread of inoculum and therefore lower disease outbreak. They suggested that a winter treatment of dormant canes with mancozeb or fluazinam should lower the carry over of disease into each season, and will reduce disease pressure on spray programs applied in spring. Dipping planting materials in these fungicides can furthermore reduce the spread of this pathogen (Castillo-Pando *et al.*, 1997). Halleen and Fourie (2005) isolated, among other trunk disease pathogens, *Phomopsis* spp. from vineyards in South Africa. They treated pruning wounds with benomyl, flusilazole, *Bacillus subtilis* and *Trichoderma* formulations and found flusilazole reduced natural *Phomopsis* infections by 53%.

PETRI AND ESCA DISEASE

Petri disease is the name describing dieback of young grapevines and was formerly known as “black goo” due to the black gum produced by the plant in response to the fungus. Not only does this disease cause dieback of grapevines, it also predisposes the wood to infections by other fungi, especially soft wood rotting fungi, leading to esca disease. Esca of grapevine is a complex of different diseases. It was proposed by Surico (2001) that when a healthy grapevine cutting gets infected in the nursery by *Phaeoconiella chlamydospora* or *Phaeoacremonium aleophilum*, the infected young plant develops symptoms described as “young esca” or “Petri disease”. Subsequent infections by wood rotting fungi, i.e. *Fomitiporia punctata*, results in

“esca proper”. Infection of a healthy young plant by *F. punctata* alone, results in classic white rot symptoms. In California, the dark, tiny spotting of the grapes is called “black measles” and in many other grape-growing areas of the world the sudden wilting of vines in summer is referred to as “apoplexy” (Mugnai *et al.*, 1999). In Australia, young esca was reported for the first time in 1999 where it affected 3- to 7-year-old vines (Edwards *et al.*, 2001). It was furthermore reported in Germany (Fischer and Kassemeyer, 2003), Spain (Armengol *et al.*, 2001) and South Africa where it was described by Ferreira *et al.* (1994) as slow dieback of grapevines caused by *Phialophora parasitica*.

Symptoms

The symptoms of Petri decline are longitudinal brown-black streaking of the vascular tissue and accumulation of black tarry material in the affected vessels. It is observed as black dots in transverse sections of the trunk. This typical exudation is not found in *Botryosphaeria* dieback, which is often confused with esca symptoms. Further symptoms can range from graft failure, weak growth, shoot dieback and slow decline of the grapevine (Edwards and Pascoe, 2004).

In the wood of trunks and arms affected with Esca, a characteristic zone of necrosis is associated with a large wound (Dubos and Larignon, 1988). A cross-sectional cut reveals a central, damaged zone, which is pale in colour and soft in texture (white rot), surrounded by an area of darker, harder wood. Sometimes the rot reaches the surface, causing cracks along the trunk; these symptoms are called “mal dello spacco” or cracking disease in Italy (Mugnai *et al.*, 1999). A longitudinal section reveals a zone of pale brown, necrotic wood, which is usually preceded by an area of hard, black wood. Sometimes the necrotic zone is sectorial because of secondary infection by esca-associated fungi in wood already invaded by *E. lata*. Various other types of wood deterioration become visible together with or even preceding white rot, i.e. small, dark brown or black spots in cross section, pink-brown or dark red-brown areas often develop from black spots mainly in the core of the trunk or on the margin of decayed or necrotic tissues and brown areas of varying shade and texture (Mugnai *et al.*, 1999).

Symptoms of esca on leaves consist of pale green or chlorotic spots between the veins or along the leaf margins that usually spread outward to the distal parts of the shoots. The spots, initially small and scattered over the lamina, gradually expand and coalesce, become partly necrotic, and ultimately leave only a narrow strip of unaffected green tissue along the main veins (Mugnai *et al.*, 1999). Foliar symptoms are probably influenced by environmental parameters

(Surico, 2001). A peculiar characteristic of esca is that visible symptoms may disappear completely during one or more successive growing seasons, making disease management more problematic. Vines that show foliar symptoms should therefore be thoroughly monitored.

Spotting of berries is common in California and southern Italy (Mugnai *et al.*, 1999). Masses of tiny spots are irregularly scattered, especially toward the distal end of the berry, therefore the name black “measles” used in California to describe this disease. Acute esca symptoms include rapid basipetal wilt of entire vines, including the clusters. This condition is called “vine apoplexy” (Edwards *et al.*, 2001) and is thought to be favoured by hot summers (Mugnai *et al.*, 1999; Edwards and Pascoe, 2004).

Etiology

Petri disease affects young vines. The organisms most consistently associated with this disease are *Phaeoconiella chlamydospora* (synonym *Phaeoacremonium chlamydosporum*) and some *Phaeoacremonium* species, particularly *Pm. aleophilum* (Mugnai *et al.*, 1999; Surico, 2001; Feliciano *et al.*, 2004; Fourie and Halleen, 2004b). *Pm. aleophilum* and *Pa. chlamydospora* were furthermore reported to be pathogenic to berries (Gubler *et al.*, 2004). Wood decay or white heart rot are also considered to be part of the esca disease complex and are caused by the basidiomycetes, *Stereum hirsutum*, *Fomitiporia punctata* and *F. mediterranea* (Mugnai *et al.*, 1999; Sparapano and Bruno, 2005). Esca and wood decay are, however, seen as two distinct diseases but may act synergistically towards decline and dieback of grapevines.

Epidemiology

Fomitiporia punctata produces its basidiocarps on many tree species and are occasionally encountered on dead vine trunks or branches left in the field or at the edge of vineyards after pruning. *Pa. chlamydospora* produces conidia during the saprobic phase on dead vine wood or other plant debris, and it was found that chlamydospores persist in the soil (Mugnai *et al.*, 1999). The inoculum can therefore be splash-dispersed and is then available to infect trunks, branches, and roots through wounds caused by pruning, grafting, or in other ways. It was also shown that *Pa. chlamydospora* and *Phaeoacremonium* spp. were present in grapevine rootstock cuttings (Fourie *et al.*, 2001) and can therefore spread in this manner (Rumbos and Rumbou, 2001). Larignon and Dubos (2000) trapped airborne *Pa. chlamydospora* conidia in a vineyard. The conidia are capable of infecting grapevine wood through wounds, therefore making management of this disease complex difficult. Sporulation of conidia were also reported on the surface of deep fissures in vine trunks and cordons (Edwards and Pascoe, 2001). This pathogen has,

however, the ability to inhabit soil. Retief *et al.* (2005) detected *Pa. chlamydospora* in South African nursery rootstock cuttings, hydration water and soil, whereas Whiteman *et al.* (2005) detected *Pa. chlamydospora* from soil used to plant rootstock mother block vines in.

Management

Cultural practices. The development of control strategies for prevention of Petri and esca disease has been restricted because little is known of how the pathogens infect and spread (Jaspers, 2001). Cortesi *et al.* (2000), however, found that *F. punctata* was unlikely to spread in the vineyard through roots or pruning tools. Contrary to these findings, Scalabrelli (2005) found that spraying the pruning tools and cut surfaces with a sodium hypochlorite (8%) solution reduced the number of esca-affected plants.

Due to the devastating effect of esca, it cannot be controlled except by trunk renewal (Calzarano *et al.*, 2004). These authors found that trunk renewal combined with fungicide injection was effective in restoring vigour of grapevines. Vigour of grapevines is very important since it is closely related to yield. Cyproconazole applied through trunk injections after renewal has a beneficial effect on vine growth and yield and is therefore considered to be part of a management strategy for esca in older vineyards (Calzarano *et al.*, 2004). However, if all the necrotic wood is not removed during trunk renewal, renewed vines can again become diseased. Therefore, the effect of trunk renewal may only be temporary. Another approach may be to make vines more resistant by applying chemicals or to apply biological agents to young established vines or to nursery vines (Di Marco *et al.*, 2004). The observation that the spread of esca tends to be along the rows of vines suggests that the tools used in grafting and pruning carry the fungal inoculum (Mugnai *et al.*, 1999). Vineyard sanitation is therefore of utmost importance.

Owing to the sometimes unsatisfactory results of chemical control, traditional cultural practices are essential to maintain vine health and vigour. Ensuring that propagation material always comes from nurseries or mother plants with no wood discolouration is of utmost importance (Mugnai *et al.* 1999). Grafting can also introduce fungi into the vine. In older vineyards showing foliar symptoms, rotted wood should be removed and the resulting wounds treated with fungicide or biological wound protectants. Pruning debris should be removed or burned, rather than being buried or chopped (Mugnai *et al.* 1999). Uprooting of all the dead vines and removal of these and other materials left over from surgery operations should be implemented. A traditional cure for esca applied in ancient times, were the cutting open of the

affected trunk and inserting a stone to keep it open (Mugnai *et al.*, 1999; Rumbos and Rumbou, 2001). The rotted wood is then exposed to air and therefore the foliar symptom development of esca is delayed for a certain time. A problem with this strategy is that bigger wounding sites are exposed, predisposing the wood to more infections.

Fourie and Halleen (2004b) concluded that a major source of *Phaeoconiella* and *Phaeoacremonium* infections during the nursery stage are not due to contaminated soil, but rather to increased colonisation of rootstocks by these fungi. Propagation material should be free of infection to prevent or eradicate contamination of material (Zanzotto *et al.*, 2001). This can be achieved by drenching material in the hydration tanks with *Trichoderma* and benomyl formulations. Hot water treatment of rootstock (30 min at 50°C) prior to grafting is also very important. Fourie and Halleen (2004b) further recommended a 1-hour cool-down period in which the cold water are amended with benomyl or tested *Trichoderma* formulations. Contrary to these findings, Rooney and Gubler (2001) found hot water treatment of grapevine cuttings to be ineffective in inhibiting *Pa. chlamydospora*, and therefore did not recommend the use of hot water as an effective control measure in California. Laukart *et al.* (2001) also confirmed these findings but furthermore suggested hot water treatment should be applied as a disinfectant during the propagation process, rather than a curative treatment. They found phosphonate to be a potential curative treatment for young vines infected with *Pa. chlamydospora*.

Treatments with fungicides, hot water and *Trichoderma* all have varying results, and therefore increasing the plant's natural defense or resistance may be a means to prevent Petri disease (Del Rio *et al.*, 2004). Phenolics are well-known antifungal and antibacterial compounds, and the accumulation of these compounds at the infection site is one of the first plant responses. *In vitro* studies by Del Rio *et al.* (2004) showed that *p*-coumaric acid (3 g l⁻¹) inhibited *Pa. chlamydospora* growth by more than 50%. They found similar results with *Pm. aleophilum* and *E. lata*. Application with 0.3% Brotomax, containing *p*-coumaric acid, to vines increased polyphenolic levels, thereby enhancing the disease tolerance or resistance of these vines (Del Rio *et al.*, 2004).

Chemical. In France, Portugal and Spain, chemical control is still carried out through the use of sodium arsenite treatments, as its use has not yet been banned in these countries (Mugnai *et al.*, 1999). In a fungicide sensitivity study done by Jaspers (2001), it was found that benomyl and carbendazim were effective in inhibiting *in vitro* mycelial growth of *Pa. chlamydospora* with EC₅₀ values of less than 0.08 µg/ml and for pyrimethanil and cyprodinil/fludioxonil, EC₅₀ values were less than 0.02 µg/ml. The author concluded that some systemic fungicides may

inhibit internal spread of *Pa. chlamydospora* within vines and that regular foliar application of systemic fungicides on young vines, especially during periods of environmental stress, may enable vines to overcome the effects of the pathogen during establishment. It was further found that hydroxyquinoline sulphate reduced germination of *Pa. chlamydospora* conidia with an EC₅₀ value of 0.0015 µg/ml. Groenewald *et al.* (2000) found benomyl, fenarimol, kresoxim-methyl, prochloraz manganese chloride and tebuconazole to be effective in mycelial inhibition of *Pa. chlamydospora* with EC₅₀ values ranging from 0.02 to 0.46 µg/ml.

Biological. Di Marco *et al.* (2004) did experiments with *Trichoderma* on the control of esca and found that hairy root development was four times as great in cuttings dipped in *Trichoderma* soon after graft callusing, as in cuttings not treated with the biological agent. A better root development would increase water and nutrient uptake therefore making the vine more resistant to stress related diseases. Similar findings were observed by Fourie *et al.* (2001) in grapevine nurseries.

CONCLUSION

Grapevine trunk diseases are complex and not yet fully understood. Although these diseases are described and studied separately, the pathogens causing the various diseases often act synergistically when causing disease symptoms. Disease diagnosis is sometimes erroneously based on symptomatology alone and potentially inaccurate diagnosis would result in the recommendation of incorrect management strategies. It is therefore obvious that these diseases should be treated as a complex of pathogens, in diagnosis, research and management.

A combination of cultural practices, pruning wound treatments and sanitation, are important in formulating an effective disease management strategy against the trunk disease complex. An understanding of wound response mechanisms is also important in developing management strategies to reduce wound susceptibility by promoting wound response and the application of wound protecting agents. Furthermore, the periodicity and extent of inoculum dose in the air should also be considered as it will have a strong effect on seasonal patterns of infection. Use of fungicides with both protectant and curative activity against trunk pathogens must be implemented.

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2. TRUNK DISEASE PATHOGENS OF TABLE GRAPEVINES: SYMPTOMATOLOGY AND DISTRIBUTION IN SOUTH AFRICA

ABSTRACT

Phaeomoniella chlamydospora, *Eutypa lata*, *Phomopsis*, *Phaeoacremonium*, and *Botryosphaeria* spp. are important trunk disease pathogens that cause premature decline and dieback of grapevines. Previous research in South Africa has focused primarily on wine grapes and the incidence and symptomatology of these pathogens on table grapes are largely unknown. A survey was therefore conducted to determine the status and distribution of these pathogens and associated symptoms in climatically diverse table grape growing regions. Fifteen farms were identified in the winter rainfall (De Doorns, Paarl and Trawal) and summer rainfall (Upington and Groblersdal) areas. Samples were taken in July and August 2004 from Dan-ben-Hannah vineyards that were 8 years and older. Distal ends of arms were removed from 20 randomly selected plants in each vineyard. These sections were dissected and isolations were made from each of the various symptom types observed: brown or black vascular streaking, brown internal necrosis, wedge-shaped necrosis, watery necrosis, esca-like brown and yellow soft wood rot, as well as asymptomatic wood. Fungal isolates were identified using molecular and morphological techniques. *Pa. chlamydospora* was most frequently isolated (46.0%), followed by *Phaeoacremonium aleophilum* (10.0%), *Phomopsis viticola* (3.0%), *Botryosphaeria obtusa* (3.0%), *B. rhodina* (2.2%), *B. parva* (2.0%), *Fusicoccum vitifusiforme* (0.6%), *B. australis*, *B. dothidea* and an undescribed *Diplodia* sp. (0.2% each), while *E. lata* was not found. Most of these pathogens were isolated from a variety of symptom types, indicating that disease diagnosis can not be based on symptomatology alone. *Pa. chlamydospora* was isolated from all areas sampled, although most frequently from the winter rainfall region. *Pm. aleophilum* was found predominantly in Paarl, while *P. viticola* only occurred in this area. Although *B. obtusa* was not isolated from samples taken in De Doorns and Groblersdal, it was the most commonly isolated *Botryosphaeria* sp., being dominant in Upington and Trawal. *B. rhodina* occurred most frequently in Groblersdal and *B. parva* in Paarl, while *B. australis* was isolated from Paarl only. The rest of the isolates (33%) consisted of sterile cultures, *Exochalara*, *Cephalosporium*,

Wangiella, *Scytalidium*, *Penicillium* spp. and two unidentified basidiomycetes, which were isolated from five samples with yellow esca-like symptoms from the Paarl area. These findings clearly illustrate that grapevine trunk diseases are caused by a complex of fungal pathogens, which has serious implications for disease diagnosis and management.

INTRODUCTION

Table grape production in South Africa is of great economic importance. In terms of exported volume, table grapes are among the top ten agricultural commodities. During the 2003/2004 seasons, a total of 427,491 ton table grapes were produced, of which 239,500 ton were exported (<http://www.oabs.co.za>). Major table grape production areas in South Africa are the Western Cape, Northern Cape, Limpopo and Mpumalanga (Fig. 1). However, the sustainability as well as yield quantity and quality of table grape production are negatively affected by grapevine trunk disease pathogens that cause premature decline and dieback. These pathogens, which have also been reported elsewhere (Armengol et al. 2001; Edwards and Pascoe 2004; Fischer and Kassemeyer 2003; Gatica et al. 2001; Larignon and Dubos 1997; Rumbos and Rumbou 2001), include *Eutypa lata* (Ferreira, Matthee, and Thomas 1989), *Phaeoconiella chlamydospora* (= *Phaeoacremonium chlamydosporum*) (Ferreira, van Wyk, and Calitz 1999; Groenewald, Bellstedt, and Crous 2000) and several species in the genera *Phaeoacremonium* (Mostert, Groenewald et al. 2005), *Botryosphaeria* (van Niekerk, Crous, and Groenewald 2004) and *Phomopsis* (Mostert et al. 2001). *Eutypa lata* causes the formation of characteristic pruning wound cankers. A cross section of the affected area on the cordon or trunk typically shows a wedge-shaped zone of necrotic sapwood extending from the point of origin of the canker. The dead wood is hard and the flow of water and nutrients is thus impeded (Sosnowski, Creaser, and Wicks 2003). Petri disease, caused by *Pa. chlamydospora* and several *Phaeoacremonium* spp., cause weak growth, shoot dieback and slow decline of young grapevines. In older grapevines, these pathogens are part of the esca disease complex, which might involve chronic decline or rapid dieback following wood rotting by basidiomycete fungi, such as *Fomitiporia* and *Stereum* spp. (Mugnai, Graniti, and Surico 1999). *Botryosphaeria* spp. were found to be associated with reduced growth and death of cordons. Internal symptoms such as brown wood streaking and wedge-shaped necrosis are commonly associated with these pathogens (van Niekerk, Crous, and Groenewald 2004; van Niekerk, Fourie et al. 2005). In South Africa, *Phomopsis viticola* is well-known as the causal organism of Phomopsis cane and leaf spot (Mostert et al., 2001), but has

recently also been isolated from symptoms associated with trunk diseases such as eutypa dieback, esca and Petri disease (Edwards and Pascoe 2004; Fischer and Kassemeyer 2003).

The incidence of these pathogens appears to be affected by climate, as regional occurrence of specific pathogenic species was reported to vary from absent to predominant. Semillon grapevine material from the Hunter region in New South Wales with dieback or decline symptoms, has been examined extensively for 8 years (Castillo-Pando et al. 2001). The authors never detected *E. lata* and the most frequently isolated fungus was *B. obtusa*. Urbez et al. (2005) studied the occurrence of *Botryosphaeria* spp. in different Californian vineyards and found the pathogen closely associated with internal wedge-shaped cankers, similar to the symptom expressed by *E. lata*. They isolated *B. obtusa* and *B. rhodina* from the hot regions and *B. dothidea* and *B. parva* from the colder areas of California. *B. obtusa* was the most commonly isolated species. Merrin et al. (1995) studied the variation of *Phomopsis* on grapevine in Australia and found Taxon 1 (*Diaporthe australafricana*) predominantly in vineyards occurring on the coastline and Taxon 2 (*P. viticola*) in vineyards occurring inland. Eskalen and Gubler (2001) trapped airborne spores of *Pa. chlamydospora*, *Pm. aleophilum* and *Pm. inflatipes* (later reported to be *P. aleophilum*) during and following winter and spring rainfall in Californian vineyards. Management of these diseases focuses on wound protection as most of these pathogens infect the vine through pruning wounds (Ferreira, Matthee, and Thomas 1989; Larignon and Dubos 2000; Mostert, Halleen et al. 2005; Mugnai, Graniti, and Surico 1999; van Niekerk, Fourie et al. 2005). However, the pathogen profiles in different climatic regions might differ and would impact on the efficacy of management strategies that are practiced. In South Africa, research on grapevine trunk diseases have focused mostly on wine grape vineyards situated in the Western Cape Province (Ferreira 1994; Ferreira, Matthee, and Thomas 1989; Ferreira, van Wyk, and Venter 1994; van Niekerk, Crous, and Groenewald 2004).

The aim of this study was therefore to determine the distribution and symptomatology of trunk disease pathogens in table grape vineyards from climatically different regions of South Africa.

MATERIALS AND METHODS

Regions. Regions that differ climatically in terms of rainfall and temperature patterns were chosen for the survey. During August and September 2004, 15 farms were sampled in De Doorns, Paarl and Trawal in the Western Cape region, Upington in the Northern Cape region

and regions in the north-eastern part of South Africa, which included Naboomspruit and Nylstroom in the Limpopo province and Groblersdal in the Mpumalanga province (Fig. 1). The latter regions are collectively treated as 'Groblersdal'. The Western Cape has a winter rainfall pattern whilst the Northern Cape and Groblersdal have summer rainfall patterns. Average monthly temperatures and rainfall for these regions are given in Figs 2 and 3.

Plant material. The table grape cultivar Dan-ben-Hannah was sampled, as this cultivar is one of the oldest established varieties in South Africa and is planted in the winter and summer rainfall areas. This cultivar is furthermore one of the largest export cultivars in South Africa with 1,695,735 cartons passed for export during the 2003/2004 season (<http://www.oabs.co.za>). All the vineyards sampled were older than 8 years. Twenty plants were randomly selected from each farm where the distal end of a cordon, containing a cordon section and spur with pruning wounds, was sampled.

Isolations. The cordon sections were cut into smaller pieces and split longitudinally to expose any internal symptoms. Symptom types were classified as black streaking, brown streaking, wedge-shaped necrosis, brown internal necrosis, watery necrosis and esca-like brown and yellow soft wood rot (Fig. 4). Sections representing each symptom type were selected and surface-sterilised by submersion in 5% NaOCl for 1 min, followed by 70% ethanol for 30 s before drying under a laminar flow hood. Wood pieces showing soft wood rotting symptoms were not surface-sterilised to facilitate isolation of basidiomycetes. For each symptom type, as well as asymptomatic wood, five 0.5 × 1-mm-sections were aseptically removed from the interface between symptomatic and asymptomatic tissue, plated onto 2% potato-dextrose agar (PDA; Biolab, Wadeville, South Africa) and incubated at 23°C for 4 weeks. Throughout this 4-week period, subcultures were made onto fresh PDA plates from wood pieces showing fungal growth.

Identification. Preliminary identification of fungal cultures to genus level was based on morphological characteristics. All cultures from *Botryosphaeria*, *Eutypa*, *Phaeoacremonium*, *Phaeoconiella*, *Phomopsis* and related genera hosting known trunk disease pathogens were selected for further identification. *Pa. chlamydospora* was identified to species level based on morphological characteristics. Single-conidial strains were obtained from other genera for molecular and morphological species identification. *Botryosphaeria* cultures were grown on water agar (WA; Biolab, Wadeville, South Africa) and single hyphal tips were transferred to PDA after 2-3 days of incubation (25°C) in order to obtain pure cultures. Cultures were stored at 18°C under water, mineral oil and glycerol, as well as PDA slants and are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U). All *Botryosphaeria*, *Phomopsis* and *Phaeoacremonium* isolates were subjected to molecular identification.

Botryosphaeria isolates were prepared for internal transcribed spacer (ITS) and translation elongation factor 1- α (EF1- α) sequence analyses as described by van Niekerk *et al.* (2004). Genomic DNA was isolated from pure cultures using the GenElute™ Plant Genomic DNA Miniprep Kit (SIGMA, Sigma-Aldrich Corporation St. Louis, USA). The primer pairs ITS1 and ITS4 or ITS6 and ITS4 were used for amplification of the internal transcribed spacer region of the nuclear rRNA, spanning the 3' end of the 18S (small subunit) rRNA gene, ITS1 region, the 5.8S rRNA gene, the ITS2 region and the 5' end of the 28S (large subunit) of the rRNA gene (White *et al.* 1990). Isolates that could not be identified with definite certainty based on ITS sequences were selected for amplification of the EF1- α gene using the primers EF1-728F and EF1-986R (Carbone and Kohn 1999). PCR conditions as used by van Niekerk *et al.* (2004) were used to amplify these gene areas. *Phomopsis* isolates were selected for ITS sequence analysis and fungal genomic DNA was extracted as described for *Botryosphaeria* spp. The primers ITS4 and ITS6 (White *et al.* 1990) were used to amplify the ITS regions of the nuclear rRNA using PCR conditions published by van Niekerk *et al.* (2005) were used for amplification. *Phaeoacremonium* spp. were selected for β -tubulin sequence analysis and fungal genomic DNA were extracted following the isolation protocol of Lee and Taylor (1990). The primer pair T1 and Bt2b were used to amplify the β -tubulin gene, using PCR conditions recommended by Mostert *et al.* (2005).

All PCR reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, California, USA) and products were separated by electrophoresis in a 1% (w/v) agarose gel in 0.5 \times TAE running buffer (0.4 M Tris, 0.05 M NaAc and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) after ethidium bromide staining. The PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, USA). Purified PCR products were sequenced in both directions using the PCR primers. The sequencing reaction was carried out with an ABI Prism Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California, USA), containing AmpliTaq DNA Polymerase, as recommended by the manufacturer. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut, USA). The ITS, EF1- α and β -tubulin sequences were aligned using Sequence Alignment Editor v2.0a11 and manually edited where necessary and compared with respective sequences deposited in GenBank (<http://www.ncbi.nlm.nih.gov>).

In order to confirm molecular identification, morphological identification of the various species was done based on colony characteristics (on PDA, malt extract agar and oatmeal agar) and other morphological characteristics, such as conidial length and width, and phialide type and length in the case of *Phaeoacremonium* species. The frequency of isolation in each region was determined for each species. Symptom types associated with each species were also calculated as a proportion of the species' frequency of isolation.

RESULTS

Isolations and fungal identification. In total, 1468 fungal isolations were made from asymptomatic and symptomatic wood in the sampled Dan-ben-Hannah cordon sections. The most frequently isolated fungus was *Pa. chlamydospora* (46%), followed by *Phaeoacremonium aleophilum* (10%), various *Botryosphaeria* spp. (8%) and *Phomopsis viticola* (3%). No *Eutypa* isolates were found in the sampled areas. The rest of the isolates (33%) consisted of sterile isolates, *Exochalara*, *Cephalosporium*, *Wangiella*, *Scytalidium* spp. (mitosporic fungi) and five basidiomycete isolates, representing two unidentified species, that were isolated from yellow esca-like symptoms in the Paarl area, as well as *Penicillium* spp. and bacteria.

Phaeomoniella chlamydospora was isolated from all the regions sampled, although most frequently from the winter rainfall areas, with brown internal necrosis and black streaking being the two most common symptoms from which it was isolated (Fig. 5). Black streaking was the only symptom type from which *Pa. chlamydospora* was isolated in all the areas. *Pa. chlamydospora* was furthermore isolated from all the symptom types described, except asymptomatic wood.

Pm. aleophilum was isolated predominantly from the Paarl area (Fig. 6) and mostly from wedge-shaped necrosis, brown soft wood rot symptoms and brown internal necrosis. No isolates were found in the Upington area, nor was it isolated from asymptomatic and watery necrotic wood.

Botryosphaeria isolates were isolated mostly from Groblersdal in the summer rainfall area (Fig. 7). These fungi were primarily isolated from wedge-shaped necrotic lesions. *B. obtusa*, the species most frequently observed, was isolated from wedge-shaped necrosis, brown internal necrosis, brown streaking, watery necrosis and brown and yellow soft rot. It was found in Western Cape areas (Trawal and Paarl) and Northern Cape areas (Upington). *B. rhodina* was

only isolated from Groblersdal (summer rainfall area) and from wedge-shaped necrosis, brown soft wood rot, black streaking and brown internal necrosis. *B. parva* occurred in the Groblersdal and Paarl areas and was isolated from brown internal necrosis and wedge-shaped necrosis. *B. australis* was isolated once only and from black streaking in the Paarl area. *B. dothidea* was also isolated once only and from wedge-shaped necrosis in the Groblersdal area. *Fusicoccum vitifusiforme* was isolated from Groblersdal and was associated with brown internal necrosis, black streaking and wedge-shaped necrosis. An undescribed *Diplodia* species was isolated in the De Doorns area from asymptomatic wood.

Phomopsis viticola was the only *Phomopsis* sp. isolated and was isolated from the Paarl area only. It was associated mostly with watery necrosis, wedge-shaped necrosis and brown soft wood rot symptoms (Fig. 9).

DISCUSSION

This study presents the first comprehensive report on the occurrence and distribution of trunk disease pathogens on table grapevines from climatically and geographically diverse regions in South Africa. In this study, fungal isolations were made from various symptom types present in cordon sections of mature grapevines. Most of these symptoms originated from wounds, which represent several years' pruning cuts. All grapevine trunk disease pathogens are reported to be wound invading pathogens (Ferreira, Matthee, and Thomas 1989; Larignon and Dubos 2000; Mostert, Halleen et al. 2005; Mugnai, Graniti, and Surico 1999; Munkvold and Marois 1995; van Niekerk, Fourie et al. 2005; van Niekerk, Groenewald et al. 2005), whereas certain opportunistic pathogens are known to cause disease or symptoms on stress-predisposed hosts only (Ferreira, van Wyk, and Calitz 1999; Larignon and Dubos 2000; Pusey 1989), and symptom expression of others can be variable between seasons (Creaser and Wicks 2001; Erincik et al. 2003; Mugnai, Graniti, and Surico 1999). Therefore, the underlying principle of this survey was that randomly sampled cordon sections would represent a non-biased indication of the occurrence of trunk disease pathogens in each area. The survey might, however, be biased towards pathogens that invade pruning wounds during the period of susceptibility. This susceptibility period has been reported to be as long as 2 weeks after pruning for *Eutypa lata* (Petzoldt, Moller, and Sall 1981), but a recent study has demonstrated that pruning wounds remained susceptible to infection by a range of trunk disease pathogens for at least 3 weeks (J.M. van Niekerk, pers. comm.). To ensure successful infection of pruning wounds, inoculum of these pathogens should therefore be present during the period of pruning wound susceptibility, which, depending on pruning time, would be between July and October. Lecomte *et al.* (2001) furthermore demonstrated the ability of *Eutypa lata* to infect desuckering wounds on cordons during spring, but later found that the *in natura* occurrence of these infections was very low (Lecomte et al. 2005). Nonetheless, the possibility of infection of desuckering wounds on sampled cordons by *Eutypa lata* and other trunk disease pathogens during spring (October to November) should be considered.

Several trunk disease causing pathogens, most notably *Eutypa lata*, *Phomopsis viticola*, *Phaeomoniella chlamydospora* and several *Botryosphaeria* and *Phaeoacremonium* spp., were previously reported from wine grapes in South Africa (Ferreira, Matthee, and Thomas 1989; Fourie and Halleen 2004; Mostert and Crous 2000; Mostert, Groenewald et al. 2005; van Niekerk, Fourie et al. 2005). In the present study, all these pathogens, except *E. lata*, were isolated. However, the incidence of these pathogens varied between regions, most likely due to differences in climatic conditions. The absence of *E. lata* from regions other than Paarl could be expected, as it was reported that this pathogen only occurs in areas where mean annual rainfall exceeds 600 mm and are unlikely to occur in areas where the annual rainfall is below 250 mm (Carter 1988). Its apparent absence from table grapes in the Paarl region was, however, unexpected as typical *Eutypa* dieback symptoms (wedge-shaped necrosis) were frequently observed, and this pathogen is reported to be of major economic importance in this winter rainfall region (Halleen, Volkmann, and H. 2001; van Niekerk, Fourie, and Halleen 2003). Its absence might be attributed to the predominance of the faster-growing *B. obtusa* and *B. parva* from these symptoms in Paarl.

Phaeomoniella chlamydospora and *Phaeoacremonium aleophilum*, the main causal organisms of Petri disease (Mugnai, Graniti, and Surico 1999), was the most commonly isolated pathogens. Contrary to previous reports from South Africa (Groenewald et al. 2001; Mostert, Groenewald et al. 2005), *Pm. aleophilum* was the only *Phaeoacremonium* sp. isolated. Apart from *Pm. aleophilum* being absent from Upington, both these Petri disease pathogens were isolated from all surveyed regions. However, these pathogens were most frequently isolated from material sampled in Paarl, which had the highest monthly rainfall from April to September (autumn to spring), and least frequently from the summer rainfall areas, Upington and Groblersdal. For *Pa. chlamydospora*, it was especially evident that distribution patterns were influenced by rainfall, as it was also less frequently isolated from the other winter rainfall regions sampled (De Doorns and Trawal), which receive three to four times less winter rain than Paarl. In general, *Pm. aleophilum* was isolated almost five times less than *Pa. chlamydospora*, which concurs with previous reports that the latter pathogen is the main Petri disease pathogen in South Africa (Fourie and Halleen 2001). Infected propagation material is reported to be the major means of long-distance distribution of these pathogens (Edwards et al. 2003; Fourie and Halleen 2004; Fourie and Halleen 2002, 2004; Mugnai, Graniti, and Surico 1999; Retief et al. 2005; Whiteman et al. 2003). Most of South Africa's nurseries are situated in the Western Cape (Vine Improvement Association, Paarl 7622, South Africa) and grapevines planted in South Africa would in all probability have originated from the same propagation material as the other regions. The initial inoculum for Petri disease would therefore have been present in all the areas surveyed, but disease progress was inhibited, most likely by the lack of winter rains and possibly also higher temperatures. Eskalen and Gubler (2001) trapped *Pa. chlamydospora* and *Pm. inflatipes* (*Pm. aleophilum*) spores during and following rainfall in late winter and early spring. They furthermore trapped spores of *Pm. aleophilum* more commonly in early to mid-summer and concluded that *Pm. aleophilum* did not infect susceptible pruning wounds during winter. In accordance with these findings, Edwards and Pascoe (2004) found *Pm. aleophilum* to be associated with hot regions. However, in our study, *Pm. aleophilum* were completely absent in the Upington area, which has a very hot and dry climate. It was shown by Adalat et al. (2000) and Gaforio et al. (2005) that *Pa. chlamydospora* is a more aggressive pruning wound invader than *Pm. aleophilum*, which might also explain the absence of *Pm. aleophilum* in Upington.

Pa. chlamydospora and *Pm. aleophilum* are primarily associated with Petri and esca disease of young (1-5 years) and mature grapevines (10 years and older), respectively (Mugnai et al.,

1999). In Petri-diseased young grapevines, these pathogens are typically associated with black vascular streaking of the wood (Mugnai *et al.*, 1999). In this survey, *Pa. chlamydospora* were consistently isolated from brown and black streaking symptoms in all the areas sampled, emphasising the pathogen's association with this symptom type. It was also isolated from more severe (i.e. progressive) symptom types, such as brown internal necrosis, watery necrosis, wedge-shaped necrosis and brown and yellow soft wood rot. The latter two symptom types are typical of esca disease (Mugnai, Graniti, and Surico 1999). In this survey, *Pm. aleophilum* was rarely isolated from Petri disease symptoms, and was mostly associated with the more progressive symptom types, which corroborates reports by Gatica *et al.* (2001) and Larignon and Dubos (1997), who associated this species mostly with soft wood rot and sectorial brown necrosis. Two unidentified basidiomycete species were also isolated from the soft wood rot symptoms, but from the Paarl region only. This is in accordance with esca disease, where several basidiomycete species in genera such as *Fomitiporia* and *Stereum* are reported to be involved in this disease complex (Mugnai *et al.*, 1999). The absence of basidiomycete fungi from soft wood rotting symptoms in other regions is unclear, and can probably be ascribed to the isolation techniques used, which might have favoured the faster-growing fungi. *Pa. chlamydospora* and *Pm. aleophilum* were not isolated from asymptomatic wood, although a biotrophic / endophytic phase has been reported for these pathogens (Chiarappa 2000; Fourie and Halleen 2002; Larignon and Dubos 2000; Mugnai, Graniti, and Surico 1999; Phillips 2002).

Eight percent of the fungal isolates obtained in this survey were identified as *Botryosphaeria* spp. Species in this genus are associated with cankers and dieback of fruit trees (Arauz and Sutton 1989; Pusey 1989), pistachio (Ma, Luo, and Michailides 2004), pine trees (Slippers *et al.* 2005) and proteas (Denman *et al.* 2004). *Botryosphaeria* spp. are increasingly reported from diseased grapevines across the world and are associated with internal wood necrosis, dieback and canker formation (Phillips 2002; Rumbos and Rumbou 2001; van Niekerk, Crous, and Groenewald 2004; Wood and Wood 2005). In a survey that consisted of symptomatic and asymptomatic grapevines, van Niekerk *et al.* (2004) confirmed the occurrence of *B. australis*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina* and a *Diplodia* sp. from grapevines in South Africa, while *Diplodia porosum*, *Fusicoccum viticlavatum* and *F. vitifusiforme* were described as new. *B. dothidea*, *B. ribis* and *B. stevensii*, which are reported as grapevine pathogens (Phillips 1998, 2002), were not found. In the present study, *B. obtusa*, *B. rhodina*, *B. parva*, *B. australis*, *F. vitifusiforme*, the undescribed *Diplodia* sp. as well as *B. dothidea* were found. Of these species, *B. obtusa*, *B. parva* and *B. rhodina* were isolated most often. *B. obtusa* and *B. parva* were found in climatically diverse areas, while *B. rhodina* was found in the subtropical Groblersdal region only. The remaining species were isolated markedly less frequently and each occurred in one region only: *B. dothidea* and *F. vitifusiforme* in Groblersdal, *B. australis* in Paarl and the *Diplodia* sp. in De Doorns.

These findings are largely in accordance with reports from California (Urbez *et al.*, 2005) and Australia (Taylor *et al.*, 2005), where *B. obtusa* was the most common species. *B. obtusa* appeared not to be very specific with regard to climatic conditions, as it was isolated from arid areas with low summer rainfall, very high summer temperatures and frosty winters (Upington), hot summers and mild winters with moderate levels of winter rainfall (Trawal) and mild winters with high winter rainfall and comparatively mild summers (Paarl). From these areas, it was isolated more frequently from Trawal, than from Upington and Paarl, roughly in a 10:4:3 ratio. It should be noted that all vineyards sampled were drip- or micro-irrigated, which might affect epidemic dynamics during the growing season, but not necessarily during the pruning period. For *B. obtusa*, optimum sporulation occurred at temperatures 18°C and 24°C (Copes and Hendrix 2004), which certainly fall within the

temperature range for all these regions. Most researchers, however, consider this species a saprophyte (Larignon and Dubos 1997; Mugnai, Graniti, and Surico 1999) or only weakly pathogenic on grapevine (Phillips 1998). Although this species was isolated from almost all symptom types described, its role as a saprophyte or pathogen remains unclear.

B. parva also occurred in climatically diverse regions: it was isolated from the subtropical Groblersdal region (summer rainfall), Paarl and Trawal region (winter rainfall) roughly at a 6:4:1 ratio. It was isolated most commonly from typical *Botryosphaeria* dieback symptoms (wedge-shaped necrosis and brown internal necrosis), but also from soft wood rotting symptoms, which is atypical for *Botryosphaeria* spp. Its association with the latter symptom types is therefore suspected to be saprophytic.

Urbez *et al.* (2005) found that *B. rhodina* was the only species occurring in the warmer regions of southern California. The optimum growth temperature for *B. rhodina* was reported as 30°C (CABI 2005) and optimum temperature for sporulation as between 12°C and 24°C (Copes and Hendrix 2004). *B. rhodina* was also the only species found on table grapes in subtropical Western Australia (Wood and Wood, 2005) where up to 90% of plants died as a result of *B. rhodina*-induced cane dieback. These authors attributed higher disease incidence mostly to increased summer rainfall. This aspect might also explain the apparent absence of *B. rhodina* from Upington, which receives on average four times less summer rainfall (November to February), than Groblersdal. *B. rhodina* was mostly associated with wedge-shaped necrosis, which corroborates findings by Wood and Wood (2005). This species was, however, also associated with soft wood rotting symptoms. As with *B. parva*, this association is supposed to be saprophytic.

The remaining *Botryosphaeria* spp., *B. australis*, *B. dothidea* and *F. vitifusiforme*, were all isolated from typical *Botryosphaeria* dieback symptoms, namely black streaking (*F. vitifusiforme*), brown internal necrosis (*B. australis* and *F. vitifusiforme*) and wedge-shaped necrosis (*B. dothidea* and *F. vitifusiforme*), while the *Diplodia* sp. was isolated from asymptomatic wood. *B. australis* was shown to be one of the most virulent *Botryosphaeria* spp. (van Niekerk *et al.*, 2004). However, this species appeared not to be widely distributed in South Africa as it was only isolated once from the Paarl region. *F. vitifusiforme* is a newly described species by van Niekerk *et al.* (2004) that was isolated from the Western Cape region. In the present survey, it was not found in this winter rainfall area, but was only isolated from the summer rainfall region Groblersdal. This species was, however, not shown to be highly virulent on green shoots, mature canes or mature wood (van Niekerk *et al.*, 2004). *B. dothidea* was only isolated once and from Groblersdal, which support findings by Doidge *et al.* (1953). The temperature range for conidium production of *B. dothidea* ranges from 6°C to 30°C (Copes and Hendrix 2004), which indicates that it might be suited to climatically diverse regions other than the subtropical Groblersdal region. The latter statement is in part supported by the fact that Urbez *et al.* (2005) found *B. dothidea* to be prevalent in the colder areas of California. The *Diplodia* sp. was isolated from De Doorns, one of the coolest areas sampled, and was furthermore the only *Botryosphaeria* related species found in that area. Van Niekerk *et al.* (2004) demonstrated it to be weakly pathogenic, and since it was isolated from asymptomatic wood in this survey, might therefore not play an important role in the trunk disease complex.

In South Africa, *Phomopsis viticola* most commonly cause cane and leaf blight (Mostert and Crous 2000; Mostert *et al.* 2001), but was recently implicated as a trunk disease pathogen (van Niekerk *et al.*, 2005b). Edwards and Pascoe (2004), Fischer and Kassemeyer (2003) and Larignon and Dubos (1997) also isolated *P. viticola* from esca-affected grapevines, while

Phillips (1998) isolated it from vines with excoiiose. Van Niekerk *et al.* (2005) isolated *P. viticola* as well as *P. amygdali*, a well-known peach pathogen, from grapevine pruning wounds and asymptomatic nursery material respectively, and determined that both species were pathogenic on green grapevine shoots. These authors furthermore distinguished 13 other *Phomopsis* spp. from grapevine, all of which appeared to be non-pathogenic. In the present study, *P. viticola* was the only *Phomopsis* spp. isolated. It was furthermore isolated from the Paarl region only, supporting reports that it is favoured by wet, cool spring weather (Hewitt and Pearson 1988; Swart and De Kock 1994). It was frequently isolated from wedge-shaped necrosis, but also from asymptomatic wood and other symptom types. Since it was isolated from such a wide variety of symptom types, the actual role of *P. viticola* as a primary trunk disease causing pathogen remains unclear.

Results from this study clearly indicate the complex nature of grapevine trunk diseases. Each region had its own unique pathogen profile that should be considered when implementing management strategies. Moreover, the symptomatology of these pathogens varied and overlapped, which indicates that diagnosis cannot be based on symptoms alone.

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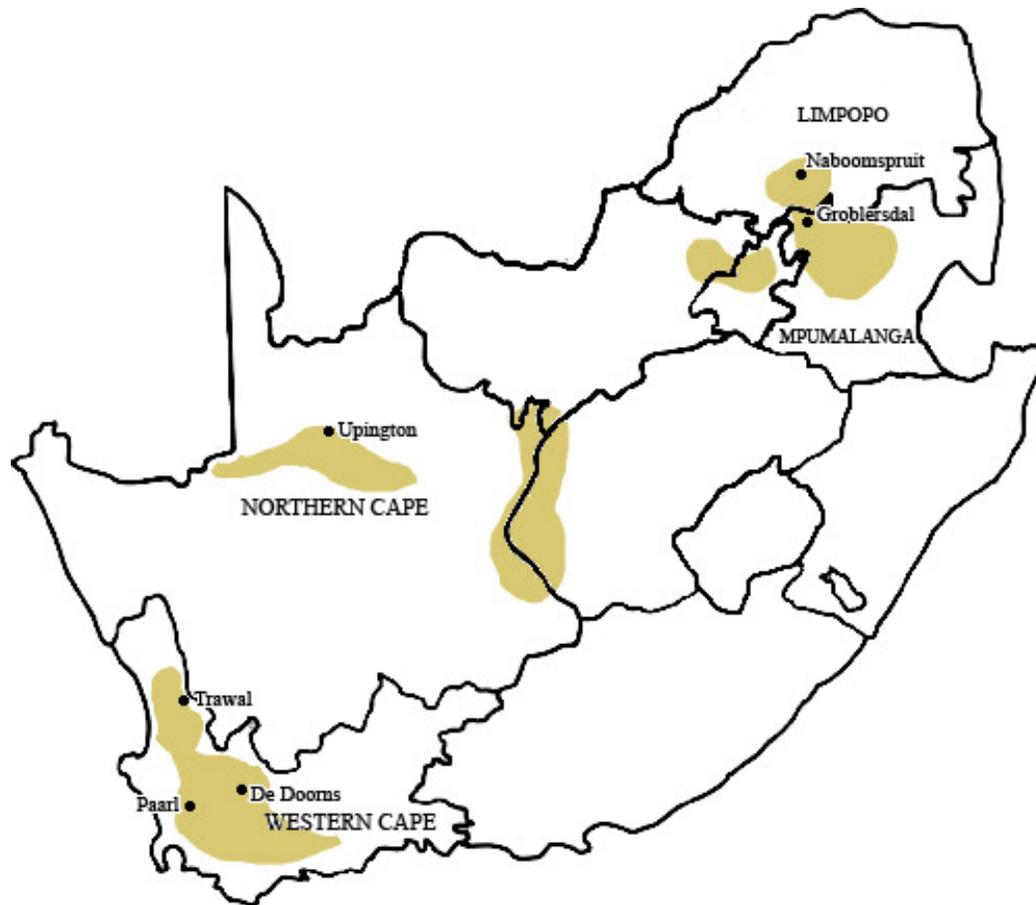


Figure 1. Map of South Africa showing the major table grape growing regions in the Western Cape, Northern Cape, Mpumalanga and Limpopo provinces.

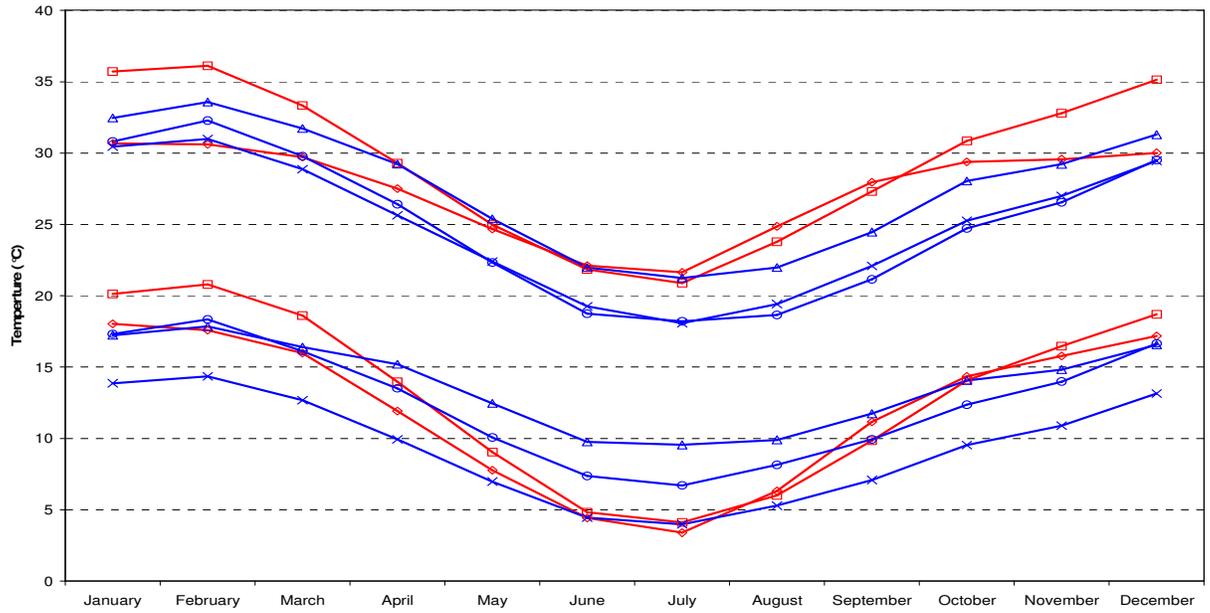


Figure 2. Average monthly minimum and maximum temperatures for the 1994-2004 period in Upington - □, Groblersdal - ◇, Trawal - △, De Doorns - X and Paarl - ○.

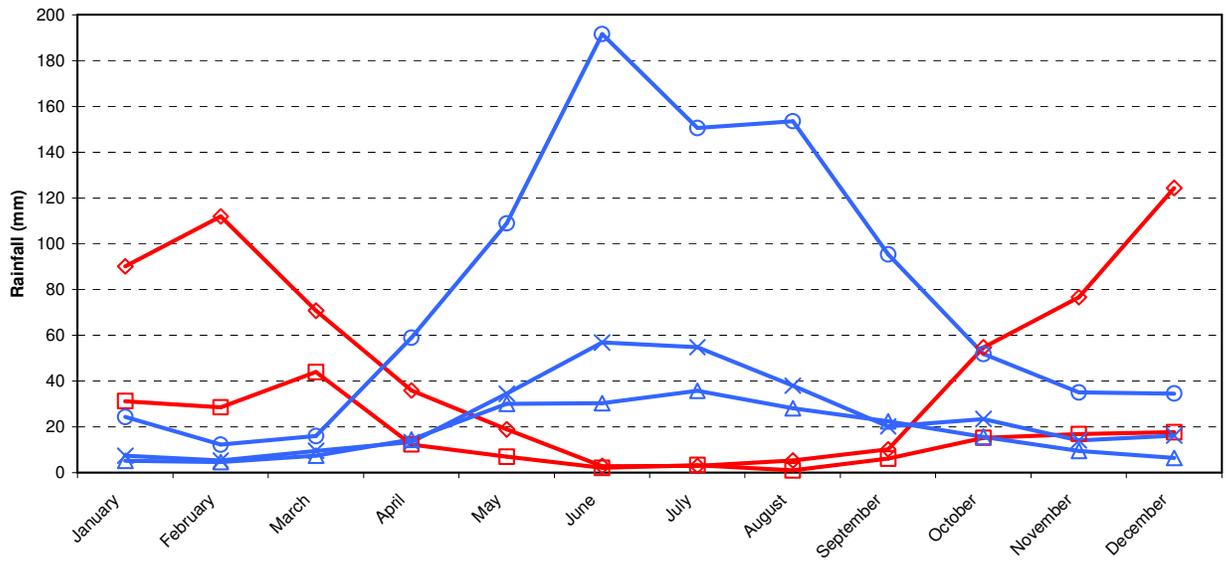


Figure 3. Average monthly rainfall patterns for the 1994-2004 period in Upington - □, Groblersdal - ◇, Trawal - △, De Doorns - X and Paarl - ○.

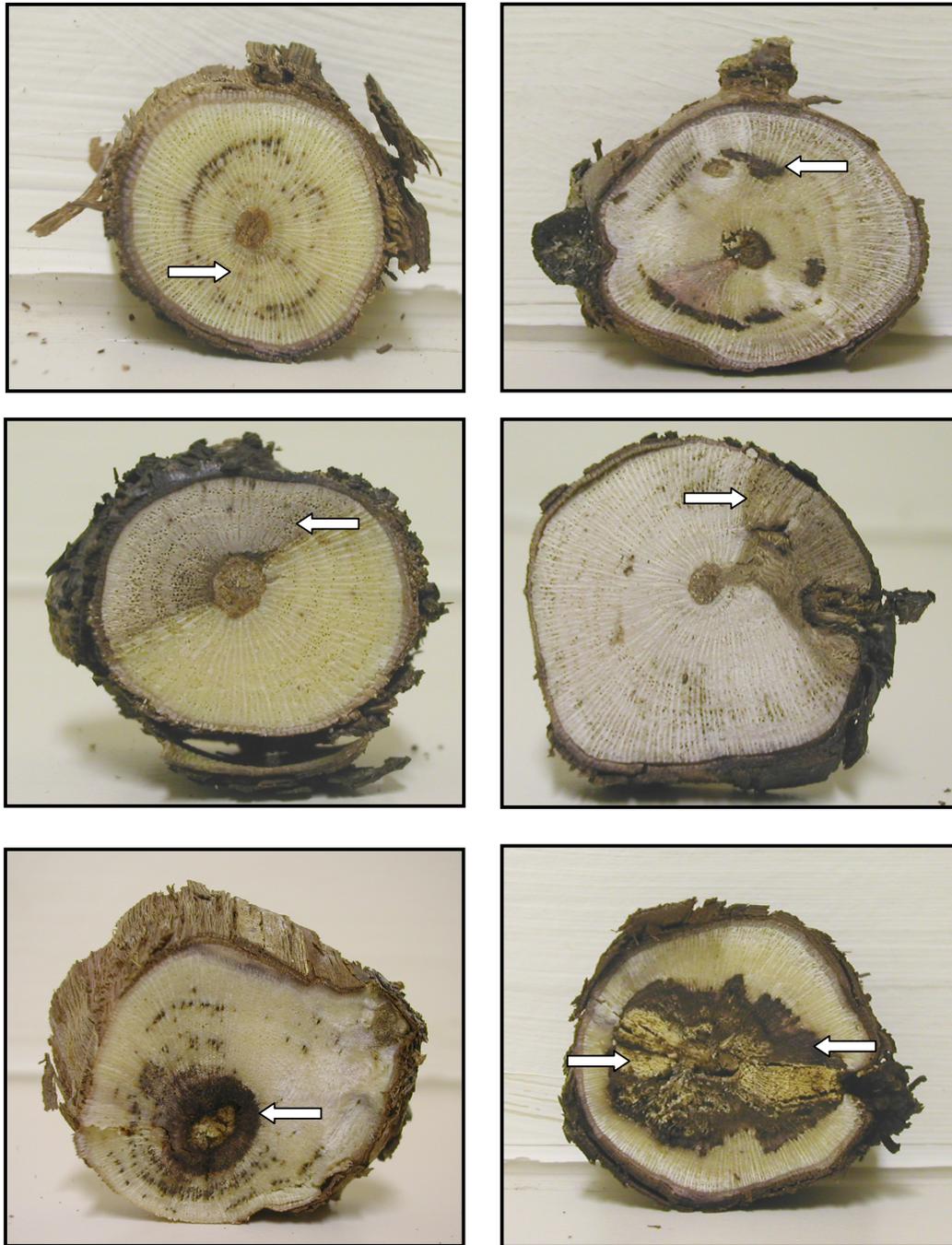


Figure 4. Cross sections made through cordon sections displaying various symptom types: A) brown streaking, B) black streaking, C) wedge-shaped necrosis, D) watery necrosis, E) brown internal necrosis, F) esca-type yellow (left) and brown (right) soft wood rot.

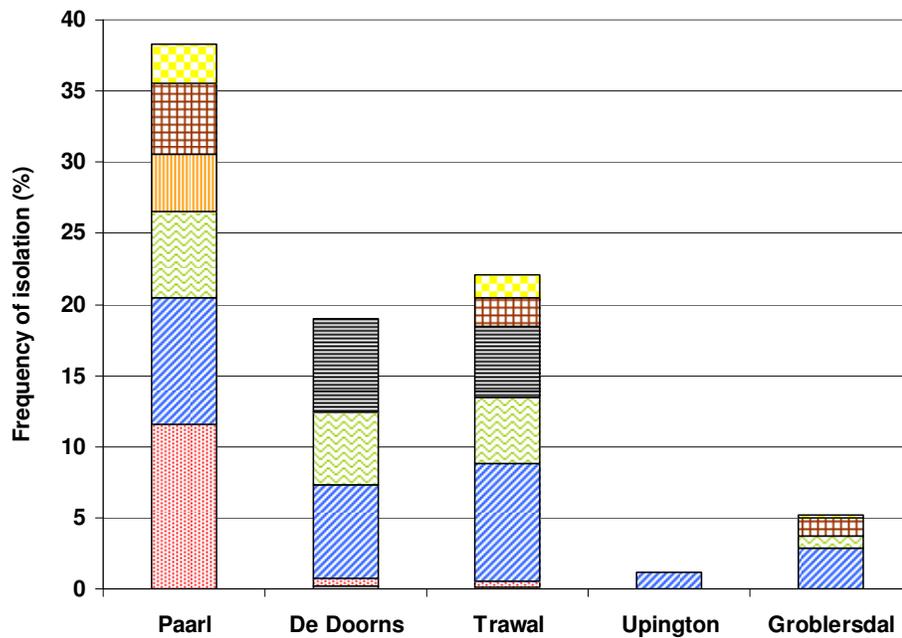


Figure 5. Frequency of isolation of *Phaeomoniella chlamydospora* and associated symptom types (asymptomatic wood - □, brown streaking - ■, black streaking - ■, brown internal necrosis - ■, watery necrosis - ■, wedge-shaped necrosis - ■, esca brown - ■, esca yellow - ■), given as a proportion of total number of isolations from cordons sampled from 15 Dan-ben-Hannah vineyards in winter (Paarl, De Doorns and Trawal) and summer (Upington and Groblersdal) rainfall areas of South Africa.

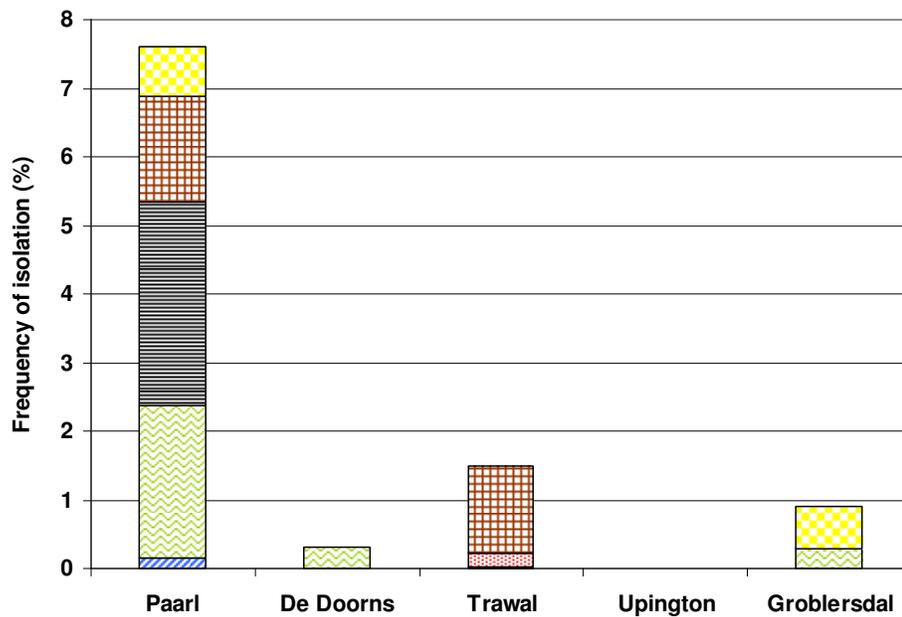


Figure 6. Frequency of isolation of *Phaeoacremonium aleophilum* and associated symptom types (asymptomatic wood - □, brown streaking - ■, black streaking - ▨, brown internal necrosis - ▩, watery necrosis - ▪, wedge-shaped necrosis - ▨, esca brown - ▩, esca yellow - ▩), given as a proportion of total number of isolations from cordons sampled from 15 Dan-ben-Hannah vineyards in winter (Paarl, De Doorns and Trawal) and summer (Upington and Groblersdal) rainfall areas of South Africa.

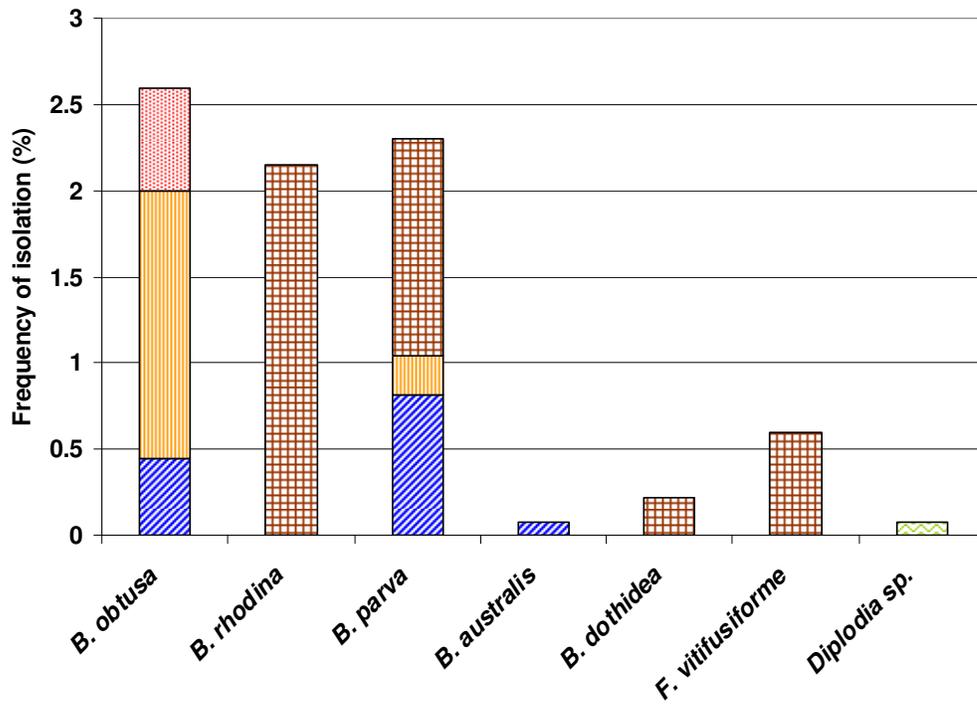


Figure 7. Frequency of isolation of *Botryosphaeria* and related species, given as a proportion of the total number of isolations made from cordons sampled from 15 Dan-ben-Hannah vineyards in winter (Paarl - , De Doorns -  and Trawal - ) and summer (Uppington -  and Groblersdal - ) rainfall areas of South Africa.

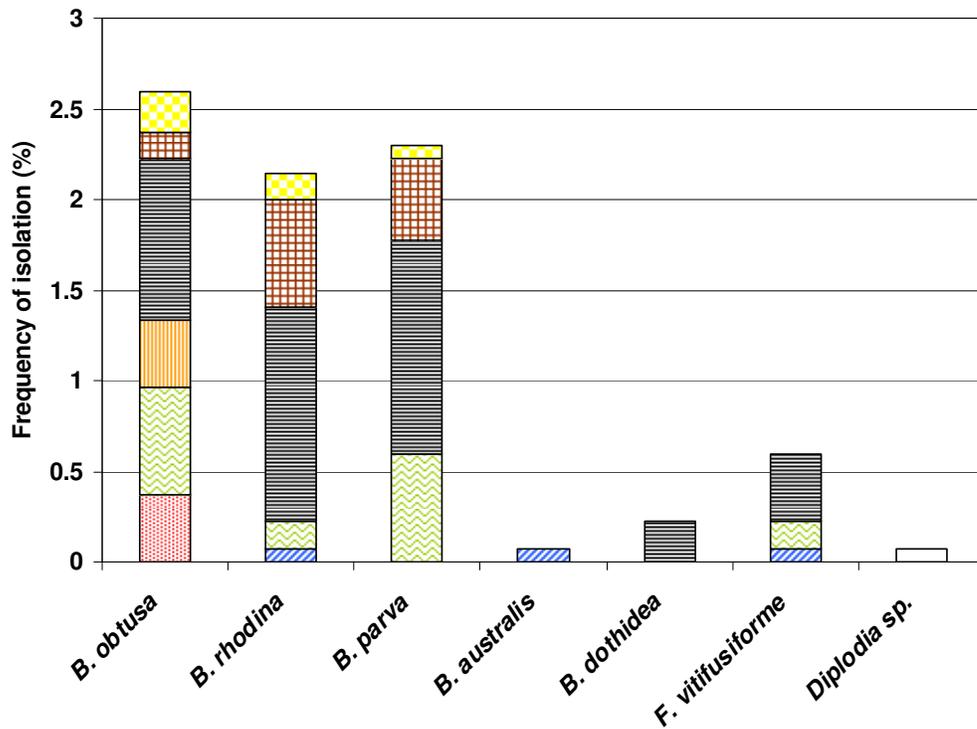


Figure 8. Frequency of isolation of *Botryosphaeria* and related species and associated symptom types (asymptomatic wood - □, brown streaking - ■, black streaking - ■, brown internal necrosis - ■, watery necrosis - ■, wedge-shaped necrosis - ■, esca brown - ■, esca yellow - ■), given as a proportion of total number of isolations from cordons sampled from 15 Dan-ben-Hannah vineyards in winter (Paarl, De Doorns and Trawal) and summer (Upington and Groblersdal) rainfall areas of South Africa.

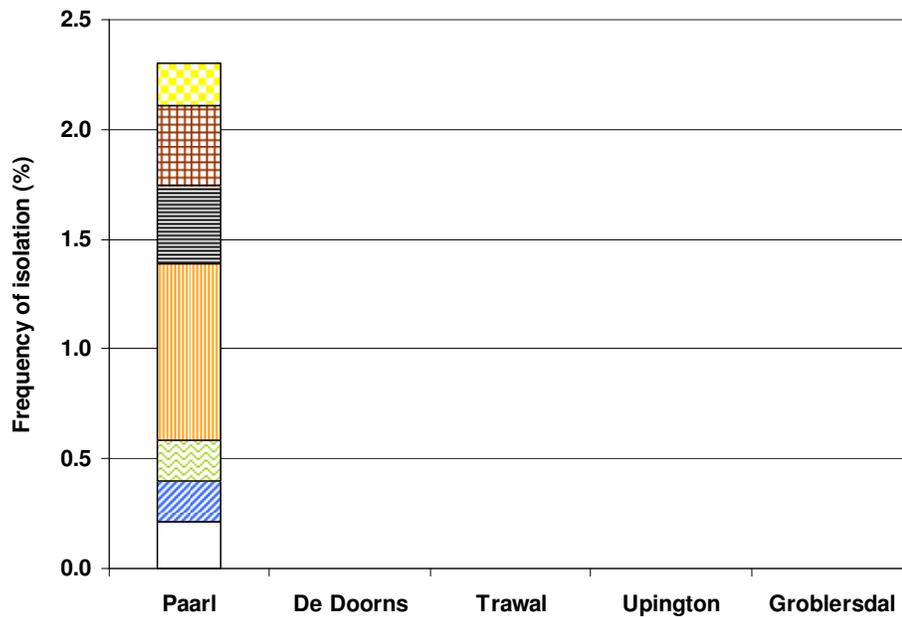


Figure 9. Frequency of isolation of *Phomopsis viticola* and associated symptom types (asymptomatic wood - □, brown streaking - ■, black streaking - ■, brown internal necrosis - ■, watery necrosis - ■, wedge-shaped necrosis - ■, esca brown - ■, esca yellow - ■), given as a proportion of total number of isolations from cordons sampled from 15 Dan-ben-Hannah vineyards in winter (Paarl, De Doorns and Trawal) and summer (Upington and Groblersdal) rainfall areas of South Africa.

3. EVALUATION OF FUNGICIDES AS POTENTIAL GRAPEVINE PRUNING WOUND PROTECTANTS AGAINST *BOTRYOSPHAERIA* SPP.

ABSTRACT

Protection of wounds against infection by trunk disease pathogens is the most efficient and cost-effective means to prevent grapevine trunk diseases. Studies done to determine the effectiveness of chemical pruning wound protectants have mostly focused on the control of *Eutypa dieback*. However, other important wound pathogens, such as *Phaeomoniella chlamydospora*, *Phomopsis* and *Botryosphaeria* species, pose just as significant a threat to sustainable grape production. Fungicide sensitivity studies have been conducted for *Pa. chlamydospora*, *P. viticola* and *Eutypa lata*. However, no such studies have been conducted for the pathogenic *Botryosphaeria* species from grapevines in South Africa. Ten fungicides were therefore tested *in vitro* for their efficacy on mycelial inhibition of the four most common or pathogenic *Botryosphaeria* species in South Africa, *B. australis*, *B. obtusa*, *B. parva* and *B. rhodina*. Iprodione, pyrimethanil, copper ammonium acetate, kresoxim-methyl and boscalid were ineffective in inhibiting the mycelial growth at the highest concentration tested (5 µg/ml; 20 µg/ml for copper ammonium acetate). Benomyl, tebuconazole, prochloraz manganese chloride and flusilazole were the most effective fungicides with EC₅₀ values for the different species ranging from 0.36-0.55, 0.07-0.17, 0.07-1.15 and 0.04-0.36 µg/ml, respectively. These fungicides, except prochloraz manganese chloride, are registered on grapes in South Africa and were also reported to be effective against *Pa. chlamydospora*, *P. viticola* and *E. lata*. Results from bioassays on 1-year-old Chenin Blanc grapevine shoots indicated that benomyl, tebuconazole and prochloraz manganese chloride were most effective in limiting lesion length in pruning wounds that were inoculated with the *Botryosphaeria* spp. after fungicide treatment. The bioassay findings were, however, inconclusive due to low and varied re-isolation incidences. Benomyl, tebuconazole, prochloraz manganese chloride and flusilazole can be identified as fungicides to be evaluated as pruning wound protectants in additional bioassays and vineyard trials against *Botryosphaeria* spp. as well as the other grapevine trunk disease pathogens.

INTRODUCTION

Several pathogens are capable of causing decline and dieback associated with grapevine trunk diseases. These include *Eutypa lata*, *Phaeoconiella chlamydospora*, *Phaeoacremonium*, *Phomopsis* and *Botryosphaeria* species (Ferreira *et al.*, 1989; Mugnai *et al.*, 1999; Groenewald *et al.*, 2001; Mostert *et al.*, 2001; van Niekerk *et al.*, 2004; Mostert *et al.*, 2005b; van Niekerk *et al.*, 2005b, Chapter 2). The occurrence of these trunk disease pathogens appear to be greatly affected by the prevailing climatic conditions (Merrin *et al.*, 1995; Munkvold and Marois, 1995; Mugnai *et al.*, 1999; Larignon and Dubos, 2000; Erincik *et al.*, 2003; Copes and Hendrix, 2004; Edwards and Pascoe, 2004; van Niekerk *et al.*, 2005a, Chapter 2) and management strategies in each area should either be specific with regard to pathogen incidence, or general but effective against all trunk disease pathogens.

Wounds, especially pruning wounds, are regarded as the primary infection site for these pathogens (Ferreira *et al.*, 1989; Mugnai *et al.*, 1999; Larignon and Dubos, 2000; Mostert *et al.*, 2005a; van Niekerk *et al.*, 2005a). At present, research on pruning wound protection has focused mainly on *E. lata* and is achieved by applying fungicides (Moller and Kasimatis, 1980; Munkvold and Marois, 1993a; Halleen *et al.*, 2001; Sosnowski *et al.*, 2004, 2005) or biological control agents (Carter and Price, 1974; Ferreira *et al.*, 1991; Munkvold and Marois, 1993b; Hunt, 2004) as pruning wound protectants. However, protection of pruning wounds against all the trunk disease pathogens that are present in a specific area is essential for effective prevention of these diseases. *In vitro* fungicide sensitivity studies have been done on *E. lata*, *Pa. chlamydospora* and *P. viticola* only. Groenewald *et al.* (2000) showed that benomyl, fenarimol, prochloraz manganese chloride and tebuconazole inhibited mycelial growth of *Pa. chlamydospora* at low concentrations. These findings were complimented by Jaspers (2001), who also found cyprodinil/fludioxonil and pyrimethanil to be effective against mycelium growth and folpet and hydroxyquinoline sulphate against spore germination. Mostert *et al.* (2000) found flusilazole to be effective against *P. viticola* and studies done by Halleen *et al.* (2001) showed flusilazole, tebuconazole, benomyl and fenarimol to be effective in inhibiting *E. lata*. Fungicide evaluation trials done in Australian vineyards showed carbendazim to be the most effective pruning wound treatment in controlling *E. lata* infections (Sosnowski *et al.*, 2004). Halleen and Fourie (2005) reported similar findings with benomyl in South African vineyards, showing that

not only benomyl, but also flusilazole limited natural infections by *P. chlamydospora*, *P. viticola* and *Botryosphaeria* spp. The specific identity of the *Botryosphaeria* spp. was, unfortunately, not determined.

In a recent survey of table grape vineyards in South Africa, it was shown that *B. obtusa*, *B. parva* and *B. rhodina* appeared to be the most common *Botryosphaeria* spp., but their incidence in climatically different regions varied from prevalent to absent (Chapter 2). Van Niekerk *et al.* (2004) demonstrated that *B. australis*, *B. parva*, and to a lesser extent *B. rhodina* and *B. obtusa*, were pathogenic on grapevine. No fungicides have been evaluated in South Africa against these *Botryosphaeria* spp. In Australia, Savocchia *et al.* (2005) found tebuconazole, flusilazole, spiroxamine and fluazinam to be effective *in vitro* in reducing mycelial growth of *B. obtusa* and *B. lutea*. EC₅₀ values for tebuconazole were 0.01 mg/l for both species and for flusilazole, values were 0.46 and 0.3 mg/l for *B. obtusa* and *B. lutea*, respectively. EC₅₀ values for spiroxamine were 0.06 and 0.38 mg/l for *B. obtusa* and *B. lutea*, respectively and 0.01 mg/l for fluazinam for both species. The fungicides were, however, not tested against *B. australis*, *B. rhodina* and *B. parva*.

The aim of this study was therefore to determine the *in vitro* efficacy of selected fungicides against the most important *Botryosphaeria* spp., *B. australis*, *B. rhodina*, *B. parva* and *B. obtusa*, occurring on grapevines in South Africa. Bioassays were also conducted with the most effective *in vitro* fungicides in order to determine their potential as pruning wound protectants.

MATERIALS AND METHODS

***In vitro* testing of fungicides.** Sixteen *Botryosphaeria* isolates, which were previously isolated from grapevine (van Niekerk *et al.*, 2004, Chapter 2) were used in these trials. These isolates included four isolates each of *B. australis* (STE-U 5040, 4598, 4416 and 4591), *B. parva* (STE-U 4589, 4420, 5253 and 5130), *B. rhodina* (STE-U 4583, 4423, 4422 and 4419) and *B. obtusa* (STE-U 5139, 4444, 4440 and 5037). The isolates are maintained at the Department of Plant Pathology culture collection at the University of Stellenbosch (STE-U). Based on previous fungicide efficacy studies on grapevine trunk disease pathogens (Groenewald *et al.*, 2000; Mostert *et al.*, 2000; Halleen *et al.*, 2001) and *B. protearum* (Denman *et al.*, 2004), eight fungicides, i.e. benomyl, fenarimol, iprodione, prochloraz manganese chloride, tebuconazole,

flusilazole, pyrimethanil and kresoxim-methyl were selected (Table 1). Boscalid, a new broad-spectrum fungicide in a novel chemical class (www.agro.basf.com) and copper ammonium acetate was also included. The latter fungicide was also included as copper-containing fungicides are recommended as pruning wound protectants against the bacterial blight pathogen, *Xylophilus ampelinus* (Panagopoulos, 1988), which is of major economic importance in the South African table grape industry (Roleen Carstens, ARC Infruitec-Nievoorbij, Stellenbosch, South Africa, pers. comm.). The fungicides were added to molten (50°C) potato dextrose agar (PDA) medium at seven different concentrations: 0 (control), 0.05, 0.1, 0.5, 1, 2.5 and 5 µg fungicide (a.i.)/ml. Copper ammonium acetate was tested at higher concentrations of active ingredient, namely at 0 (control), 0.5, 1, 2.5, 5, 10 and 20 µg/ml. Mycelial plugs (5 mm in diameter) obtained from actively growing margins of the *Botryosphaeria* cultures were placed on the amended PDA plates. Three plates per concentration were used. These were incubated at 25°C and the radial mycelial growth of the colonies measured after 24 and 48 hours. Each colony's diameter was measured twice perpendicularly for each of the three replicates and the control. For each isolate × fungicide × concentration combination, the percentage inhibition was calculated relative to the respective control treatment. The function, Logistic Dose Response (% Inhibition = $b/1 + (\text{fungicide concentration}/c)^d$), with the intercept (a) equal to 0, was fitted to the data. From these regression lines, EC₅₀ and EC₉₀ values (the fungicide concentration where colony growth are inhibited by 50% or 90% compared to the control, respectively) were calculated.

Glasshouse bioassays. Subsequent to the *in vitro* study, the four most effective fungicides were selected for use in the bioassays. Copper ammonium acetate was also included. Dormant 1-year-old Chenin Blanc vine shoots (each containing 5 internodes) were hot water treated (30 min at 50°C), followed by submersion in cold water containing a suspension of Sporekill (1.5 ml/l). The shoots were then placed in a custom-built hydroponic system (Fig. 1) at approximately 27°C and allowed to bud. At the woolly bud stage, the distal ends of the shoots were aseptically pruned off at a distance of 1 cm above the second bud. These pruning wounds were immediately sprayed with 1 ml of the selected fungicides at the registered concentrations (Table 1). After 3 days, spore suspensions (1×10^4 conidia/ml) of three isolates each of the different *Botryosphaeria* spp., i.e. *B. australis* (STE-U 4416, 4591 and 5040), *B. rhodina* (STE-U 4583, 4419 and 4423), *B. obtusa* (STE-U 4440, 4444 and 5139) and *B. parva* (STE-U 4420, 5130 and 4589), were sprayed onto the treated wounds. Spore suspensions were prepared from pycnidia of these isolates that were produced on sterilised pine needles on water agar as

described by Van Niekerk *et al.* (2004). Inoculated control treatments consisted of shoots that were treated with sterile deionised water and an unsprayed, inoculated control. The water inside the tubes was replaced with fresh water, amended with Chemicult (1 g/l l) at weekly intervals. The treated shoots were incubated at a temperature range of 20-32°C (average 27°C). After 3 months, the distal internode was removed from each shoot, split longitudinally and lesion formation in the xylem tissue measured. Data was submitted to statistical analysis of variance and means compared using Student's t-test for least significant ($P < 0.05$) differences (Snedecor and Cochran, 1967). From each shoot, isolations were also made from the interface between healthy and symptomatic xylem wood. Four small (1 × 0.5 mm) wood sections were aseptically removed from this zone, placed on PDA medium and incubated a 23°C for 3 weeks. Identification of the fungal cultures was made to genus level, based on morphological characteristics. The incidence of re-isolated *Botryosphaeria* spp. was calculated for each cutting.

RESULTS

In vitro testing of fungicides. Iprodione, pyrimethanil, copper ammonium acetate, kresoxim-methyl and boscalid were ineffective in inhibiting mycelial growth at the concentrations tested and the EC₅₀ and EC₉₀ values for these fungicides could not be calculated. Data for these treatments were therefore not included in the analysis. Analyses of variance of the EC₅₀ and EC₉₀ values for benomyl, tebuconazole, flusilazole, prochloraz mc and fenarimol showed a significant interaction between *Botryosphaeria* spp. and fungicides ($P < 0.0001$; ANOVA table not shown), indicating that the species reacted differently to the fungicides. EC₅₀ and EC₉₀ values are given in Table 2. Benomyl, tebuconazole and flusilazole were the most effective fungicides against all four species tested. Prochloraz mc and fenarimol exhibited significantly lower EC₅₀ values for *B. australis* (1.15 and 1.74 µg/ml, respectively) compared with the other fungicides (0.13 to 0.55 µg/ml). Compared with the other fungicides, fenarimol also effected significantly lower EC₅₀ values for *B. obtusa* (1.50 µg/ml vs. 0.14 to 0.36 µg/ml) and *B. rhodina* (3.01 µg/ml vs. 0.08 to 0.36 µg/ml). Fenarimol EC₉₀ values could not be determined for *B. australis*, *B. obtusa* and *B. rhodina* and for *B. parva* it was significantly higher (3.09 µg/ml) than EC₉₀ values for the other fungicides (0.53 to 2.05 µg/ml). EC₉₀ values of the other fungicides for *B. australis* (0.70 to 1.28 µg/ml), *B. obtusa* (0.78 to 2.05 µg/ml), *B. parva* (0.47 to 1.58 µg/ml) and *B. rhodina* (1.40 to 1.97 µg/ml) were fairly similar, except for the

benomyl \times *B. australis* (4.39 $\mu\text{g/ml}$), prochloraz mc \times *B. parva* (2.05 $\mu\text{g/ml}$) and prochloraz mc \times *B. rhodina* (3.87 $\mu\text{g/ml}$) combinations, which had significantly higher EC_{90} values.

Glasshouse bioassays. Black vascular streaking and necrotic lesions were observed in the longitudinally dissected shoots. Apart from the general wound response, no lesions were observed in the uninoculated shoots. Analysis of variance of the lesion length data showed no species \times treatment interaction ($P = 0.7360$). Significant differences were, however, observed between the mean lesion lengths for species ($P < 0.0001$) and treatments ($P = 0.0209$; ANOVA table not shown). Significantly longer lesions were measured in the shoots that were inoculated with *B. parva* (6.40 mm), compared with those inoculated with *B. rhodina* (4.98 mm), *B. australis* (4.82 mm) and *B. obtusa* (4.76 mm), while statistically shorter lesions were measured on the control shoots that were not inoculated (3.59 mm). The longest lesion lengths were measured on shoots that were treated with water prior to inoculation (6.19 mm). These lesion lengths did not differ significantly ($P < 0.05$) from those measured on shoots that were treated with copper ammonium acetate (5.67 mm) or flusilazole (5.21 mm), but significantly shorter lesions were measured on shoots treated with tebuconazole (4.89 mm), benomyl (4.77 mm), prochloraz mc (4.62 mm) and the uninoculated control (3.59 mm).

The incidence of *Botryosphaeria* spp. that were isolated from lesions in the water-treated controls varied, and ranged from 0% to 39.4%. The data were therefore not statistically analysed. No *Botryosphaeria* spp. were isolated from the uninoculated control shoots. The incidence of *Botryosphaeria* isolated from *B. obtusa*-inoculated shoots declined from relatively high levels in water-treated shoots (39.4%) to moderate levels in copper ammonium acetate-treated shoots (24.9%), low levels in flusilazole-, prochloraz mc- (12.5% each) and tebuconazole-treated shoots (7.7%), and very low levels in benomyl-treated shoots (1.0%). From the other treatments, re-isolation data did, however, not conform to this expected trend. From shoots that were inoculated with *B. australis*, positive isolations were made only from lesions in flusilazole- (83.3%) and tebuconazole-treated (16.7%) shoots. Few *Botryosphaeria* spp. were isolated from water-treated shoots that were inoculated with *B. parva* (6.0%), whereas higher levels (15.1% to 24.1%) were isolated from the fungicide-treated shoots. For the *B. rhodina* treatment, the fungus was isolated from the water- (38.5%), tebuconazole- (38.5%) and prochloraz mc-treated shoots (23.1%) only.

DISCUSSION

This study presents the first report on the *in vitro* fungicide efficacy of *B. rhodina*, *B. parva* and *B. australis*. In this study, I calculated mean EC₅₀ values for benomyl (0.44 µg/ml), tebuconazole (0.13 µg/ml), prochloraz mc (0.55 µg/ml) and flusilazole (1.68 µg/ml), that were similar to the respective values of 0.45, 0.28, 0.5 and 1.08 µg/ml reported by Denman *et al.* (2004) for *B. protearum*, the canker pathogen of *Protea magnifica*. Savocchia *et al.* (2005) also reported tebuconazole and fluazinam to be the most effective fungicides for reducing *in vitro* mycelial growth of *B. obtusa* and *B. lutea*. Fluazinam is, however, not available in South Africa. Differences in the EC₅₀ values were observed between the different *Botryosphaeria* spp. tested in this study. Benomyl and tebuconazole showed relative consistent inhibitory action, whereas prochloraz mc, flusilazole and fenarimol varied considerably between the tested species. Tebuconazole were the most effective fungicide *in vitro*. *B. parva* was the species most inhibited by the fungicides tested, with tebuconazole, prochloraz mc and flusilazole showing the best mycelial inhibitory action *in vitro*. *B. rhodina* and *B. obtusa* were also inhibited at low concentrations, except two isolates against which fenarimol did not show good inhibition (results not shown). *B. australis* was the least inhibited by the fungicides. These fungicides, except prochloraz mc, are registered on grapes in South Africa and were also reported to be effective against *Pa. chlamydospora*, *P. viticola* and *E. lata* (Groenewald *et al.*, 2000; Mostert *et al.*, 2000; Halleen *et al.*, 2001).

Results from the bioassays indicated that benomyl, tebuconazole and prochloraz mc were the most effective pruning wound protectants against the *Botryosphaeria* spp. tested. However, due to the low and varied re-isolation incidences, these findings cannot be considered as conclusive. One reason for the low re-isolation incidences might have been the relatively short incubation period of 3 months, during which time the pathogen did not have adequate time to become established inside the grapevine wood. *Botryosphaeria* spp. are furthermore reported to be stress-related pathogens (Van Niekerk *et al.* 2005a) and the absence of stress on the grapevine shoots might therefore also have resulted in inadequate pruning wound colonisation. It was, however, clear that treatment of pruning wounds with these fungicides did not completely prevent infection by the *Botryosphaeria* spp., and application of these chemicals at higher dosages should be investigated.

Halleen and Fourie (2005) demonstrated the efficacy of flusilazole and benomyl as pruning wound protectants against *E. lata* in Cabernet Sauvignon vineyards, and also the efficacy of these fungicide treatments against natural infection by *Botryosphaeria* spp. Sosnowski *et al.* (2005) also found benomyl, among other pruning wound treatments, to be effective in reducing *E. lata* infection in Australian Cabernet Sauvignon vineyards. From my findings, as well as those reported in literature, benomyl, tebuconazole, flusilazole and prochloraz mc can be identified as fungicides to be evaluated as pruning wound protectants in additional bioassays and vineyard trials against *Botryosphaeria* spp. as well as the other grapevine trunk disease pathogens.

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Table 1. Fungicides selected for sensitivity testing.

Active ingredient	Trade name	Manufacturer	Formulation ^a	Registered concentration in South Africa ^b
Benomyl	Benlate	Dow AgroSciences	500 g/kg WP	50 g/100 l (grapes)
Boscalid	Cantus	BASF	500 g/kg WG	Not registered
Fenarimol	Rubigan	Klub M5	120 g/l EC	20 ml/100 l (grapes)
Iprodione	Rovral Flo	Bayer	255 g/l SC	200 ml/100 l (grapes)
Prochloraz mc	Octave	Bayer	500 g/kg WP	25 g/100 l (apricots)
Tebuconazole	Folicur	Bayer	250 g/l EW	20 ml/100 l (grapes)
Flusilazole	Olymp	DuPont	100 g/l EW	50 ml/100 l (grapes)
Pyrimethanil	Scala	Bayer	400 g/l SC	120 ml/100 l (grapes)
Copper ammonium acetate	Copper Count-N	Hygrotech	316 g/l SL	500 ml/100 l (grapes)
Kresoxim-methyl	Stroby	BASF	500 g/kg WG	15 g/100 l (grapes)

^aWP = Wettable powder, WG = Water dispersible granule, EC = Emulsifiable concentrate, SC = Suspension concentrate, EW = Emulsion, oil in water, SL = Soluble concentrate.

^bAccording to Nel *et al.* (2003).

Table 2. EC₅₀ and EC₉₀ values calculated for each *Botryosphaeria* species and fungicide treatment interaction following *in vitro* mycelium growth studies on fungicide-amended PDA.

Treatment	EC ₅₀ (µg/ml)*	EC ₉₀ (µg/ml)*
<i>B. australis</i>		
Benomyl	0.55 d	4.39 h
Tebuconazole	0.13 ab	0.98 d
Prochloraz mc	1.15 e	1.28 d
Flusilazole	0.21 abc	0.70 d
Fenarimol	1.74 g	-
<i>B. obtusa</i>		
Benomyl	0.39 bcd	0.78 d
Tebuconazole	0.14 ab	1.88 de
Prochloraz mc	0.36 bcd	2.05 e
Flusilazole	0.36 bcd	1.67 de
Fenarimol	1.50 f	-
<i>B. parva</i>		
Benomyl	0.47 cd	1.58 d
Tebuconazole	0.07 a	0.53 d
Prochloraz mc	0.07 a	2.05 e
Flusilazole	0.04 a	0.47 d
Fenarimol	0.45 cd	3.09 f
<i>B. rhodina</i>		
Benomyl	0.36 bcd	1.40 d
Tebuconazole	0.17 ab	1.75 de
Prochloraz mc	0.60 d	3.87 g
Flusilazole	0.08 a	1.97 e
Fenarimol	3.01 h	-
	LSD=0.282	LSD=0.447

*Means followed by the same letter do not differ significantly ($P < 0.05$)



Figure 1. Hydroponics system showing treated shoots after incubation period of 3 months.