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The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate

Jan M. VAN NIEKERK[1], Wilma BESTER[1], Francois HALLEEN[1, 2], Pedro W. CROUS[1,3] and Paul H. FOURIE[1,4]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa
[2]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[3]CBS-KNAW, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
[4]Citrus Research International, P.O. Box 2201, Stellenbosch 7602, South Africa

Summary. Grapevine trunk diseases, caused by a range of phytopathogenic fungi, represent a serious impediment to wine and table grape production wherever these crops are cultivated. Previous studies have shown that the distribution of these pathogens is influenced by climate and that they are associated with a variety of internal wood decay symptoms. Little was known, however, about the influence of climate on the disease symptomatology of the different pathogens in a specific area. To address this, a survey was conducted in 30 wine and table grape vineyards in summer, marginal and winter rainfall regions of South Africa. Apart from *Eutypa lata*, which occurred only in the winter rainfall region, *Phaeomoniella chlamydospora*, species of *Phaeoacremonium*, Botryosphaeriaceae and *Phomopsis* occurred in all regions surveyed. The incidence of the fungal genera and species associated with trunk disease varied between regions, with overlapping symptom profiles that differed based on the climatic region. These findings suggest that symptom-based disease diagnosis alone is unreliable and that distribution and symptomatology of grapevine trunk pathogens are strongly influenced by climatic conditions in a specific production region.

Key words: Petri disease, Eutypa, Bot canker.

Introduction

Trunk disease pathogens associated with decline and dieback of grapevines include members of the Botryosphaeriaceae (van Niekerk *et al.,* 2004; 2010; Pitt *et al*., 2010), *Phaeoacremonium* (*Pm.*) (Mostert *et al.,* 2006b), *Phomopsis* (van Niekerk *et al.,* 2005), various basidiomycete species (Fischer, 2006), as well as *Phaeomoniella* (*Pa.*) *chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams (Mugnai *et al.,* 1999) and *Eutypa lata* (Pers.) Tul. & C. Tul. (Munkvold *et al.,* 1994). These pathogens often occur together in declining grapevines, with different pathogens often associated with the same type of decay symptoms (Larignon and Dubos, 1997). Apart from overlapping symptomatology, pathogen distribution appears to be influenced by climate (Magarey and Carter, 1986; Merrin *et al.,* 1995; Úrbez-Torres *et al.,* 2006; Pitt *et al*., 2010).

Botryosphaeriaceae species may be cosmopolitan in nature or occur only in certain climates (Taylor *et al*., 2005; Pitt *et al*., 2010). *Diplodia seriata* De Not. is found in climatically diverse grape growing areas around the world (Castillo-Pando *et al.,* 2001), while *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips have been reported from cooler production areas, and *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. is found predominantly in warmer areas

Corresponding author: P.H. Fourie
Fax: +27 865 717273
E-mail: phf@cri.co.za

(Úrbez-Torres *et al.,* 2006). A similar phenomenon has been observed with *Phomopsis* spp. associated with grapevines in Australia where *Diaporthe australafricana* Crous & Van Niekerk and *Phomopsis viticola* (Sacc.) Sacc. were observed to occur predominantly in coastal vineyards and higher in inland vineyards, respectively (Merrin *et al.,* 1995).

Eutypa dieback, caused by *E. lata*, occurs in all major grape-growing countries. However, its distribution is restricted to areas with an annual rainfall exceeding 350 mm or where overhead irrigation is applied regularly (Magarey and Carter, 1986). Sosnowski *et al*. (2007) found that the variation in foliar symptom severity from year to year might be influenced by climatic factors rather than vineyard practices, with a high rainfall in the previous year being linked with an increase in foliar symptoms the next year. An increase in spring temperatures was related to a decrease in foliar symptoms. *Phaeomoniella chlamydospora*, causal organism of Petri disease of grapevines, is also cosmopolitan in its distribution and has been reported from almost all grape-growing countries (Mugnai *et al.,* 1999). However, the various *Phaeoacremonium* spp. associated with Petri disease and esca have been found to vary in their geographical distribution (Mostert *et al.,* 2006b); of the species occurring on grapevines, *Pm. aleophilum* was found to have the widest geographical distribution.

Several basidiomycete species are involved in esca disease of grapevines, causing characteristic white rot symptoms (Surico, 2001). Initially these species, their distribution and associated symptoms were studied in Europe but in recent years several species have also been reported from North and South America, South Africa, Australia and New Zealand (Edwards *et al*., 2001b; Fischer, 2001, 2006, 2007; Gatica *et al*., 2004). These studies also showed that, although some species occurred in many grape producing countries, some species had limited geographical and climatic distribution.

From the above it is clear that climate influences the distribution of grapevine trunk pathogens. However, in South Africa such information was lacking and the effect of climate on the symptomatology of these diseases had not been elucidated. The aim of this study was, therefore, to study the influence of climate on distribution of grapevine trunk pathogens and disease symptomatology.

Materials and methods

Regions surveyed

During 2003 and 2004, 15 wine and 15 table grape vineyards were surveyed in 11 production areas in the Western Cape, Northern Cape, Limpopo and Mpumalanga provinces of South Africa. Based on annual rainfall and the dominant rainfall season, production areas were grouped into regions of summer (annual rainfall 250–750 mm; average temperature June–September, 13.3–14.8°C); winter (annual rainfall 450–700 mm; average temperature June–September, 12.1–13.7°C); and marginal rainfall (annual rainfall 250–350 mm; average temperature June–September, 13.1–16.1°C) (Figure 1). Areas included in the summer rainfall region were Upington (table and wine grapes) in the Northern Cape province, Mookgopong (table grapes) and Modimolle (table grapes) in the Limpopo province, and Groblersdal (table grapes) in Mpumalanga province. The winter rainfall region consisted of the De Doorns (table grapes), Paarl (table grapes), Malmesbury (wine grapes) and Stellenbosch (wine grapes) areas. Included in the marginal rainfall region were the Trawal (table grapes), Vredendal (wine grapes) and Robertson (wine grapes) areas that receive summer and winter rainfall.

Symptoms and isolation

The vineyards sampled were more than 8 years old, as trunk disease pathogens such as *E. lata*, Botryosphaeriaceae and fungi associated with esca are more prevalent in older vineyards (Munkvold *et al.,* 1994). In the Robertson, Vredendal, Malmesbury and Stellenbosch areas, the wine grape cultivar Cabernet Sauvignon was sampled. In the Upington area, 'Ruby Cabernet' vineyards were sampled, as no 'Cabernet Sauvignon' vineyards of sufficient age were available. In the Trawal, Mookgopong, Modimolle, Groblersdal, Upington, De Doorns and Paarl areas the table grape cultivar Dan-ben-Hannah was sampled. In each vineyard, 20 visually healthy plants were selected randomly and sampled by removing the distal end of one cordon containing a cordon section and a spur with pruning wounds.

The cordon sections were dissected into 1-cm cross-sections to expose any internal symptoms, and these were grouped into 'symptom types'. Sections representing each symptom type were selected and surface sterilised by submersion in 3.5% NaOCl for 1 min, followed by 70% ethanol for 30 s before drying in a laminar flow hood. Wood sections showing esca-like soft wood rot symptoms were not surface sterilised, to facilitate isolation of basidiomycete fungi. For each symptom type, as well as for asymptomatic tissue located at least 1 cm from any symptomatic tissue, five 0.5×1 mm pieces of wood were aseptically removed from the border between symptomatic and asymptomatic tissue and plated in 90 mm Petri dishes containing potato-dextrose agar (PDA) (Biolab, Wadeville, South Africa) amended with 0.04 g L^{-1} streptomycin sulphate to inhibit bacterial growth. Petri dishes were subsequently incubated at 25°C in an 8 h light, 16 h dark regime for 4 weeks. Cool fluorescent light was provided during the 8 h light cycle by 65 W semi-resonant lamps. Throughout this 4-week period, mycelium emerging from pieces of isolated wood was subcultured by hyphal tipping to obtain pure cultures for morphological and molecular identification.

Morphological and molecular identification

Preliminary identification of fungal cultures to generic level was based on cultural characteristics including colony growth pattern, colour, mycelial and other morphological characteristics, such as conidial shape, size and colour as recorded by Crous and Gams (2000), Mostert *et al.* (2006a)

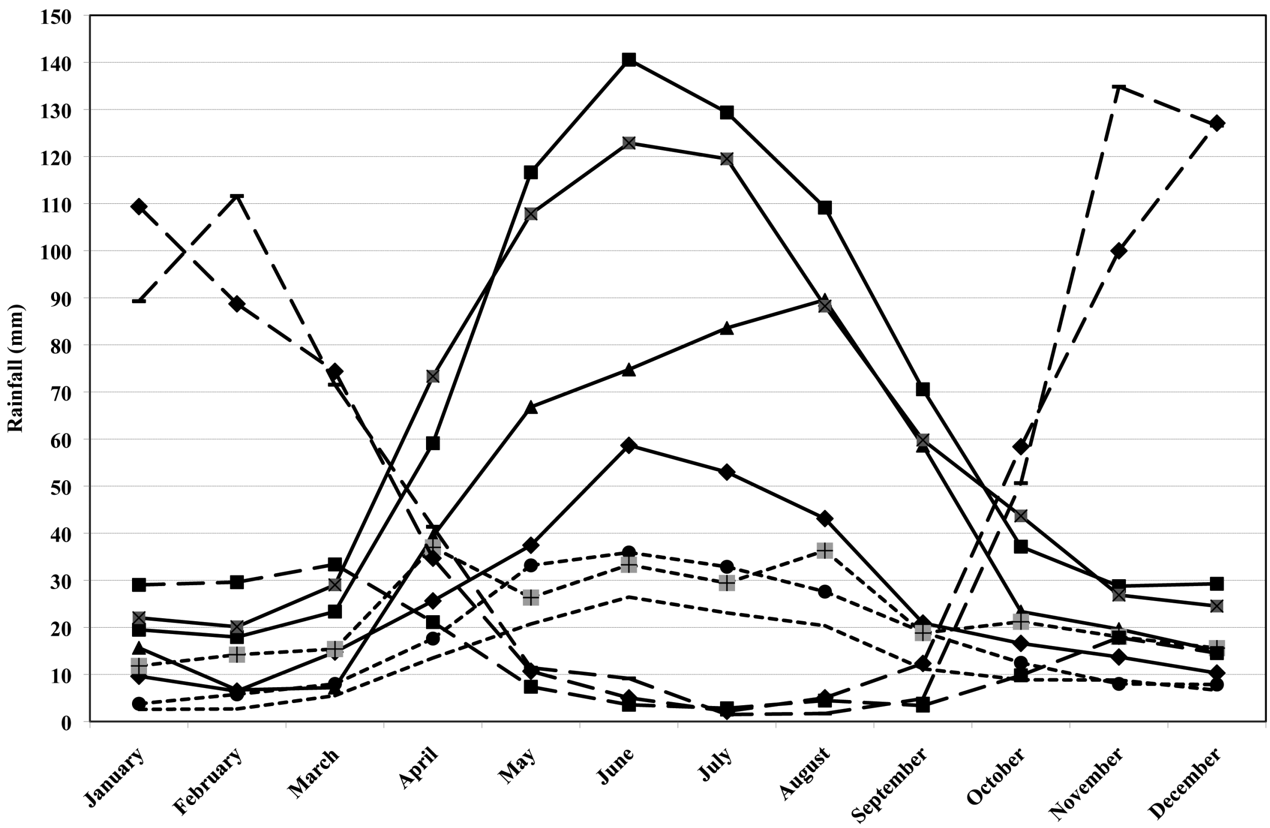

Figure 1. Long term average monthly rainfall patterns for De Doorns ( ), Paarl ( ), Malmesbury ( ), Stellenbosch ( ) Trawal ( ), Robertson ( ), Vredendal ( ), Mookgopong ( ), Modimolle ( ) and Upington ( ), South Africa.

and van Niekerk *et al*. (2004, 2005). Isolates of species in *Phaeoacremonium*, Botryosphaeriaceae and *Phomopsis* were selected for molecular identification to species level, based on differences in cultural characteristics, associated symptoms and area of origin. Basidiomycete isolates were not identified to genus or species level in this study. Selected isolates have been deposited in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U).

Internal transcribed spacer (ITS) and translation factor 1–α (EF1-α) sequence analyses for the Botryosphaeriaceae and *Phomopsis* isolates were conducted as described by van Niekerk *et al.* (2004, 2005). In the case of the *Phaeoacremonium* isolates, analysis of the β–tubulin gene was as recommended by Mostert *et al.* (2006a). For all selected isolates, genomic DNA was extracted from pure cultures grown on PDA, using the GenElute™ Plant Genomic DNA Miniprep Kit (SIGMA, Sigma-Aldrich Corporation, St Louis, MI, USA). The resulting ITS, EF1–α and β–tubulin sequences were manually aligned and edited before being compared with the respective nucleotide sequences deposited in GenBank (www.ncbi.nlm.nih.gov) for species identification.

To support molecular identification, morphological and cultural characteristics were studied and compared with reported characteristics of the respective genera and species (van Niekerk *et al.*, 2004, 2005; Mostert *et al.*, 2006a).

Data analysis

The isolation frequency in each region was determined for each genus/species as well as the symptom profile of the respective genera/species in each region. These data were subsequently subjected to analysis of variance (ANOVA) using SAS (SAS Institute Inc., NC, USA) to compare the frequency of the different pathogens in the various regions from the different grape types and their associated symptom profiles in the various regions.

Results

Symptoms and isolations

Symptom types were classified as black streaking (Figure 2a), brown streaking (Figure 2b), wedge-shaped necrosis (Figure 2c), brown internal necrosis (Figure 2d), watery necrosis (Figure 2e), and esca-like brown and yellow soft wood rot (Figure 2f). Black streaking, brown streaking and brown internal necrosis were most common on samples from the winter rainfall region, followed by the marginal and summer rainfall regions. Esca-like brown soft wood rot had the highest incidence in the winter rainfall region, followed by the summer and marginal rainfall region. Esca-like yellow soft wood rot, on the other hand, was most frequently observed in samples from the summer rainfall region followed by the winter and then the marginal rainfall region. Wedge-shaped and watery necroses were the only symptom types that occurred most frequently in the marginal rainfall region, followed by the winter and summer rainfall regions.

In total, 8605 pieces of wood were aseptically cultured from symptomatic and asymptomatic wood. Of the fungi isolated, *Pa. chlamydospora* was the most frequently isolated (13.9%), followed by a variety of *Phaeoacremonium* spp. (13.1%), Botryosphaeriaceae (9.8%), and *Phomopsis* species (3.2%). *Eutypa lata* was isolated only five times and in all cases in the winter rainfall region from wine grapes and from esca-like soft brown wood rot. Along with all these trunk pathogens, several basidiomycete isolates (1.3%) were also obtained from esca-like brown and yellow soft wood rot, brown internal necrosis, black streaking, and asymptomatic wood. The rest of the isolates (60%) obtained from the various symptom types consisted of sterile isolates, yeasts, bacteria and several genera not known as trunk pathogens.

Morphological and molecular identification

Analyses of variance of isolation data after morphological identification of the pathogens isolated from wine- and table grapes in the different climatic regions revealed a significant region × grape type × symptom × pathogen (P= 0.0025) interaction (ANOVA not shown). However, due to the complex nature of this four-factor interaction, the results are discussed with reference to the next tier of significant interactions, *viz*. region × symptom × pathogen (P= 0.0004), grape type × pathogen (P< 0.0001) and region × pathogen (P< 0.0001).

Phaeomoniella chlamydospora

The incidence of *Pa. chlamydospora* was signif-

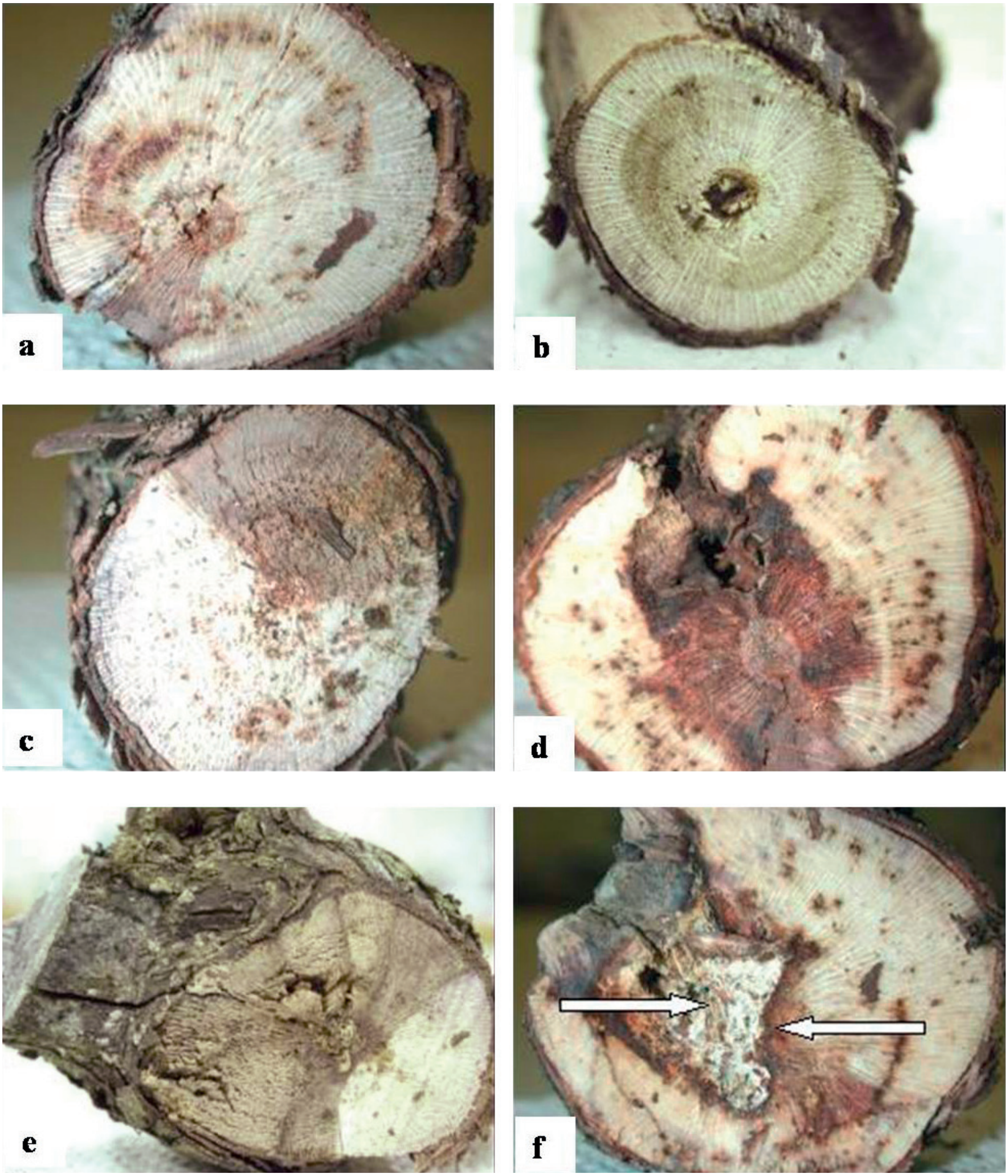

Figure 2. Cross sections made through cordon sections displaying various symptom types: a. black streaking, b. brown streaking, c. wedge-shaped necrosis, d. brown internal necrosis, e. watery necrosis, f. esca-like brown (left) and yellow soft wood rot (right).

icantly higher in the winter rainfall region (24.5%) than in the marginal (8.7%) and summer rainfall (5.4%) regions (Table 1). The incidence of this pathogen in the winter rainfall region was significantly higher than the other trunk disease pathogens occurring in this region. Results from the region × symptom × pathogen interaction showed that *Pa. chlamydospora* had the widest symptom profile in the winter rainfall region (Table 2). It was isolated most commonly from watery necrosis, brown internal necrosis, black and brown streaking, esca-like brown soft rot (between 29.7% and 42.9%), but also from wedge-shaped necrosis and esca-like yellow soft rot (between 16.9% and 19.9%). In the marginal and summer rainfall regions, *Pa. chlamydospora* was mostly isolated from black streaking (20.9% and 19.9% respectively). In the case of the marginal rainfall region it was also isolated from brown internal necrosis (15.8%) and wedge-shaped necrosis (12.5%). The grape type × pathogen interaction showed that table grapes yielded three times more isolates of *Pa. chlamydospora* isolates than did wine-grapes (21.2 compared with 6.9%; Table 3).

Phaeoacremonium spp.

The incidence of *Phaeoacremonium* spp. was highest in the winter rainfall region (16.0%), significantly higher than in the summer rainfall region (9.9%) (Table 1). *Phaeoacremonium* spp.was isolated from all symptom types in the winter rainfall region (Table 4), most commonly from black streaking, followed by wedge-shaped necrosis, esca-like brown wood rot, brown internal necrosis, brown streaking, watery necrosis (27.5–15.0%) and rarely from esca-like yellow soft rot and asymptomatic wood (1.9–1.0%; Table 4). In the marginal rainfall region, the highest incidence of *Phaeoacremonium* spp. was encountered from watery necrosis (60.0%), followed by black streaking (36.6%). Incidence of isolation from the remaining symptom types was relatively low (up to7.6%; Table 4). In the summer rainfall region, these pathogens were mostly isolated from black streaking, wedge-shaped necrosis, brown internal necrosis and esca-like yellow soft rot (28.5–8.0%) and at a low incidence from asymptomatic wood and brown streaking (0.4–4.4%; Table 4). *Phaeoacremonium* spp. were more often isolated from wine grapes than from table grapes (22.5% *vs* 3.5%; Table 3).

A total of 159 *Phaeoacremonium* isolates was selected for further identification using DNA sequence data. These isolates were representative of all symptom types in all the different rainfall regions. The β–tubulin sequences generated for all these isolates showed 100% nucleotide homology with *Pm. aleophilum* (Mostert *et al.,* 2006a), thus all isolates were confirmed as *Pm. aleophilum*.

Phomopsis spp.

Phomopsis spp. were equally common in the winter and marginal rainfall regions (4.3–4.5%) with a very low incidence in the summer rainfall region (0.2%; Table 1). However, despite the similarity in incidence in the first two regions, *Phomopsis* spp. had a much broader symptom profile in the winter rainfall region, being isolated with the highest incidence from wedge-shaped necrosis (14.2%) and then at 0.6–6.7% from all the other symptom types. *Phomopsis* spp. were also isolated from asymptomatic wood in the winter rainfall region (0.6%, Table 5). In the marginal rainfall region, *Phomopsis* spp. were isolated from wedge-shaped necrosis (21.8%), brown internal necrosis and brown streaking (up to 4.5%) (Table 5). In the summer rainfall region, *Phomopsis* spp. were isolated only from wedge-shaped necrosis and brown internal necrosis (Table 5). Wine grapes yielded the most *Phomopsis* isolates (5.5%), almost eight times more than table grapes (0.7%; Table 3).

A total of 63 *Phomopsis* isolates, representative of all regions and symptom types, was selected for identification to species level. ITS sequences generated for the selected *Phomopsis* isolates were identical and shared a 99% homology with *P. viticola* (van Niekerk *et al.,* 2005).

Botryosphaeriaceae spp.

Species of Botryosphaeriaceae were the only group of pathogens in this survey that occurred most often in the marginal rainfall region (16%); significantly higher than in the summer and winter rainfall regions (9.5% and 5.2% respectively; Table 1). The Botryosphaeriaceae spp. also had the broadest symptom profile in the marginal rainfall region (Table 6). The symptom types in the marginal rainfall region yielding most isolates of Botryosphaeriaceae were watery necrosis (50%), wedge-shaped necrosis (46%) and brown internal necrosis (37.7%). The isolation inci-

Table 1. Mean incidence of *Phaeomoniella chlamydospora*, *Eutypa lata* and species in the Botryosphaeriaceae, *Phomopsis* and *Phaeoacremonium* isolated from vines in the summer, marginal and winter rainfall regions of South Africa.

Region	Pathogen [a]				
	Pa. chlamydospora	*Phaeoacremonium* spp.	*Phomopsis* spp.	Botryosphaeriaceae spp.	*E. lata*
Summer	5.4 def	9.9 cd	0.2 gh	9.5 cde	0 h
Marginal	8.7 cdef	12.6 bc	4.5 efgh	16.0 b	0 h
Winter	24.5 a	16.0 b	4.3 fgh	5.2 defg	0.2 gh
LSD			5.04		

[a] Means followed by the same letter are not significantly different (P=0.05).

Table 2. Mean incidence of *Phaeomoniella chlamydospora* in isolates from vines displaying different symptoms in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region[a]		
	Summer	Marginal	Winter
Brown internal necrosis	7.8 de	15.8 bcde	36.2 ab
Black streaking	19.9 bcde	20.9 bcde	33.7 abc
Brown streaking	0.0 e	3.2 e	27.9 abcd
Esca brown rot	6.0 de	4.0 e	29.7 abc
Esca yellow rot	1.0 e	2.7 e	16.9 bcde
Wedge-shaped necrosis	1.1 e	12.5 cde	19.9 bcde
Watery necrosis	0.0 e	0.0 e	42.9 a
Asymptomatic wood	0.0 e	0.8 e	0.4 e
LSD		21.88	

[a] See Table 1.

Table 3. Mean incidence of *Phaeomoniella chlamydospora*, *Eutypa lata* and species in the Botryosphaeriaceae, *Phomopsis* and *Phaeoacremonium* among isolates from wine and table grape vines in South Africa.

Pathogen	Wine grape[a]	Table grape[a]
Botryosphaeriaceae spp.	12.7 b	6.9 c
Pa. chlamydospora	6.9 c	21.2 a
Phaeoacremonium spp.	22.5 a	3.5 cd
Phomopsis spp.	5.5 c	0.7 d
E. lata	0.2 d	0.0 d
LSD	4.08	

[a] See Table 1.

Table 4. Mean incidence of *Phaeoacremonium* spp. in isolates from vines displaying different symptoms in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region[a]		
	Summer	Marginal	Winter
Brown internal necrosis	11.9 cde	9.6 cde	18.4 bcde
Black streaking	28.5 bc	36.6 b	27.5 bcd
Brown streaking	4.4 e	7.6 de	15.0 cde
Esca brown rot	0.0 e	5.0 e	19.5 bcde
Esca yellow rot	8.0 cde	0.0 e	1.9 e
Wedge-shaped necrosis	14.3 cde	3.8 e	26.9 bcd
Watery necrosis	0.0 e	60.0 a	15.0 cde
Asymptomatic wood	0.4 e	0.8 e	1.0 e
LSD		20.76	

[a] See Table 1.

Table 5. Mean incidence of *Phomopsis* spp. in isolates from vines displaying different symptoms in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region [a]		
	Summer	Marginal	Winter
Brown internal necrosis	0.1 c	3.3 bc	6.7 bc
Black streaking	0.0 c	4.5 bc	3.5 bc
Brown streaking	0.0 c	1.8 c	1.0 c
Esca brown rot	0.0 c	0.0 c	1.8 c
Esca yellow rot	0.0 c	0.0 c	0.5 c
Wedge-shaped necrosis	1.4 c	21.8 a	14.2 ab
Watery necrosis	0.0 c	0.0 c	5.0 bc
Asymptomatic wood	0.0 c	0.0 c	0.6 c
LSD		11.80	

[a] See Table 1.

dence from the other symptom types ranged from 0.6% to 3.9%, with asymptomatic wood yielding the fewest isolates. In the summer rainfall region, isolates of the Botryosphaeriaceae were obtained from all the symptom types except watery necrosis. Here, the highest incidence came from wedge-shaped necrosis (29.5%), esca-like yellow soft rot (22.4%) as well as esca-like brown wood rot (14.7%). In the winter rainfall region, this group of pathogens was not isolated from watery necrosis or brown streaking and seldom from black streaking, esca-like brown wood rot and asymptomatic wood. The highest incidence of isolation was from wedge-shaped necrosis (22%), followed by esca-like yellow soft rot (9%) and brown internal necrosis (5.7%). Wine grapes yielded more Botryosphaeriaceae isolates (12.7%) than table grapes (6.9%; Table 3).

Table 6. Mean incidence of species in the Botryosphaeriaceae in isolates from vines displaying different symptoms in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region [a]		
	Summer	Marginal	Winter
Brown internal necrosis	6.9 efg	37.7 abc	5.7 efg
Black streaking	2.9 g	1.6 g	0.6 g
Brown streaking	1.2 g	3.9 fg	0.0 g
Esca brown rot	14.7 defg	2.0 g	0.5 g
Esca yellow rot	22.4 cde	1.3 g	9.0 efg
Wedge-shaped necrosis	29.5 bcd	46.0 ab	22.0 cdef
Watery necrosis	0.0 g	50.0 a	0.0 g
Asymptomatic wood	1.7 g	0.6 g	0.8 g
LSD		18.17	

[a] See Table 1.

Visual analysis of the EF1–α sequences generated for the 150 selected isolates of Botryosphaeriaceae spp. displayed great variation. The majority of the isolates were identified as *D. seriata* (van Niekerk *et al.,* 2004). This species occurred in all the rainfall areas and was isolated from all symptom types on wine and table grapes (results not shown). *Neofusicoccum parvum* occurred in all the rainfall areas, but was isolated only from table grapes and from brown internal necrosis and wedge-shaped necrosis. *Neofusicoccum australe* occurred only in the winter rainfall region, where it was isolated from both wine and table grapes and from brown internal necrosis and black streaking (results not shown).

Lasiodiplodia theobromae, *L. crassispora*, *B. dothidea* and *N. vitifusiforme* were also identified based on their DNA sequence data (van Niekerk *et al.,* 2004; Burgess *et al.,* 2006). These four species occurred only in the summer rainfall region, where they were isolated from wine and table grapes. *Lasiodiplodia theobromae* was isolated from five symptom types, wedge-shaped necrosis, brown internal necrosis, black streaking, esca-like brown wood rot and asymptomatic wood. *Lasiodiplodia crassispora* was isolated only from brown internal necrosis observed on wine grapes. *Botryosphaeria dothidea* was isolated from table grapes in association with wedge-shaped necrosis. *Neofusicoccum vitifusiforme* was also isolated only from table grapes and in association with brown internal necrosis, wedge-shaped necrosis and black streaking (results not shown).

Basidiomycetes spp. were also found to occur at varying incidences in all the rainfall regions surveyed, and were isolated only from the esca-like symptoms of hard brown rot and yellow soft rot on wine and table grapes. The highest incidence of this group of fungi was recorded in the winter rainfall region (0.87%) followed by the summer (0.23%) and marginal (0.19%) rainfall regions (results not shown).

Discussion

Given the opportunistic nature of trunk disease pathogens (Ferreira *et al.,* 1999) and the variability in expression of foliar symptoms (Creaser and Wicks, 2001; Marchi *et al.,* 2006; Sosnowski *et al.*, 2007), the sampling method followed here was considered a reliable means of determining the demography of trunk disease pathogens in climatically diverse regions. However, this sampling method might be regarded as biased towards pathogens invading pruning wounds during their susceptible period, which can vary between 2 weeks (Petzoldt *et al.,* 1981) and 16 weeks (Gubler *et al.,* 2001). Apart from pruning wounds, *E. lata* can also infect desuckering wounds on cordons in spring. Although the occurrence of these infections is low, their contribution to trunk disease infections cannot be discounted as they could have contributed to the pathogen incidence in specific regions (Creaser *et al*., 2003; Úrbez-Torres *et al*., 2006).

Except for *E. lata,* all known grapevine trunk pathogens were encountered in all the regions surveyed. *E. lata* was encountered only in wine grape samples from the winter rainfall region, which was surprising as annual rainfall in two of the rainfall regions, over 600 mm per annum, exceeded the minimum of 350 mm reported by Magarey and Carter (1986) as conducive for Eutypa dieback. It was furthermore isolated only from esca-like soft brown wood rot, which is not typically associated with this pathogen. Typical Eutypa dieback wood decay symptoms were observed in samples from all the regions, and the low incidence of *E. lata* in this survey was therefore surprising. It is possible that fast-growing species in the Botryosphaeriaceae and *Phomopsis* often isolated from this symptom type in all the regions outgrew *E. lata* in culture. However, it is also possible that Diatrypaceae species other than *E. lata* were isolated, as Trouillas *et al*. (2010) showed that several genera and species morphologically indistinguishable from *E. lata* occurred in grapes in the warm, dry regions of California.

Epidemics caused by *Phomopsis viticola* are associated with abundant rain that is conducive to spore release (Cucuzza and Sall, 1982). In the winter and marginal rainfall regions, where *Phomopsis* isolates were most prevalent, these conditions occur predominantly at the time when pruning is done and abundant susceptible pruning wounds are available for infection, leading to the high incidence of *Phomopsis* spp. in this region. Very little or no rainfall occurs in the summer rainfall region during the time when susceptible pruning wounds or green tissue are present, thus little inoculum would result in low incidence of infection by *Phomopsis* spp.

The symptom profile of *P. viticola* differed with the region. In the winter rainfall region, it was isolated from all symptom types, while in the marginal and summer rainfall regions it was isolated from only four and two symptom types respectively. These observations correspond with earlier findings of Fischer and Kassemeyer (2003), and indicate that this pathogen is an important causal organism of different internal wood decay symptoms, and cannot exclusively be regarded as a pathogen of green grapevine tissue (Cucuzza and Sall, 1982). The difference in symptom profile between regions can possibly be explained by the mechanisms involved in wound repair and the period of wound susceptibility. Munkvold and Marois (1995) reported a strong positive correlation between higher mean temperature after pruning and the rate of suberin accumulation, and therefore wound repair, in grapevine pruning wounds. In the winter rainfall region, the average temperature during the grapevine pruning period (June to September) is lower than in the marginal and summer rainfall regions, which would have influenced the rate of healing and plant defence reactions, thereby influencing incidence and symptom profiles.

Consistent with earlier reports (Denman *et al.,* 2000) indicating the wide geographical distribution and ability of this family of pathogens to infect various hosts under varied climatic conditions, Botryosphaeriaceae occurred in all the rainfall regions. These characteristics can therefore also explain the similar symptom profiles observed in the different rainfall regions. Infected alternative hosts, such as stone fruit, as shown by Damm *et al*. (2007), in the vicinity along with infected propagation material probably supplied the primary inoculum for infection of grapevines in the various regions.

The incidence of Botryosphaeriaceae spp. was highest in the marginal rainfall region. In the marginal region, rainfall, which triggers spore release of these pathogens, occurs throughout most of the year making inoculum available not to infect only fresh pruning wounds but also other mechanical wounds, which are reported by Lehoczky (1974) to be the main infection portals.

Species in the Botryosphaeriaceae have increasingly been reported from diseased grapevines in South Africa, associated with internal wood necrosis, dieback and canker formation (van Niekerk *et al.,* 2004; 2010). In line with the findings of Castillo-Pando *et al*. (2001), the Botryosphaeriaceae spp. were most often isolated from brown internal necrosis, wedge-shaped and watery necrosis and at a much lower incidence from black and brown streaking that originated from old pruning wounds. They were also recorded from atypical symptoms such as esca-like brown or yellow soft wood rot. The presence of Botryosphaeriaceae spp. in the yellow soft wood rot may be a result of their saprophytic nature (Smith *et al.,* 1996). Several isolates were also obtained from asymptomatic tissue, supporting reports of the ability of some of these species to survive endophytically in their host, causing disease only when the host is predisposed by stress (Smith *et al.,* 1996).

Numerous Botryosphaeriaceae spp. are associated with grapevines (van Niekerk *et al.,* 2004; 2010). *Diplodia seriata*, a species known to occur in climatically diverse areas, was recorded in this study in all the different rainfall regions from both grape types, similar to the findings of Úrbez-Torres *et al*. (2006) in California and Pitt *et al*. (2010) in Australia. *Neofusicoccum parvum*, previously reported in South Africa from asymptomatic tissue and pruning debris (van Niekerk *et al.,* 2004), also occurred in all the rainfall regions, but was isolated only from table grapes with brown internal and wedge-shaped necrosis. Recently, it was reported for the first time to be associated with declining grapevines in California, where it was also isolated from wedge-shaped necrosis and black streaking (Úrbez-Torres *et al.,* 2006). Of all the Botryosphaeriaceae species encountered in this study, *N. australe* was the only species to occur only in the winter rainfall region, isolated from brown internal necrosis and black streaking on wine and table grapes.

The other four Botryosphaeriaceae species identified during this survey, *L. theobromae*, *L. crassispora*, *B. dothidea* and *N. vitifusiforme*, occurred only in the summer rainfall region, where they were isolated from wine and table grapes. *L. theobromae* has been reported from a number of grape-growing countries (van Niekerk *et al.,* 2004; Úrbez-Torres *et al.,* 2006, Pitt *et al*., 2010), associated with a wide range of symptoms, including wedge- and arch-shaped necrosis, cane dieback and fruit rots (Úrbez-Torres *et al.,* 2006). In the present study, this species was also isolated from different symptom types, which included wedge-shaped and brown internal necrosis, black streaking, esca-like brown wood rot and asymptomatic wood. This species is also very specific in its climatic "preferences". In California and Australia, it was reported only from areas with a warm and humid climate (Úrbez-Torres *et al.,* 2006, Pitt *et al*., 2010), which corresponds to its presence only in the summer rainfall regions of South Africa, where the climate ranges from hot and relatively dry summers and cold winters to hot, wet and humid summers and mild winters. *L. crassispora* was recently isolated from cankered wood of *Santalum album* in Western Australia and as an endophyte from *Eucalyptus urophylla* in Venezuela (Burgess *et al.,* 2006). As for *L. theobromae*, this species was reported to have an optimum growth temperature of 30°C (Burgess *et al.,* 2006), which would also explain its presence in the summer rainfall region, where it was isolated from brown internal necrosis on wine grapes.

In the current study, *B. dothidea* was isolated from wedge-shaped necrosis and was recently reported for the first time from grapevines in California, where it was isolated from wedge-shaped necrosis and black vascular streaking (Úrbez-Torres *et al.,* 2006). *Neofusicoccum vitifusiforme*, first reported in South Africa from black streaking and brown internal necrosis occurring in South African grapevines (van Niekerk *et al.,* 2004), was isolated during the current study from brown internal and wedge-shaped necrosis. *Pa. chlamydospora* occurred at various levels in all the rainfall regions. It has been reported from almost all grape-growing countries (Mugnai *et al.,* 1999), indicating its ability to grow and infect in a wide range of climatic conditions. As *Pa. chlamydospora* is reported to be specific to grapevines (Crous and Gams, 2000), infected propagation material was probably the main source of inoculum in all regions.

The incidence of *Pa. chlamydospora* varied markedly between rainfall regions, perhaps due to the influence of regional climatic differences. It is a known invader of pruning wounds (Larignon and Dubos, 2000) and spores of this pathogen have been trapped throughout the year in French vineyards during or after rainfall (Larignon and Dubos, 2000). The high incidence recorded in the winter and marginal regions is therefore probably due to the presence of inoculum during the grapevine pruning period in the winter that would lead to numerous infections of fresh wounds. In the summer rainfall region, the infections would be limited to wounds made during viticultural practices employed during summer when inoculum of the pathogen is present after rain, thus explaining the low incidence recorded in this area.

Phaeomoniella chlamydospora and several *Phaeoacremonium* spp. are associated with Petri disease of young vines (1–5 years) and esca of older vines (10 years and older) (Mugnai *et al.*, 1999). In young vines, these pathogens are often isolated from black vascular tissue, which is a typical symptom of this disease (Mugnai *et al.,* 1999). In studies by Larignon and Dubos (1997) and Fischer and Kassemeyer (2003), *Pa. chlamydospora* was isolated from black streaking, brown necrosis and the firm dark brown margin surrounding the white rot in the trunks of esca diseased vines. In our study, *Pa. chlamydospora* was isolated from all the different symptom types, showing a much broader symptom profile than previously reported.

Phaeoacremonium spp. were isolated from samples obtained in all the regions. However, these fungi have been reported to occur on woody hosts other than grapevines (Mostert *et al.,* 2006b), indicating that infected propagation material should not be regarded as the sole source of primary *Phaeoacremonium* inoculum in a region. Despite similarity in the symptom profile of this pathogen group in different regions, variation in incidence between the regions was also observed, which could also be attributed to the climatic preferences of this genus.

Spore release of *Phaeoacremonium aleophilum* peaks after rainfall (Larignon and Dubos, 2000). Therefore, the high incidence of *Phaeoacremonium* spp. in the winter rainfall region and its slightly lower incidence in the marginal rainfall region was expected. However, the high incidence recorded in the summer rainfall region was surprising. Larignon and Dubos (2000) also found *Phaeoacremonium* inoculum to be present during the vegetative period of French vineyards, while Gubler *et al.* (2001) found that pruning wounds remained susceptible to infection by *Phaeoacremonium* spp. for up to 4 months. It is thus possible that when rain occurs in spring and/or early summer in the summer rainfall region, pruning wounds made during late winter or desuckering and girdling wounds might become infected.

Phaeoacremonium spp. were isolated from the typical Petri disease symptoms of brown and black vascular streaking. However, as with the other pathogens encountered in this study, it was also isolated from more progressive wood decay symptoms that could have developed from initial vascular infections. The isolates selected were identified as being representative of *Pm. aleophilum*, a species regarded as one of the most cosmopolitan amongst the 14 *Phaeoacremonium* spp. associated with decline diseases of grapevines around the world (Mostert *et al.,* 2006b). In accordance with these reports, this species occurred in all the regions surveyed and in association with symptoms similar to those reported by Larignon and Dubos (1997). Both *Pa. chlamydospora* and *Phaeoacremonium* spp. were isolated from asymptomatic tissue, supporting reports of a biotrophic/endophytic phase for these pathogens (Mugnai *et al.,* 1999; Larignon and Dubos, 2000).

Several basidiomycete isolates were obtained from white rot symptoms associated with esca-affected vines (Fischer, 2006). Larignon and Dubos (1997) found these fungi to be associated not only with white rot, but also with the brown margin surrounding the rot and brown internal necrosis. In our study, basidiomycetes were recorded from all the areas and were isolated from the yellow esca-like rot, and from vascular streaking and more progressive necrotic symptoms.

The results of this study highlight the complex nature of grapevine trunk diseases. All pathogens except *E. lata* occurred in all the surveyed regions. However, the incidence and symptom profiles of the pathogens varied greatly between regions. This indicates that rainfall and temperature influence not only the distribution of pathogens but also the symptomatology of the pathogens in a climatic region. The results furthermore showed that the pathogens overlapped in terms of symptomatology, making symptom-based diagnosis of these diseases and their causal organisms unreliable. Therefore, management strategies for the different pathogens in a specific region should be aimed at the whole complex of trunk disease pathogens.

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