Phytopathol. Mediterr. (2011) 50, 204–223

Characterisation of the fungi associated with esca diseased grapevines in South Africa

CHANA-LEE WHITE[1], FRANCOIS HALLEEN[1, 2], MICHAEL FISCHER[3] and LIZEL MOSTERT[1]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa
[2]Plant Protection Division, ARC Infruitec-Nietvoorbji, Private Bag X5026, Stellenbosch, 7599, South Africa
[3]Julius-Kühn-Institut, Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, D-76833 Siebeldingen, Germany

Summary. During the period from 2001 to 2008, grapevines showing foliar and/or internal symptoms of esca were collected from various grape-growing regions in South Africa. Isolations were made from typical internal wood symptoms associated with esca, and fungal isolates were characterized by cultural growth patterns, morphology and phylogenetic inference. The gene regions sequenced included the internal transcribed spacers and the 5.8S rRNA gene (ITS) for the basidiomycetes and *Phomopsis* isolates, the partial β-tubulin and actin genes for *Phaeoacremonium* isolates and the partial translation elongation 1-α gene and the ITS for the Botryosphaeriaceae isolates. The fungi identified included *Phaeomoniella chlamydospora* and six species of *Phaeoacremonium* including *Pm. aleophilum*, *Pm. alvesii*, *Pm. parasiticum*, *Pm. iranianum*, *Pm. mortoniae* and *Pm. sicilianum*, of which the latter three are reported for the first time in South Africa. The following taxa were also identified: *Eutypa lata*, *Phomopsis viticola*, *Phomopsis theicola*, *Diaporthe ambigua*, *Diplodia seriata*, *Neofusicoccum australe* and *N. parvum*. The basidiomycete isolates were distributed over ten well supported monophyletic clades among genera of the Hymenochaetales. Two of these clades could be identified as species of *Fomitiporia* and *Phellinus*.

Key words: basidiomycetes, Botryosphaeriaceae, Hymenochaetales, *Phaeomoniella chlamydospora*, *Phaeoacremonium*, *Phomopsis*.

Introduction

Esca is a well-known disease of grapevines that causes decline and loss of productivity of vines. The disease has been studied in various grapevine producing countries, including Australia, Austria, France, Germany, Greece, Italy, Portugal, Spain and the USA (Chiarappa, 1959; Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pascoe and Cottral, 2000; Reisenzein *et al*., 2000; Armengol *et al*., 2001; Rumbos and Rumbou, 2001; Fischer and Kassemeyer, 2003; Sofia *et al*., 2006). In South Africa only a few incidences of esca diseased grapevines have been reported (Marais, 1981).

Corresponding author: L. Mostert
Fax: +27 21 8084956
E-mail: lmost@sun.ac.za

Esca is a complex disease which is caused by a combination and/or succession of different fungi. *Phaeomoniella* (*Pa.*) *chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams and various species of *Phaeoacremonium* causes Petri disease, which is generally seen as the precursor disease of esca (Larignon and Dubos, 1997; Mugnai *et al*., 1999). The white wood rot symptoms associated with esca are caused by basidiomycete fungi such as *Fomitiporia* (*F.*) *mediterranea* M. Fischer, *F. polymorpha* M. Fischer and *F. australiensis* M. Fisch., J. Edwards, Cunnington & Pascoe (Fischer, 2002; Fischer and Kassemeyer, 2003; Fischer and Binder, 2004; Fischer *et al*., 2005; Fischer, 2006). *Phaeomoniella chlamydospora*, *Phaeoacremonium* species, and the basidiomycetes, have traditionally been seen as the causal agents of esca (Mugnai *et al*., 1999).

Other trunk disease fungi have also been isolated from esca diseased vines, making the etiol-

ogy of the disease more complex. Fungi regularly isolated from esca diseased vines include species of the Botryosphaeriaceae, *E. lata* Tul. & C. Tul. and *Ph. viticola* (Sacc.) Sacc. (Larignon and Dubos, 1997; Fischer and Kassemeyer, 2003; Calzarano and Di Marco, 2007; Péros *et al*., 2008). Their role in esca is not clear, since they cause Botryosphaeria canker, Eutypa dieback and Phomopsis cane and shoot blight, respectively (Munkvold *et al*., 1994; van Niekerk *et al*., 2004, 2005, 2006).

Various studies on South African grapevines have investigated Petri disease fungi (Crous *et al*., 2000; Groenewald *et al*., 2001; Mostert *et al*., 2006b), the Botryosphaeriaceae (van Niekerk *et al*., 2004), *Phomopsis* species (Mostert *et al*., 2001; van Niekerk *et al*., 2005) and the Diatrypaceae (Mostert *et al*., 2004; Safodien, 2007). In a review of basidiomycete taxa from grapevines worldwide, ten basidiomycete isolates from esca diseased grapevines in South Africa were included (Fischer, 2006). The ITS phylogeny revealed that the South African isolates formed three unrelated and new clades. One was closely related to *F. mediterranea* and two were related to *Inocutis sensu lato*. Prior to the study of Fischer (2006), *Stereum hirsutum* and *Phellinus igniarius* were believed to be causal organisms associated with esca (Marais, 1981), although no study has confirmed this.

No prior comprehensive study has been carried out in South Africa to isolate and identify the fungi associated with typical esca diseased grapevines. Also, limited information is available on the basidiomycete taxa associated with the white rot of esca in South Africa. Therefore, the aim of this study was to characterize the different fungi associated with esca diseased vines collected from geographically different grape growing regions in South Africa.

Materials and methods

Sampling of esca diseased vines

Vineyards showing leaf symptoms of esca, general decline or dieback were identified and sampled from all the major grapevine producing areas in South Africa from 2001 until 2008. Vines showing typical symptoms (external and/or internal) were removed and taken to the laboratory, where transverse sections of the wood were made. Esca diseased vines were identified as having brown-black internal discoloration accompanied by white rot.

Fungal isolations

Cross and longitudinal sections were made at various places in the cordons and trunk of each plant to investigate internal necrosis. For fungal isolations, wood sections with internal necrosis were selected and cut into two smaller sections adjacent to each other, in order to obtain two mirror images of the same symptom type. This was also done to facilitate the use of two sterilization techniques to ensure fungal isolation from soft, spongy material. The one section was flame sterilized by holding the wood with sterile forceps, lightly spraying it with 70% ethanol and passing it through a flame. The other piece was triple sterilized as follows: 30 s in 70% ethanol, 2 min in 3.5% NaOCl and 30 s in 70% ethanol. Twelve small sections of wood (1 × 1 × 2 mm) from each of the different symptom types were then aseptically removed with a scalpel and placed onto potato dextrose agar (PDA, Biolab, Midrand, South Africa) plates containing 250 mg chloramphenicol (four tissue sections per plate). Plates were incubated at 23–25°C for approximately 4 weeks. The growth of fungi from tissue pieces was monitored daily.

Morphological characterization

Isolates were identified according to morphological and cultural characteristics as species of basidiomycetes (Fischer, 2002), Botryosphaeriaceae (Van Niekerk *et al*., 2004; Crous *et al*., 2006; Damm *et al*., 2007; Phillips *et al*., 2008), *Eutypa* (Glawe and Rogers, 1982), *Phaeoacremonium* (Mostert *et al*., 2006b; Essakhi *et al*., 2008), *Phomopsis* (Mostert *et al*., 2001; Van Niekerk *et al*., 2005) or *Phaeomoniella chlamydospora* (Crous and Gams, 2000). The cultures were purified through hyphal tipping or single sporing, if possible. All of the basidiomycete isolates and a selection of isolates of the other genera were deposited in the fungal culture collection at the ARC Infruitec-Nietvoorbij in Stellenbosch and the Department of Plant Pathology, University of Stellenbosch (Table 1). The total number of isolates obtained for each fungal taxon is reported in White *et al*. (2011). This paper reports the characterization of a selected number of isolates within each taxon.

The cultural growth patterns were determined for 38 isolates of *Phaeoacremonium* on PDA, malt extract agar (MEA; 2% malt extract, Oxoid Ltd., Basingstoke, England; 1.5% agar (Difco, Le Pont de

Table 1. Details of origin, host cultivars and ages, collection dates and GenBank accession numbers for *Phaeoacremonium, Phomopsis,* Botryosphaeriaceae, *Eutypa* and basidiomycete isolates obtained from esca affected grapevines (*Vitis vinifera*) in South Africa.

Taxon	STE-U Number	Origin	Cultivar	Age of vine (years)	Collection date[1]	GenBank acc. No.
Phaeoacremonium						**TUB, ACT**
Pm. aleophilum	6986	Hermanus	Chardonnay	21	2008/02/13	JQ038909, JQ038920
	6991	Vredendal	Colombar	± 35	2008/01/30	JQ038910, JQ038921
	6996	Wellington	Cabernet Sauvignon	13	2008/02/18	
	6997	Calitzdorp	Hanepoot	37	2008/02	
	7002	Calitzdorp	Hanepoot	37	2008/02	
Pm. alvesii	6988	Klawer	Chenin blanc	41	2008/01/31	JQ038914, JQ038925
	6989	Klawer	Chenin blanc	41	2008/01/31	JQ038915, JQ038926
	7000	De Rust	Chenin blanc	38	2008/02/06	
	7001	De Rust	Chenin blanc	38	2008/02/06	
Pm. iranianum	6998	Calitzdorp	Chenin blanc	44	2008/02/06	JQ038911, JQ038922
	6999	Calitzdorp	Chenin blanc	44	2008/02/06	JQ038912, JQ038923
Pm. mortoniae	6987	Hermanus	Chardonnay	21	2008/02/13	JQ038913, JQ038924
Pm. parasiticum	6990	Klawer	Chenin blanc	41	2008/01/31	JQ038917, JQ038928
	6993	De Rust	Fransdruif	33	2008/02/07	JQ038916, JQ038927
Pm. sicilianum	6992	Oudtshoorn	Colombar	31	2008/02/07	JQ038918, JQ038929
	6994	Calitzdorp	Hanepoot	37	2008/02	JQ038919, JQ038930
	6995	Calitzdorp	Hanepoot	37	2008/02	
Phomopsis/ Diaporthe						**ITS**
Diaporthe ambigua	7003	Porterville	Colombar	15	2005/03/01	JQ038884
Phomopsis theicola	7010	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	JQ038885
	7016	Stellenbosch	Sauvignon blanc	25	2005/06/02	JQ038886
Ph. viticola	7004	Stellenbosch	Cabernet Sauvignon	Unknown	2005/05/05	JQ038887
	7005	Stellenbosch	Cabernet Sauvignon	Unknown	2005/05/05	JQ038888
	7006	Stellenbosch	Cabernet Sauvignon	Unknown	2005/02/17	
	7007	Stellenbosch	Cabernet Sauvignon	Unknown	2005/05/05	
	7008	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7009	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7011	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7012	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7013	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7014	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7015	Stellenbosch	Sauvignon blanc	25	2005/06/02	
	7017	Lutzville	Colombar	22	2008/01/30	
	7018	Somerset West	Cabernet Sauvignon	31	2008/02/20	
	7019	Ashton	Sauvignon blanc	20	2008/02/29	
Botryosphaeriaceae						**ITS, EF**
Diplodia seriata	7020	Paarl	Chenin blanc	18	2005/02/03	JQ038878, JQ038872
	7026	Porterville	Colombar	15	2005/03/01	JQ038879, JQ038873
	7031	Stellenbosch	Sauvignon blanc	25	2005/06/02	
	7032	Stellenbosch	Sauvignon blanc	25	2005/06/21	
	7033	Klawer	Fransdruif	35	2008/01/30	
	7034	Tulbagh	Chenin blanc	24	2007/11/06	
	7035	Rawsonville	Chenin blanc	20	2007/11/28	

continues

Table 1. *continued*

Taxon	STE-U Number	Origin	Cultivar	Age of vine (years)	Collection date[1]	GenBank acc. No.
Neofusicoccum australe	7024	Paarl	Hanepoot	22	2005/02/14	JQ038882, JQ038876
	7025	Porterville	Colombar	15	2005/03/01	JQ038883, JQ038877
	7028	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7029	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7030	Stellenbosch	Pinotage	28	2005/05/25	
	7021	Paarl	Chenin blanc	18	2005/02/14	
	7022	Paarl	Chenin blanc	18	2005/02/14	
	7023	Paarl	Chenin blanc	18	2005/02/14	
	7027	Porterville	Colombar	15	2005/03/02	
Neofusicoccum parvum	7036	Darling	Chenin blanc	21	2007/10/22	JQ038880, JQ038874
	7037	Constantia	Sauvignon blanc	25	2007/10/16	JQ038881, JQ038875
Diatrypaceae						**ITS**
Eutypa lata	5699	Stellenbosch	Sauvignon blanc	23	2003/03/13	JQ038891
	5700	Stellenbosch	Sauvignon blanc	23	2003/03/13	JQ038892
	5692	Stellenbosch	Chenin blanc	26	2002/11/25	
	5693	Stellenbosch	Chenin blanc	26	2002/11/25	
	5694	Stellenbosch	Chenin blanc	26	2002/11/25	
	5695	Stellenbosch	Chenin blanc	26	2002/11/25	
	5696	Stellenbosch	Chenin blanc	26	2002/11/25	
	5697	Stellenbosch	Chenin blanc	26	2002/11/25	
	5698	Stellenbosch	Chenin blanc	26	2002/11/25	
Basidiomycetes						**ITS**
Taxon 1	7038	Stellenbosch	Sauvignon blanc	23	2003/01/29	JQ038891
	7039	Stellenbosch	Sauvignon blanc	23	2003/01/29	JQ038892
	7045	Stellenbosch	Sauvignon blanc	23	2003/03/13	
	7046	Porterville	Colombar	15	2004/11/15	
	7047	Porterville	Colombar	15	2004/11/15	
	7048	Paarl	Chenin blanc	18	2005/02/03	
	7051	Paarl	Chenin blanc	18	2005/02/14	
	7054	Porterville	Dan Ben Hanna	19	2003/11/27	
	7058	Porterville	Colombar	15	2005/03/02	
	7059	Porterville	Colombar	15	2005/03/02	
	7060	Porterville	Colombar	15	2005/03/02	
	7061	Porterville	Colombar	15	2005/03/02	
	7062	Porterville	Colombar	15	2005/03/02	
	7063	Porterville	Colombar	15	2005/03/02	
	7064	Porterville	Colombar	15	2005/03/02	
	7065	Porterville	Colombar	15	2005/03/01	
	7066	Porterville	Colombar	15	2005/03/01	
	7067	Slanghoek	Hanepoot	40	2005/03/02	
	7070	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7071	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7073	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7074	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7075	Stellenbosch	Cabernet Sauvignon	19	2005/05/24	
	7078	Stellenbosch	Sauvignon blanc	25	2005/06/02	

continues

Table 1. *continued*

Taxon	STE-U Number	Origin	Cultivar	Age of vine (years)	Collection date[1]	GenBank acc. No.
	7079	Stellenbosch	Sauvignon blanc	25	2005/06/07	
	7080	Stellenbosch	Sauvignon blanc	25	2005/06/07	
	7083	Stellenbosch	Cabernet Sauvignon	19	2005/06/23	
	7084	Slanghoek	Hanepoot	40	2005/07/08	
	7088	Rawsonville	Chenin blanc	11	2005/11/03	
	7092	De Doorns	Sultana	18	2007/07/07	
	7107	Constantia	Sauvignon blanc	25	2007/10/16	
	7110	Constantia	Sauvignon blanc	18	2007/10/16	
	7112	Bonnievale	Sauvignon blanc	20	2007/10/07	
	7113	Bonnievale	Sauvignon blanc	20	2007/10/07	
	7117	Durbanville	Shiraz	21	2007/09/27	
	7118	Durbanville	Shiraz	21	2007/09/27	
	7120	Durbanville	Sauvignon blanc	23	2007/09/27	
	7123	Durbanville	Sauvignon blanc	23	2007/09/27	
	7130	Malmesbury	Chenin blanc	36	2007/10/22	
	7141	Riebeeck Kasteel	Chenin blanc	20	2007/11/06	
	7142	Tulbagh	Chenin blanc	24	2007/11/06	
	7144	Tulbagh	Chenin blanc	28	2007/11/06	
	7145	Rawsonville	Chenin blanc	20	2007/11/28	
	7146	Rawsonville	Chenin blanc	20	2007/11/28	
	7148	De Rust	Chenin blanc	38	2008/02/06	
	7149	De Rust	Chenin blanc	38	2008/02/06	
	7150	De Rust	Red muskadel	31	2008/02/06	
	7151	De Rust	Red muskadel	31	2008/02/06	
	7152	De Rust	Fransdruif	33	2008/02/07	
	7156	Lutzville	Colombar	22	2008/01/30	
	7157	Klawer	Fransdruif	35	2008/01/30	
	7158	Klawer	Chenin blanc	41	2008/01/31	
	7159	Klawer	Chenin blanc	41	2008/01/31	
	7160	Klawer	Chenin blanc	41	2008/01/31	
	7161	Klawer	Chenin blanc	41	2008/01/31	
	7162	Klawer	Chenin blanc	41	2008/01/31	
	7172	Somerset West	Cabernet Sauvignon	32	2008/02/19	
	7175	Ashton	Shiraz	30	2008/02/29	
	7176	Montagu	Colombar	27	2008/02/29	
Taxon 2	7147	Oudtshoorn	Pinotage	29	2008/02/06	JQ038893
	7154	Calitzdorp	Hanepoot	37	2008/02	JQ038894
	7155	Calitzdorp	Hanepoot	37	2008/02	
Taxon 3	7109	Constantia	Sauvignon blanc	18	2007/10/16	JQ038895
	7136	Grabouw	Sauvignon blanc	15	2007/11/08	JQ038896
	7174	Ashton	Sauvignon blanc	20	2008/02/29	
	7178	Montagu	Colombar	27	2008/02/29	
Taxon 4	7042	Stellenbosch	Chenin blanc	26	2002/11/25	
	7043	Stellenbosch	Chenin blanc	26	2002/11/25	
Taxon 5	7125	Darling	Chenin blanc	23	2007/10/22	JQ038899
	7126	Darling	Chenin blanc	23	2007/10/22	JQ038900
	7127	Darling	Chenin blanc	21	2007/10/22	

continues

Table 1. *continued*

Taxon	STE-U Number	Origin	Cultivar	Age of vine (years)	Collection date[1]	GenBank acc. No.
	7128	Darling	Chenin blanc	21	2007/10/22	
	7129	Darling	Chenin blanc	21	2007/10/22	
	7131	Malmesbury	Pinotage	36	2007/10/22	
	7132	Malmesbury	Pinotage	36	2007/10/22	
	7143	Tulbagh	Chenin blanc	24	2007/11/06	
	7153	Ladismith	Chenin blanc	28	2008/02/06	
	7177	Montagu	Colombar	27	2008/02/29	
Taxon 6	7133	Malmesbury	Pinotage	36	2007/10/22	JQ038901
	7134	Malmesbury	Pinotage	36	2007/10/22	JQ038902
Taxon 7	7076	Stellenbosch	Pinotage	28	2005/05/25	
	7090	Stellenbosch	Ruby Cabernet	22	2007/08/02	
	7106	Constantia	Sauvignon blanc	25	2007/10/16	
	7165	Franschhoek	Chenin blanc	25	2008/02/13	
	7173	Somerset West	Cabernet Sauvignon	31	2008/02/20	
Taxon 8	7138	Botrivier	Chenin blanc	20	2007/11/08	
	7139	Botrivier	Chenin blanc	20	2007/11/08	
Fomitiporia sp.	7040	Stellenbosch	Sauvignon blanc	23	2003/01/29	
	7041	Stellenbosch	Sauvignon blanc	23	2003/01/29	
	7050	Paarl	Hanepoot	22	2005/02/14	
	7052	Paarl	Chenin blanc	18	2005/02/14	
	7053	Paarl	Chenin blanc	18	2005/02/14	
	7056	Stellenbosch	Hanepoot	12	2005/02/25	
	7057	Stellenbosch	Malbec	12	2005/02/25	
	7069	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7072	Stellenbosch	Cabernet Sauvignon	15	2005/05/17	
	7077	Stellenbosch	Sauvignon blanc	25	2005/06/02	
	7081	Stellenbosch	Sauvignon blanc	25	2005/06/20	
	7082	Stellenbosch	Sauvignon blanc	25	2005/06/21	
	7086	Klaas voogds	Red Globe	10	2005/09/12	
	7093	Wellington	Chenin blanc	20	2007/09/07	
	7094	Wellington	Chenin blanc	20	2007/09/07	
	7095	Wellington	Chenin blanc	20	2007/09/07	
	7096	Franschhoek	Chenin blanc	40	2007/09/19	
	7097	Somerset West	Sauvignon blanc	16	2007/09/26	
	7108	Constantia	Sauvignon blanc	18	2007/10/16	
	7115	Durbanville	Chenin blanc	26	2007/09/27	
	7119	Durbanville	Sauvignon blanc	23	2007/09/27	
	7121	Durbanville	Sauvignon blanc	23	2007/09/27	
	7122	Durbanville	Sauvignon blanc	23	2007/09/27	
	7124	Darling	Chenin blanc	23	2007/10/22	
	7135	Grabouw	Chardonnay	15	2007/11/08	
	7137	Botrivier	Chenin blanc	20	2007/11/08	
	7140	Riebeeck Wes	Chenin blanc	19	2007/11/06	
	7163	Franschhoek	Cabernet Sauvignon	14	2008/02/12	
	7164	Franschhoek	Cabernet Sauvignon	14	2008/02/12	
	7166	Hermanus	Chardonnay	21	2008/02/13	
	7167	Hermanus	Chardonnay	21	2008/02/13	
	7168	Hermanus	Chardonnay	21	2008/02/13	

continues

Table 1. *continued*

Taxon	STE-U Number	Origin	Cultivar	Age of vine (years)	Collection date[1]	GenBank acc. No.
	7169	Wellington	Cabernet Sauvignon	13	2008/02/18	
	7170	Wellington	Cabernet Sauvignon	13	2008/02/18	
	7171	Somerset West	Tinta Barroca	28	2008/02/19	
Phellinus sp.	7055	Marken	Prime seedless	5	2003/11/27	JQ038907
	7098	Kanon Eiland	Sultana	Unknown	2007/09/15	JQ038908
	7099	Kanon Eiland	Sultana	Unknown	2007/09/15	
	7100	Keimoes	Chenin blanc	18	2007/09/15	
	7101	Keimoes	Colomino	18	2007/09/15	
	7102	Keimoes	Colomino	18	2007/09/15	
	7103	Marchand	Sultana	40	2007/09/15	
	7104	Marchand	Sultana	40	2007/09/15	
	7105	Marchand	Sultana	40	2007/09/15	
	7179	Keboes	Sultana	Unknown	2008/02/27	
	7180	Prieska	Sultana	Unknown	2008/04/17	

[1]Isolates were collected by Francois Halleen and Chana-Lee White.

Claix, France) and on Oatmeal Agar (OA, Difco) at 25°C. After 8 days the radial growth of the colonies was measured on MEA. After 16 days, the colour of the colonies was determined on all the media (Rayner, 1970). From these results, 17 *Phaeoacremonium* isolates were selected for further identification of morphological structures formed on MEA.

The Botryosphaeriaceae and *Phomopsis* isolates were plated onto PDA and incubated at 25°C. After 2 weeks 18 out of 137 isolates of Botryosphaeriaceae and 17 *Phomopsis* isolates were selected on the basis of different cultural growth patterns. Botryosphaeriaceae isolates were grown on sterile pine needles on water agar (WA, Biolab) to induce the formation of pycnidia (Crous *et al*., 2006). The *Phomopsis* isolates formed pycnidia on PDA.

Microscope slide mounts in lactic acid were made of the selected Botryosphaeriaceae, *Pa. chlamydospora*, *Phomopsis* and *Phaeoacremonium* isolates. Conidial dimensions were measured under a light microscope (Axioskop, Zeiss, Oberkochen, Germany). Twenty-four spores were measured from each isolate and 95% confidence intervals were calculated for mean dimensions of spores.

The basidiomycetes were plated onto PDA and incubated at 25°C. After 4 weeks, a selection of 134 of the 350 basidiomycete isolates was made. The isolates represented all of the sampled geographical regions, and included all of the different fungal growth patterns in the cultures. A selection of 31 basidiomycete isolates was made, representative of the different phylogenetic clades. A growth study was done with these isolates with mycelial plugs (2 mm diam.) taken from the margins of the colonies, plated onto PDA and incubated at 25°C. After 14 days, the colony diameters were measured.

DNA isolation and amplification

Genomic DNA was extracted from fresh fungal mycelia obtained from PDA plates not older than 14 days from 134 basidiomycete, 18 Botryosphaeriaceae, 17 *Phomopsis* and 17 *Phaeoacremonium* isolates. The CTAB based DNA extraction method was used as described by Damm *et al*. (2008).

The partial β-tubulin region (TUB) was amplified for the *Phaeoacremonium* isolates with the primers T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995). The actin gene (ACT) was amplified using primers ACT-512F and ACT-783R (Carbone and Kohn, 1999). PCR conditions as stated in Mostert *et al*. (2006b) were used for these two gene areas.

For the Botryosphaeriaceae isolates, the elongation factor-1α was amplified with the primers EF1-728F and EF1-986R (Carbone and Kohn, 1999). The internal transcribed spacers 1 and 2 and the 5.8S rRNA gene were amplified with the ITS1 and ITS4 primers (White *et al*., 1990) for the Botryospha-

eriaceae and *Phomopsis* isolates. The PCR reaction contained 1 μL of undiluted DNA, 1 x PCR buffer, 4 pmol of each primer, 0.2 mM of each dNTP, 1.5 U of Taq Polymerase, 2.5 mM $MgCl_2$, and the reaction was made up to a total volume of 25 μL with sterile water. The PCR amplification cycles included a denaturing step at 95°C for 8 min followed by 35 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 60 s, followed by a final extension step at 72°C for 5 min.

The ITS region was also amplified for the basidiomycetes with the primers ITS1 and ITS4. Due to the presence of heterokaryotic mycelium of basidiomycete fungi (Clark and Anderson, 2004), the PCR products were cloned to ensure sequencing of a single copy. A PCR reaction for each isolate was performed to amplify the ITS region using 2 μL undiluted DNA, 0.5 mM of each primer, 0.2 mM of each dNTP, 1 × PCR buffer without $MgSO_4$ (Fermentas Life Sciences, St. Leon-Rot, Germany), 0.5 U of Pfu Taq Polymerase (Fermentas Life Sciences), 4 mM $MgSO_4$ (Fermentas Life Sciences), and the reaction was made up to a total volume of 25 μL with sterile water. The parameters used were a denaturing step at 95°C for 3 min, followed by 40 cycles of 95°C for 1 min, 45°C for 1 min and 72°C for 2 min, followed by a final extension step at 72°C for 5 min.

The PCR reactions were run on a GeneAmp PCR System 9700. All PCR products were visualized under UV light on a 1% agarose gel stained with ethidium bromide. The PCR products were cleaned using the MSB Spin PCRapase kit (Invitek, Berlin, Germany). The ITS products of the basidiomycetes were cloned using the CloneJET™ PCR cloning kit (Fermentas Life Sciences) according to manufacturer's instructions. Colonies were selected and a PCR reaction performed to obtain a product which was then cleaned using the MSB Spin PCRapase kit.

The cleaned products were sequenced in both directions using an ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) with the primers used in the initial PCR reactions. The products were then analyzed on an ABI Prism 3130XL DNA sequencer (Perkin-Elmer, Norwalk, CN).

Phylogenetic analyses

Consensus sequences were made using Geneious Pro v3.6.2 (Biomatters Ltd., Auckland, New Zealand). Reference sequences representing the relevant species for Botryosphaeriaceae (van Niekerk *et al*., 2004), *Phaeoacremonium* (Mostert *et al*., 2006b; Essakhi *et al*., 2008), *Phomopsis* (van Niekerk *et al*., 2005) and the basidiomycetes (Fischer, 2006) were obtained from GenBank (http://www.ncbi.nlm.gov) and included in the different gene alignments. The sequences were automatically aligned using MAFFT v6 (Katoh *et al*., 2002) and further manual alignment was performed using Sequence alignment editor v2.0a11 (Rambaut, 2002).

The congruencies of the TUB and ACT dataset for *Phaeoacremonium* and the EF and ITS dataset for the Botryosphaeriaceae were tested with partition homogeneity using PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford, 2003). Maximum parsimony analyses were performed with PAUP, using the heuristic search option, with ten random taxon additions for all the datasets. Tree bisection and reconstruction was used as the branch swapping algorithm. All characters were unordered and of equal weight. Gaps were treated as missing data. Bootstrap support values were calculated from 1000 heuristic search replicates. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and the rescaled consistency index (RC) values.

Results

Phenotypic characterization

The growth patterns of the *Phaeoacremonium* isolates are reported in Table 2. The colony textures were, in most cases, felty on MEA and PDA, and woolly on OA. Some variation in colony color was observed among the isolates of *Pm. aleophilum*, *Pm. alvesii* L. Mostert, Summerb. & Crous, *Pm. iranianum* L. Mostert, Gräfenhan, W. Gams & Crous and *Pm. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf. full stop, but in general color was similar to the respective species. Conidial dimensions were measured for 17 isolates representing the different cultural growth patterns. The shape of the aerial conidia was similar to that reported for the respective species (Mostert *et al*. 2006; Essakhi *et al*. 2008) Commas. The conidia of *Pm. aleophilum* isolates were mostly oblong-ellipsoidal or cylindrical, occasionally reniform. Those of *Pm. alvesii* were mainly ovoid or oblong-ellipsoidal and sometimes reniform to allantoid. Conidia of *Pm. iranianum* and *Pm. mortoniae* Crous & W.

Table 2. Cultural growth characteristics of *Phaeoacremonium* isolates grown on malt extract agar (MEA), potato dextrose agar (PDA) and oatmeal agar (OA).

Species	No. of isolates	Colony colour[a]			Radial growth after 8 days (mm)
		MEA	PDA	OA	MEA
Pm. aleophilum	14	Greyish sepia (15'''i)	Smoke grey (21''''f)	Vinaceous buff (17'''d)	5 – 10
Pm. aleophilum	1	Isabelline (17''i)	Pale mouse grey (15''''d)	Greyish sepia	5
Pm. aleophilum	1	Dark mouse grey (13''''K) with yellow pigment	Smoke grey	Isabelline (17''i)	13
Pm. alvesii	4	Venacious purple (65'''b); Pale venacious (5'''f)	Pale venacious; buff (19''f)	Fawn (13'''); Venacious purple (65'''b)	10 – 14.5
Pm. alvesii	1	Buff	Buff	Greyish sepia	10
Pm. iranianum	1	Buff	Buff	Buff	8.5
Pm. iranianum	1	Venacious buff	Greyish sepia	Smoke grey	13
Pm. mortoniae	1	Buff	Buff yeast like growth	Buff	12
Pm. parasiticum	3	Pale purplish grey (1''''d)	Greyish sepia	Pale mouse grey	12
Pm. parasiticum	3	Venacious buff mixed with buff	Greyish sepia	Mouse grey (13'''')	12
Pm. sicilianum	6	Venacious buff mixed with Pale mouse grey	Greyish sepia	Pale mouse grey	12 – 14
Pm. sicilianum	2	Pale mouse grey	Greyish sepia	Pale mouse grey	13.5

[a] Colony colour descriptions according to Rayner (1970).

Gams were oblong-ellipsoidal, but of *Pm. mortoniae* were sometimes reniform Full stop Conidia of *Pm. parasiticum* were mostly oblong-ellipsoidal and sometimes allantoid to broadly oblong. Conidia of *Pm. sicilianum* Essakhi, L. Mugnai, Surico & Crous were mainly allantoid, with some being subcylindrical. The conidium dimensions were not a distinguishing feature due to the overlap among the different *Phaeoacremonium* species.

Only seven isolates of *Phomopsis* formed pycnidia. The alpha conidial dimensions and shape were similar to that of *Ph. viticola* (Mostert *et al*., 2001). Identification of the other isolates was achieved with the phylogenetic analysis.

The colony colour of the 18 Botryosphaeriaceae isolates varied from pale grey to dark grey or olivaceous coloured, and the colony textures were mostly woolly. The colony colour of *Diplodia seriata* De Not isolates included pale mouse grey (15''''d) to mouse grey (13''''i), olivaceous grey (21''''i) or pale olivaceous grey (21''''d). *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips isolates had pale olivaceous grey colonies. Four isolates of *D. seriata* and one of *N. parvum* formed pycnidia. Conidia of *N. parvum* were hyaline, aseptate and measured (13-)15–18(–19) x 6-8 µm, while those of *D. seriata* were brown and aseptate with the inner walls appearing rough in texture and measured (20-)22–25 x (8-)9–10(–12) µm.

The basidiomycete isolates were grouped into 15 cultural growth patterns outlined in Table 3. Variable colony characters occurred within several of the taxa. Colony colour did not always remain the same after subculturing. The different taxa, as determined by the phylogenetic analysis, comprised various cultural growth patterns. However, Taxa 7 and 8 were distinctly only orange-brown in colour with fluffy mycelial growth. Colony diameters were measured for selected isolates of each taxon (Table 4). Taxon 4 was the slowest growing taxon with a

Table 3. Description of the cultural growth patterns of the basidiomycete isolates after 4 weeks on PDA.

Taxa	STE-U No.	Origin	Cultural growth patterns
1	7038; 7039; 7045; 7046; 7047; 7048; 7051; 7058; 7059; 7060; 7061; 7062; 7063; 7064; 7065; 7066; 7067; 7070; 7071; 7073; 7074; 7075; 7078; 7079; 7080; 7083; 7084; 7088; 7092; 7107; 7110; 7112; 7113; 7117; 7118; 7120; 7120; 7123; 7130; 7141; 7142; 7144; 7145; 7146; 7148; 7149; 7150; 7151; 7152; 7156; 7157; 7158; 7159; 7160; 7161; 7162; 7172; 7175; 7176	Ashton; Bonnievale; Constantia; De Doorns; De Rust; Durbanville; Klawer; Lutzville; Malmesbury; Montagu; Paarl; Porterville; Rawsonville; Riebeeck Kasteel; Slanghoek; Somerset West; Stellenbosch; Tulbagh	Cotton white/ pale yellow; Flat brown; Flat orange-yellow; Flat white/ pale yellow (cream); Fluffy cream with dark mycelial strands; Slow growing clear shades of brown with sparse mycelium; Sparse white; Speckled white/ yellow/ brown; Tufty orange/ brown; Tufty white; White brown/ radiating growth streaky growth; Woolly light brown; Woolly sparse white
2	7147; 7154; 7155	Oudtshoorn; Calitzdorp	Flat orange-yellow; Speckled white/yellow/ brown
3	7109; 7136; 7174; 7178	Ashton; Constantia; Grabouw; Montagu	Flat white/ pale yellow (cream); Slow growing shades of brown with sparse mycelium; Wooly light brown; Woolly sparse white
4	7042; 7043	Stellenbosch	Flat various yellow/ brown/ white tones; Slow growing clear shades of brown with sparse mycelium
5	7125; 7126; 7127; 7128; 7129; 7131; 7132; 7143; 7153; 7177	Darling; Ladismith; Malmesbury; Montagu; Tulbagh	Cotton white/ pale yellow; Flat brown; Flat orange-yellow; Flat white/ pale yellow (cream); Speckled white/ yellow/ brown
6	7133; 7134	Malmesbury	Flat brown; Speckled white/ yellow/ brown
7	7076; 7090; 7106; 7165; 7173	Constantia; Franschhoek; Somerset West; Stellenbosch	Fluffy orange-brown
8	7138; 7139	Botrivier	Fluffy orange-brown
Fomitiporia	7040; 7041; 7049; 7050; 7052; 7053; 7056; 7057; 7069; 7072; 7077; 7081; 7082; 7086; 7093; 7094; 7095; 7096; 7097; 7108; 7115; 7119; 7121; 7122; 7124; 7135; 7137; 7140; 7163; 7164; 7166; 7167; 7168; 7169; 7170; 7171	Botrivier; Constantia; Darling; Durbanville; Fomitiporia; Franschhoek; Grabouw; Hermanus; Klaas Voogds; Paarl; Riebeeck Wes; Somerset West; Stellenbosch; Wellington	Flat brown; Flat various yellow/ brown white tones; Flat white/ pale yellow (cream); Speckled white/ yellow/ brown; Tufty orange/ brown; Woolly light brown
Phellinus	7055; 7098; 7099; 7100; 7101; 7102; 7103; 7104; 7105; 7179; 7180	Kanon Eiland; Keboes; Keimoes; Marchand; Marken; Prieska	Flat brown; Speckled white/ yellow/ brown; Tufty orange/ brown

Table 4. Colony diameters of the basidiomycete isolates grown on PDA at 25°C after 14 days.

STE-U No.	Taxa	Average diameter (mm)
7038	1	75 ± 2
7058		52 ± 7
7084		60 ± 15
7141		63 ± 17
7148		70 ± 15
7147	2	85 ± 0
7154		83 ± 3
7155		85 ± 0
7109	3	80 ± 1
7136		72 ± 10
7174		63 ± 1
7042	4	41 ± 2
7043		29 ± 22
7126	5	43 ± 6
7143		51 ± 4
7153		70 ± 6
7133	6	85 ± 0
7134		78 ± 2
7090	7	45 ± 2
7106		51 ± 4
7165		57 ± 3
7138	8	85 ± 0
7139		71 ± 7
7069	*Fomitiporia* sp.	46 ± 14
7096		65 ± 23
7122		85 ± 0
7135		77 ± 2
7168		83 ± 2
7055	*Phellinus* sp.	79 ± 2
7098		85 ± 0
7105		85 ± 0

colony diameter ranging from 29 to 41 mm after 14 days. Taxon 7 was also slow growing (45 to 57 mm in diameter), but overlapped with the growth ranges of Taxon 1, Taxon 5 and *Fomitiporia* sp.

Phaeomoniella chlamydospora isolates were identified on the basis of their distinct olive green to white yeast-like growth on PDA, pigmented conidiophores and small oblong-ellipsoidal conidia (Crous and Gams, 2000). The cultures of *Pa. chlamydospora* were not characterized further, since previous studies have shown that only one species, which has very little variation, occurs on grapevine (Pottinger *et al*., 2002; Mostert *et al*., 2006a).

Isolates of *Eutypa* were identified by the typical white to cream, cottony colonies on PDA, lacking fruit bodies.

Phylogenetic analyses

The combined TUB and ACT alignment of the *Phaeoacremonium* isolates included 1042 nucleotides of which 508 nucleotides were parsimony-informative. A maximum parsimony analysis was conducted and isolates were grouped in well-supported clades with known *Phaeoacremonium* species (Figure 1). Five of the isolates grouped with *Pm. aleophilum* sequences, three isolates with *Pm. sicilianum* sequences, two isolates with *Pm. iranianum* sequences, two isolates with *Pm. parasiticum* sequences and one isolate with *Pm. mortoniae* sequences, all with a bootstrap support of 100%. Four isolates grouped in the *Pm. alvesii* clade, with a bootstrap support of 75%.

The parsimony analysis for the *Phomopsis* isolates included 494 nucleotides of which 111 were parsimony-informative. Fourteen of these isolates were identified as *Ph. viticola*, as they grouped with the reference sequences with a bootstrap support of 100% (Figure 2). Two isolates grouped with *Ph. theicola*, with a bootstrap support of 71%. One isolate grouped with *Diaporthe ambigua* Nitschke, with a bootstrap support of 100%.

The EF and ITS alignment of the Botryosphaeriaceae included 559 nucleotides of which 391 were parsimony-informative. *Diplodia seriata* was the most predominant species found and seven isolates grouped with the reference sequences with a bootstrap support of 63% (Figure 3). Five isolates grouped with reference sequences of *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, with a bootstrap support of 99%. Six isolates grouped with isolates of *N. parvum*, with a bootstrap support of 100%.

The ITS alignment of the 134 basidiomycete isolates included 931 nucleotides of which 548 were parsimony-informative. The sequences grouped into eight well-supported monophyletic clades (Taxa 1 to 8), of which the genus identity is uncertain (Figure 4). Two clades clustered with the genera *Phellinus* and *Fomitiporia*. Taxa 1, 2, 3, 4, 5, 7, 8, *Phellinus* sp. and *Fomitiporia* sp. each had a bootstrap support of 100%. Taxon 6 had a bootstrap support of 82%. Taxa 1 to 4 grouped with cf.

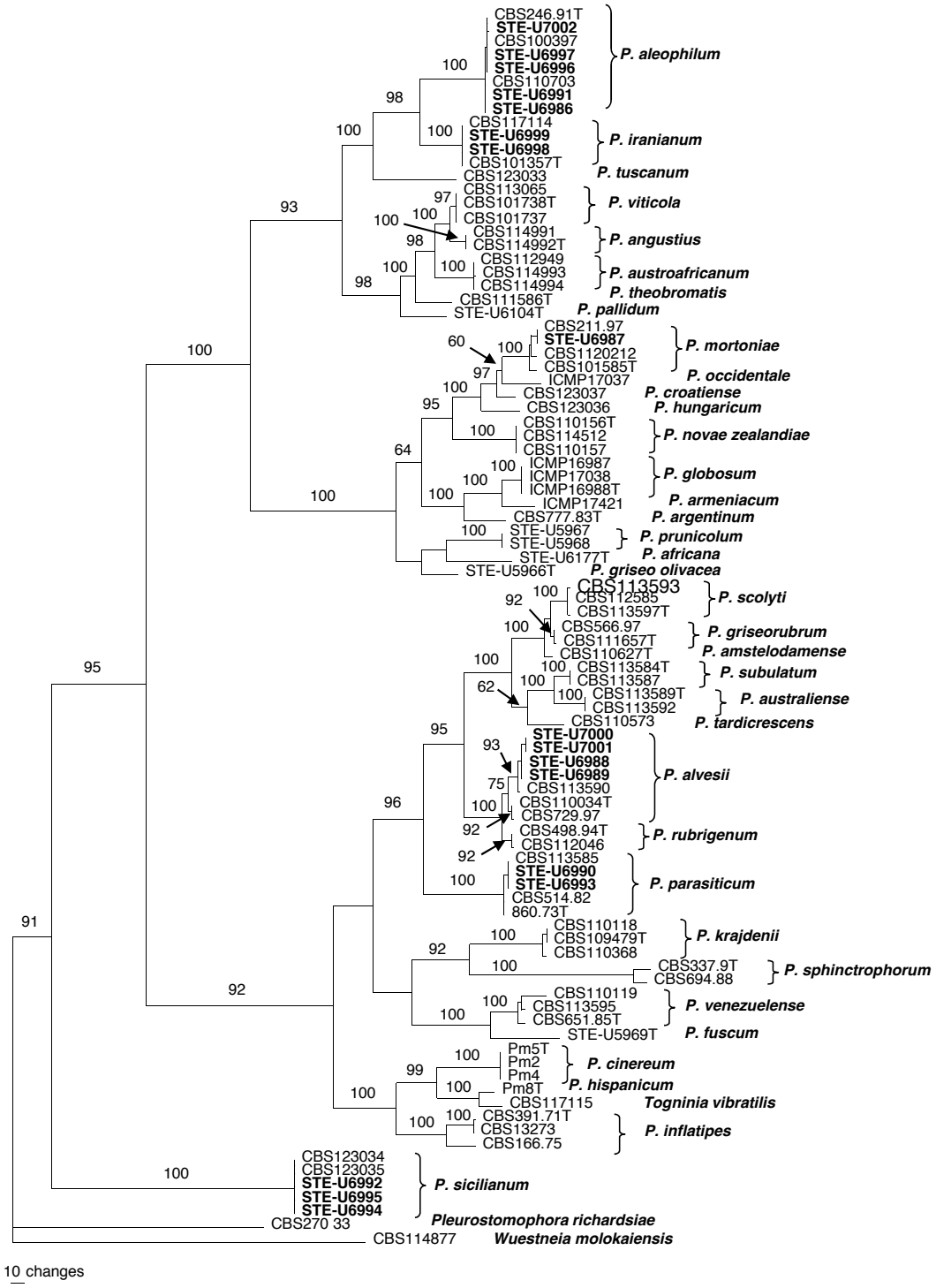

Figure 1. One of 100 most parsimonious trees obtained from heuristic searches of the combined β-tubulin and actin sequences (length: 2621steps; CI: 0.473; RI: 0.858; RC: 0.406) of *Phaeoacremonium* isolates. Bootstrap support values above 60% are shown above the nodes. The outgroups were *Pleurostomophora richardsiae* and *Wuestneia molokaiensis*. Isolates in bold print are from this study.

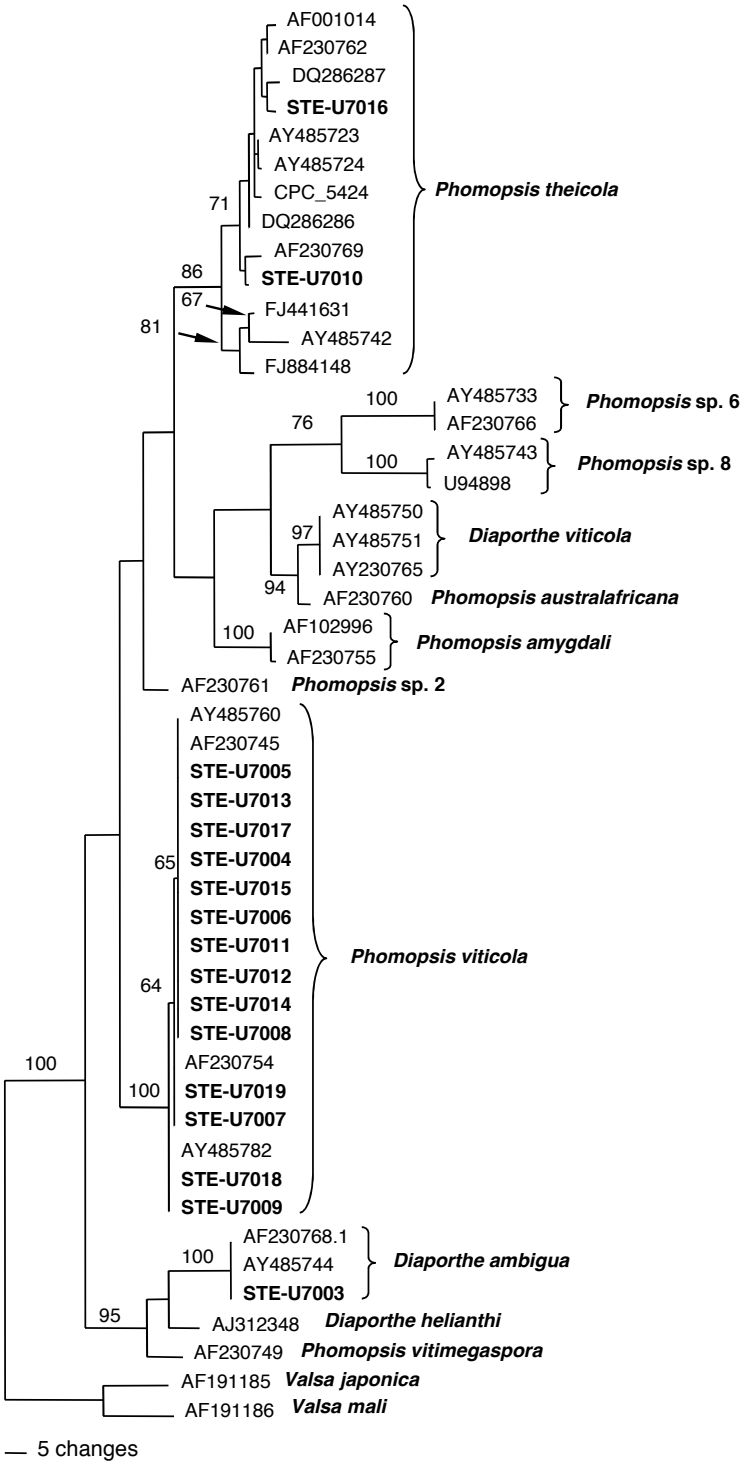

Figure 2. One of 22 most parsimonious trees obtained from heuristic searches of the ITS sequences (length: 325 steps; CI: 0.578; RI: 0.847; RC: 0.490) of *Phomopsis* isolates. Bootstrap support values above 60% are shown above the nodes. The outgroups used were *Valsa japonica* and *Valsa mali*. Isolates in bold print are from this study.

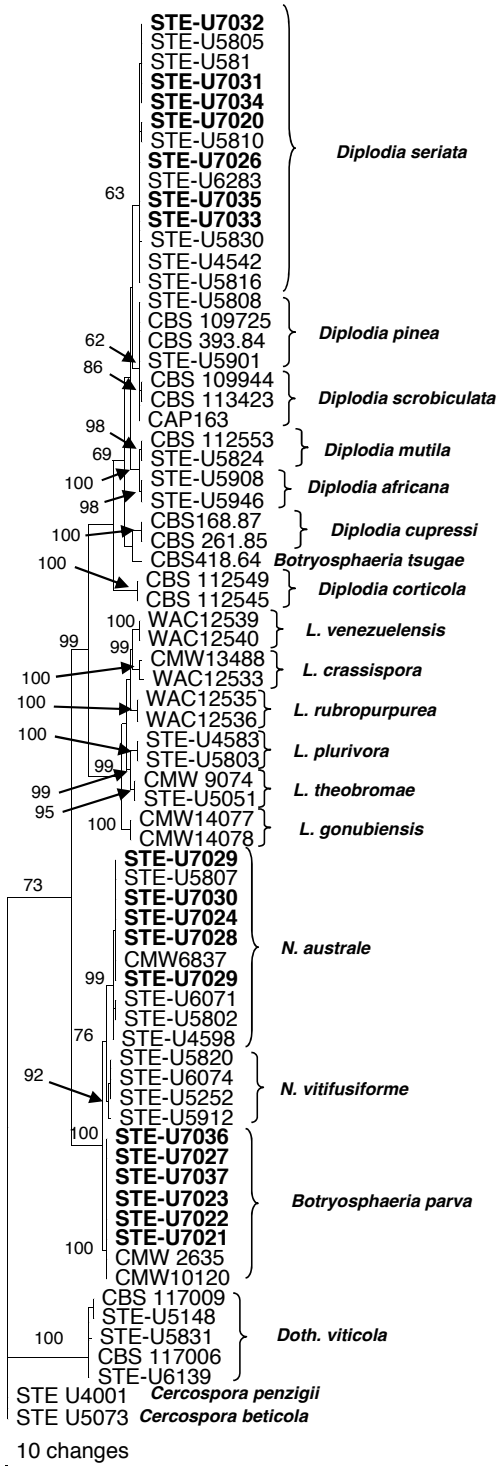

Figure 3. One of 70 most parsimonious trees obtained from heuristic searches of the combined EF and ITS sequences (length: 759 steps; CI: 0.735; RI: 0.950; RC: 0.698) of Botryosphaeriaceae isolates. Bootstrap support values above 60% are shown above the nodes. The outgroups used were *Cercospora penzigii* and *Cercospora beticola*. Isolates in bold print are isolates from this study.

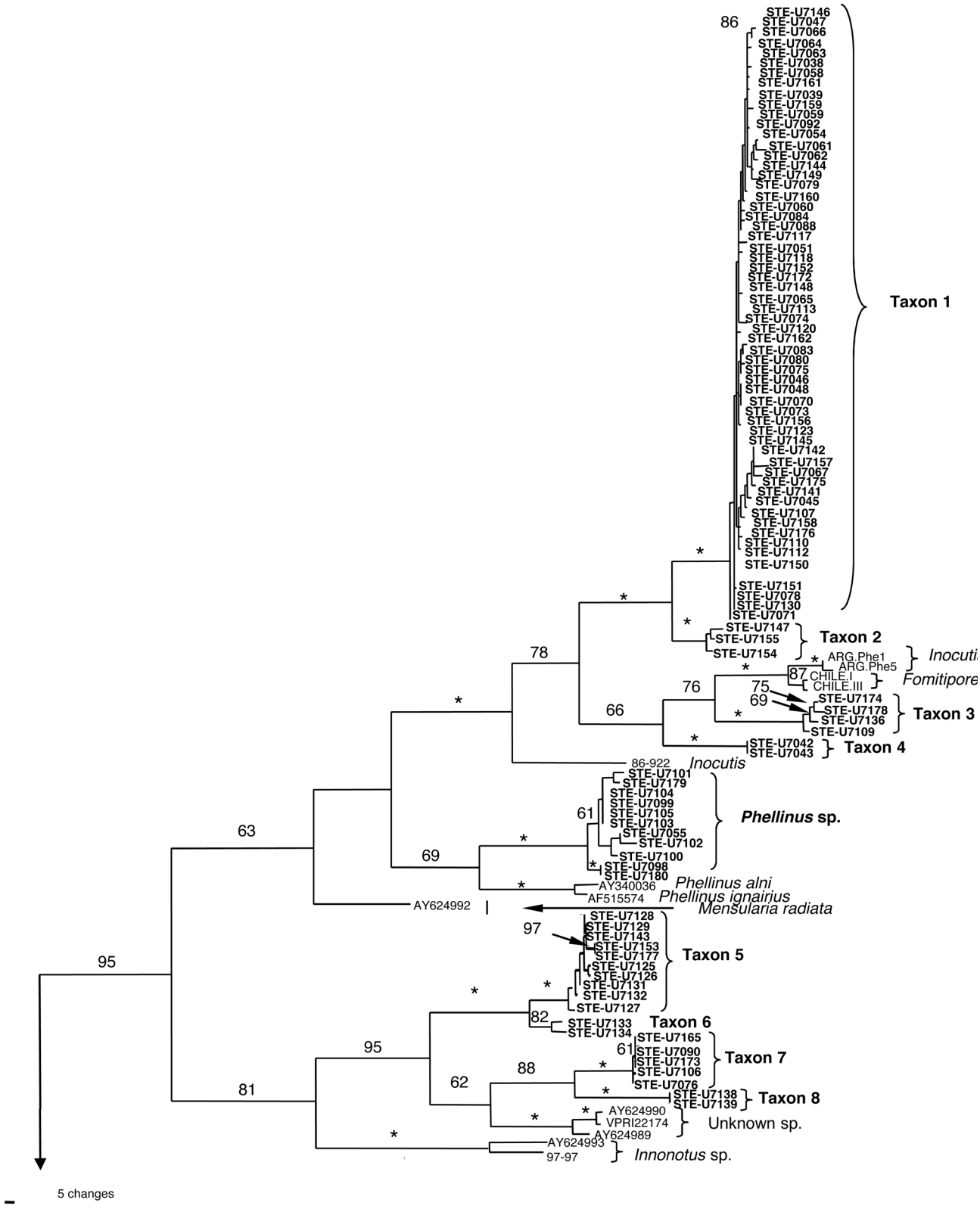

Figure 4. *continues*

Figure 4. *continued*

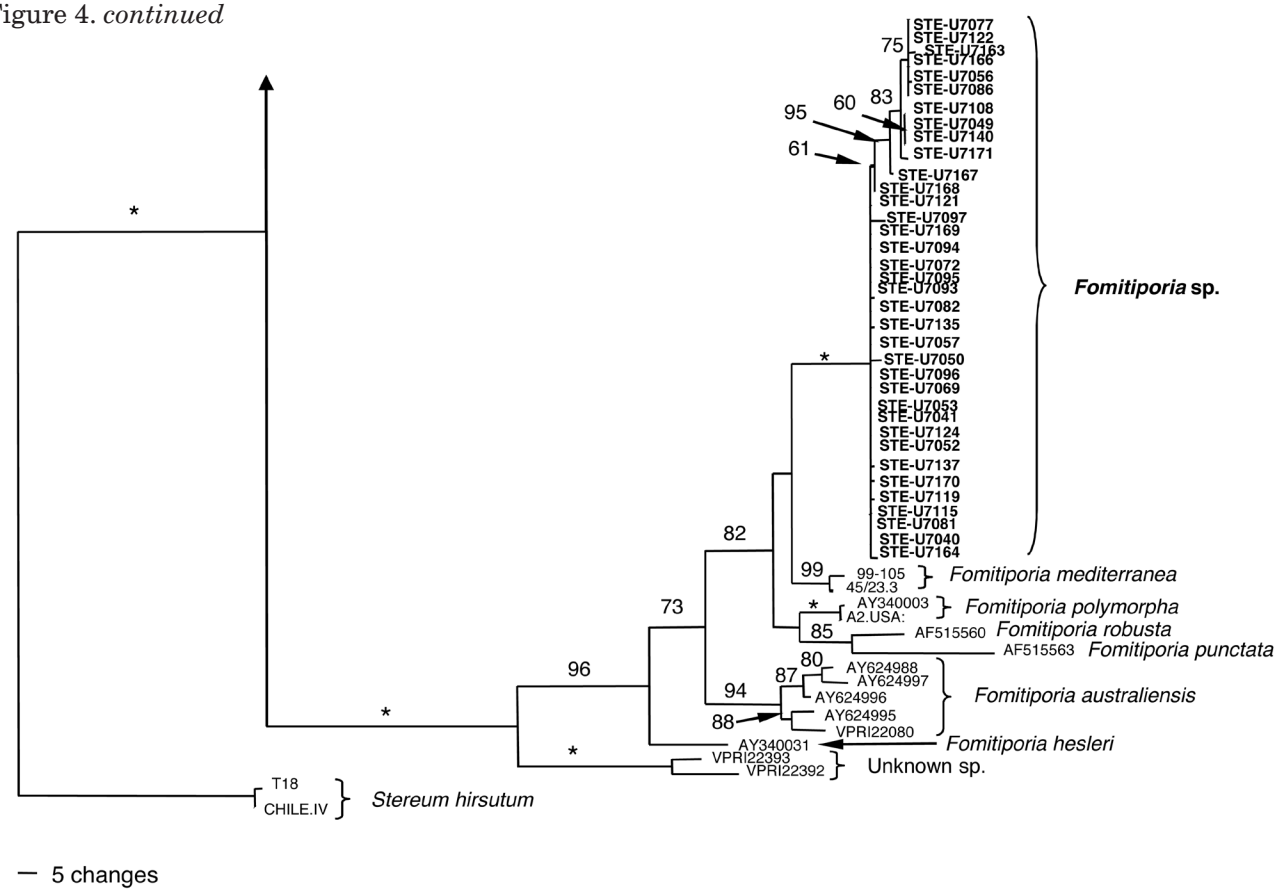

Figure 4. One of ten most parsimonious trees obtained from heuristic searches of the ITS sequences (length: 2050 steps; CI: 0.560; RI: 0.938; RC: 0.526) of basidiomycete isolates. Bootstrap support values are shown above the nodes and bootstrap values of 100% are indicated by an asterisk (*). The outgroups used were *Stereum hirsutum* isolates T18 and Chile IV. Isolates in bold print are from this study.

Fomitiporella cf. and cf. *Inocutis* cf., and Taxa 5 to 8 grouped with *Inonotus*. Taxon 1 represented 42% of the basidiomycete isolates, followed by *Fomitiporia,* which comprised 25% of the isolates. *Phellinus* was the next largest taxon (8% of isolates) followed by taxon 5 (7%), taxon 7 (4%), taxon 3 (3%) and taxon 2 (2%). Taxa 4, 6 and 8 were the least frequently isolated, and each comprised 1% of the isolates. The majority of basidiomycete taxa included isolates from different regions and were not restricted to specific locations. Four taxa were restricted to a specific locality. Taxon 4 came only from Stellenbosch, taxon 6 from Malmesbury and taxon 8 from Botrivier, but only two isolates of each were found. *Phellinus* sp. isolates were all obtained from Keimoes, Kanon Eiland, Prieska, Marchand and Upington (Northern Cape) and Marken in Limpopo. In a few cases, up to three basidiomycete taxa were found within one plant.

The identity of the nine *Eutypa* isolates was determined as *E. lata,* using ITS phylogenic analysis (Safodien, 2007).

Discussion

Different fungi are associated with esca diseased vines in South Africa. These fungi include species of basidiomycetes and Botryosphaeriaceae*, E. lata, Phaeoacremonium* spp.*, Phaeomoniella chlamydospora* and *Phomopsis* spp.

Six species of *Phaeoacremonium* were isolated in this study, and include *Pm. aleophilum, Pm.*

alvesii, Pm. iranianum, Pm. mortoniae, *Pm. parasiticum* and *Pm. sicilianum*. There are 25 species of *Phaeoacremonium* world-wide that have been isolated from either Petri diseased or esca grapevines (Crous *et al*., 1996; Mostert *et al*., 2006b; Essakhi *et al*., 2008; Graham *et al*., 2009; Gramaje *et al*., 2009). *Phaeoacremonium aleophilum* is the most common species on grapevines (Crous *et al*., 1996; Mostert *et al*., 2006b), followed by *Pm. parasiticum* (Mostert *et al*., 2006b), which has also been confirmed in this study. In South Africa, *Pm. aleophilum*, *Pm. austroafricanum*, *Pm. krajdenii*, *Pm. parasiticum*, *Pm. scolyti*, *Pm. subulatum*, *Pm. viticola* and *Pm. venezuelense* have previously been isolated from grapevines (Mostert *et al*., 2006b). This is the first report of *Pm. mortoniae*, *Pm. iranianum* and *Pm. sicilianum* in South Africa.

In the current study, *Ph. viticola*, *Ph. theicola*, and *Diaporthe ambigua* were found to be associated with esca. Van Niekerk *et al*. Comma (2005) showed that 15 *Phomopsis* species occur on grapevines in South Africa. Of these, *Ph. viticola* is commonly found on grapevines and is associated with Phomopsis cane and leaf blight (Mostert *et al*., 2001; van Niekerk *et al*., 2005). *Phomopsis theicola*, previously known as *Phomopsis* sp. 1 (Santos and Phillips, 2009), has a wider host range, including *Protea* sp., *Pyrus* sp. and *Prunus* sp. (Mostert *et al*., 2001; van Niekerk *et al*., 2005). *Diaporthe ambigua* rarely occurs on grapevine and is more commonly associated with cankers on *Malus* sp., *Prunus* sp. and *Pyrus* sp. (Smit *et al*., 1996; Crous *et al*., 2000; van Niekerk *et al*., 2005).

Three species of the Botryosphaeriaceae, namely *D. seriata*, *N. parvum* and *N. australe*, were found associated with esca symptoms. However, twelve species of the Botryosphaeriaceae have previously been isolated from grapevines in South Africa (van Niekerk *et al*., 2004, 2006, 2010). Of these, *D. seriata, Neofusicoccum parvum* (Crous *et al*., 2006) and *Lasiodiplodia theobromae* were the most common (van Niekerk *et al*., 2003, 2004). *Neofusiccocum australe* (Crous *et al*., 2006) was also commonly found and was the most pathogenic species on South African grapevines (van Niekerk *et al*., 2004). *Lasiodiplodia crassispora* was recently identified from grapevines and found to be highly pathogenic (van Niekerk *et al*., 2010). Several species of the Botryosphaeriaceae, including *Diplodia seriata*, *N. parvum* and *N. australe*, are associated with grapevines in other countries, such as Australia, USA (California), Portugal and Spain (Phillips, 2002; Taylor *et al*., 2005; Úrbez-Torrez *et al*., 2006; Sánchez-Torres *et al*., 2008).

The phylogeny of the EF and ITS region gave low bootstrap support for *D. seriata* (63%). Other analyses have also shown low bootstrap support for *Diplodia* species. In a study using only the ITS region no support was found for the *D. pinea* clade, which grouped basal to *D. seriata* and *D. scrobiculata* (Phillips *et al*., 2007). The combined EF and ITS analysis of Damm *et al*. (2007) also gave low bootstrap support for the *D. seriata* (67%) and *D. pinea* clades (62%). *Diplodia pinea*, *D. scrobiculata* and *D. seriata* are phylogenetically closely related, and also share morphological features including aseptate conidia that become pigmented within pycnidia (Phillips *et al*., 2007).

Phaeomoniella chlamydospora is frequently associated with esca-diseased vines or declining grapevines worldwide (Mostert *et al*., 2006a). In the current study, *Pa. chlamydospora* was commonly associated with esca diseased vines. Even though *Pa. chlamydospora* has recently been isolated from *Convolvulus arvensis*, a weed that can be found in vineyards (Agustí-Brisach *et al*., 2011), it has not yet been isolated from other woody hosts. Six additional species of *Phaeomoniella* have been found on other hosts. *Phaeomoniella zymoides* and *Pa. pinifoliorum* have been found on pine needles (Lee *et al*., 2006), and *Phaeomoniella dura*, *Pa. effusa*, *Pa. prunicola*, *Pa. tardicola* and *Pa. zymoides* have been found on *Prunus* spp. trees in South Africa (Damm *et al*., 2010).

Genera of the Diatrypaceae that occur on grapevines include *Cryptosphaeria*, *Cryptovalsa*, *Diatrype*, *Diatrypella*, *Eutypa* and *Eutypella* (Trouillas *et al*., 2010). In South Africa *Cryptovalsa ampelina*, *E. lata*, *Eutypa leptoplaca* and *Eutypella vitis* have been found on grapevines (Mostert *et al*., 2004; Safodien, 2007). In the present study, only *E. lata* was found to be associated with esca symptoms (Safodien, 2007). *Eutypa lata* has also been found on esca affected vines in Italy, Germany, Spain and France (Mugnai *et al*., 1999; Fischer and Kassemeyer, 2003; Martin and Cobos, 2007; Péros *et al*., 2008).

Ten different basidiomycete taxa, not corresponding with known species, were found in the current study. Two taxa could be linked to the genera of *Fomitiporia* and *Phellinus*. The other taxa

could possibly be species of *Inonotus* or *Inocutis*. Two of the taxa, *Fomitiporia* sp. and taxon 1, contained the majority of the basidiomycete isolates we obtained. Phylogenetic species recognition using the ITS region was used to identify the different taxa (Fischer and Binder, 2004; Sánchez-Torres *et al*., 2008). For formal description of these phylogenetic taxa, the basidiocarps need to be linked to the sequence identity. Only a few basidiocarps were found in the current study (not reported) and will be used in further work to establish the identity of these ten taxa.

A diversity of basidiomycete fungi causing white rot have been found from grapevines including *Armillaria mellea*, *Flammulina velutipes*, *Pleurotus pulmonarius*, *Inonotus hispidus*, *Stereum hirsutum*, *Trametes hirsuta*, *Trametes versicolor* (Fischer and Kassemeyer, 2003). *Peniophora incarnata* and *Hirneola aruculae-judae* have also been found on grapevines, but their association with white rot is uncertain (Fischer and Kassemeyer, 2003). However, the diversity of basidiomycete taxa found from esca diseased vines is generally less, and taxa are often restricted to a specific area. *Fomitiporia mediterranea* is the most common species in Europe (Fischer, 2006). *Fomitiporia australiensis* together with two unknown taxa is restricted to Australia and *F. polymorpha* to North America (Fischer, 2005, 2006). *Inocutis jamaicensis* (Murrill) Gottlieb, J.E. Wright & Moncalvo and *Fomitiporella vitis* Auger, Aguilera & Esterio (no formal description of this species has been published), associated with 'hoja de malvon' and chlorotic leaf roll, respectively, occur on grapevines in South America (Fischer, 2006; Lupo *et al*., 2006). *Stereum hirsutum* (Willd.: Fr.) Pers. has sometimes been isolated from esca diseased vines in Europe (Larignon and Dubos, 1997; Martin and Cobos, 2007; Sánchez-Torres *et al*., 2008), although its role within the esca complex is uncertain. The perception that basidiomycetes are not a threat to grapevines has limited the research on basidiomycetes associated with esca (Fischer, 2006).

In the present study, ten basidiomycete taxa were found, possibly due to the wide area of investigation which consisted of different climatic regions. Most of the taxa were found in the Western Cape province. Stellenbosch had the highest diversity, with four taxa present (taxa 1, 4, 7 and *Fomitiporia* sp.). However, this could be due to the bias in number of samples analyzed from this location. Some of the taxa were restricted to a specific area. In the Northern Cape or Limpopo provinces, which are known for their warm climate, only *Phellinus* sp. (11 isolates) was found. These areas are also geographically isolated from the other grapevine production areas in the Western Cape. Taxon 2 (three isolates) was only found in Oudtshoorn and Calitzdorp, which is about 400 km from the Cape Peninsula.

In South Africa, esca of grapevines is associated with different fungi, including ten basidiomycete taxa, *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. Additionally *E. lata*, three species of *Phomopsis* spp. and three species of the Botryosphaeriaceae were also found. Pathogenicity studies on field grapevines are underway to assess the relevance of the different basidiomycete taxa as grapevine pathogens. Knowledge regarding the fungi associated with esca diseased vines will aid in further research to understand the co-occurrence and the role of the different trunk disease fungi in grapevines.

Acknowledgements

The authors would like to thank the Agricultural Research Council, Department of Plant Pathology, University of Stellenbosch, Winetech (Project WW06/37), National Research Foundation (NRF) and the Technology and Human Resources for Industry Programme (THRIP) for financial support. Technical assistance by Zane Sedeman, Carine Vermeulen, Linda Nel, Julia Marais and Adoration Shubane is greatly appreciated.

Literature cited

Agustí-Brisach C., D. Gramaje, M. León, J. García-Jiménez and J. Armengol, 2011. Evaluation of vineyard weeds as potential hosts of black foot and Petri disease pathogens. *Plant Disease* 95, 803–810.

Armengol J., A. Vicent, L. Torné, F. García-Figueres and J. García-Jiménez, 2001. Fungi associated with esca and grapevine decline in Spain: a three-year survey. *Phytopathologia Mediterranea* 40, 325–329.

Calzarano F. and S. Di Marco, 2007. Wood discoloration and decay in grapevines with esca proper and their relationship with foliar symptoms. *Phytopathologia Mediterranea* 46, 96–101.

Carbone I. and L.M. Kohn, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91, 553–556.

Chiarappa L., 1959. Wood decay of the grapevine and its relationship with black measles disease. *Phytopathology* 49, 510–519.

Clark T.A. and J.B. Anderson, 2004. Dikaryons of the basidiomycete fungus *Schizophyllum commune* and evolution in long-term culture. *Genetics* 167, 1663–1675.

Crous P.W. and W. Gams, 2000. *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea* 39, 112–118.

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

Crous P.W., A.J.L. Phillips and A.P. Baxter, 2000. *Phytopathogenic Fungi from South Africa*. University of Stellenbosch Printers, Stellenbosch, South Africa, 358 pp.

Crous P.W., B. Slippers, M.J. Wingfield, J. Rheeder, W.F.O. Marasas, A.J.L. Phillips, A. Alves, T. Burgess, P. Barber and J.Z. Groenewald, 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* 55, 235–253.

Damm U., P.W. Crous and P. Fourie, 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* 99, 664–680.

Damm U., L. Mostert, P.W. Crous and P.H. Fourie, 2008. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia* 20, 97–102.

Damm U., P.H. Fourie and P.W. Crous, 2010. *Coniochaeta* (*Lecythophora*), *Collophora* gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. *Persoonia* 24, 60–80.

Essakhi S., L. Mugnai, P.W. Crous, J.Z. Groenewald and G. Surico, 2008. Molecular and phenotypic characterization of novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine. *Persoonia* 21, 119–134.

Fischer M., 2002. A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress* 1, 315–324.

Fischer M., 2006. Biodiversity and geographic distribution of basidiomycetes causing esca-associated white rot in grapevine: a worldwide perspective. *Phytopathologia Mediterranea* 45, S30–S42.

Fischer M. and M. Binder, 2004. Species recognition, geographic distribution and host-pathogen relationships: a case study in a group of lignicolous basidiomycetes, *Phellinus* s.l. *Mycologia* 96, 799–811.

Fischer M. and H-H. Kassemeyer, 2003. Fungi associated with esca disease of grapevine in Germany. *Vitis* 42, 109–116.

Fischer M., J. Edwards, J.H. Cunnington and I.G. Pascoe, 2005. Basidiomycetous pathogens on grapevine: a new species from Australia – *Fomitiporia australiensis*. *Mycotaxon* 92, 85–96.

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

Glawe D. A., 1982. Observations on the anamorphs of six species of *Eutypa* and *Eutypella*. *Mycotaxon* 14, 334–346.

Graham A.B., P.R. Johnston and B.S. Weir, 2009. Three new *Phaeoacremonium* species on grapevine in New Zealand. *Australasian Plant Pathology* 38, 505–513.

Gramaje D., J. Armengol, H. Mohammadi, Z. Banihashemi and L. Mostert, 2009. Novel *Phaeoacremonium* species associated with Petri disease and esca of grapevine in Iran and Spain. *Mycologia* 101, 920–929.

Groenewald M., J. Kang, P.W. Crous and W. Gams, 2001. ITS and β-tubulin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. *Mycological Research* 105, 651–657.

Katoh K., K. Misawa, K. Kuma and T. Miyata, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30, 3059–3066.

Larignon P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology* 103, 147–157.

Lee H.B., J.Y. Park, H.S. Jung and R.C. Summerbell, 2006. Two new *Phaeomoniella* species: *Phaeomoniella zymoides* and *Phaeomoniella pinifoliorum* spp. nov., new acid-tolerant epiphytic fungi isolated from pine needles in Korea. *Mycologia* 98, 598–611.

Lupo S., L. Bettucci, A. Pérez, S. Martínez, C. Césari, G. Escoriaza and M. Gatica, 2006. Characterization and identification of the basidiomycetous fungus associated with 'hoja de malvón' grapevine disease in Argentina. *Phytopathologia Mediterranea* 45, S110–S116.

Martin M.T. and R. Cobos, 2007. Identification of fungi associated with grapevine decline in Castilla y León (Spain). *Phytopathologia Mediterranea* 46, 18–25.

Marais P.G., 1981. Grapevine diseases and abnormalities. In: "*Wingerdbou in Suid Afrika*". (Burger J., Deist J., ed.), VORI, 7660 Stellenbosch, South Africa, 404–405.

Mostert L., E.C.A. Abeln, F. Halleen and P.W. Crous, 2006a. Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. *Australasian Journal of Plant Pathology* 35, 453–460.

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

Mostert L., F. Halleen, P.W. Crous and M.L. Creaser, 2004. *Cryptovalsa ampelina*, a forgotten shoot and cane pathogen of grapevines. *Australasian Journal of Plant Pathology* 33, 295–299.

Mostert L., J.Z. Groenewald, W. Gams, R.C. Summerbell and P.W. Crous, 2006b. Taxonomy and pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* anamorphs. *Studies in Mycology* 54, 1–115.

Mugnai L., A. Graniti and G. Surico, 1999. Esca (Black

measles) and brown wood-streaking: Two old and elusive diseases of grapevines. *Plant Disease* 83, 404–418.
Munkvold G.P., J.A. Duthie and J.J. Marois, 1994. Reductions in yield and vegetative growth of grapevines due to Eutypa dieback. *Phytopathology* 84, 186–192.
O'Donnell K. and E. Cigelnik, 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7, 103–116.
Pascoe I. and E. Cottral, 2000. Developments in grapevine trunk disease research in Australia. *Phytopathologia Mediterranea* 39, 68–75.
Péros J.-P., G. Berger and I. Jamaux-Despréaux, 2008. Symptoms, wood lesions and fungi associated with esca in organic vineyards in Languedoc-Roussillon (France). *Journal of Phytopathology* 156, 297–303.
Phillips A.J.L., 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. *Phytopathologia Mediterranea* 41, 3–18.
Phillips A.J.L., P.W. Crous, 2007. *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. *Fungal Diversity* 25, 141–155
Phillips A.J.L., A. Alves, S.R. Pennycook, P.R. Johnston, A. Ramaley, A. Akulov and P.W. Crous, 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21, 29–55.
Rambaut A., 2002. *Sequence Alignment Editor*. Version 2.0. Department of Zoology, University of Oxford, Oxford, UK.
Reisenzein H., N. Berger and G. Nieder, 2000. Esca in Austria. *Phytopathologia Mediterranea* 39, 26–34.
Rumbos I. and A. Rumbou, 2001. Fungi associated with esca and young grapevine decline in Greece. *Phytopathologia Mediterranea* 40, S330–S335.
Safodien S., 2007. The molecular identification and characterization of Eutypa dieback and a PCR-based assay for the detection of *Eutypa* and Botryosphaeriaceae species from grapevine in South Africa. M.Sc. Thesis, University of Stellenbosch, Stellenbosch, South Africa.
Sánchez-Torres P., R. Hinarejos, V. González and J.J. Tuset, 2008. Identification and characterization of fungi associated with esca in vineyards of the Comunidada Valencia (Spain). *Spanish Journal of Agricultural Research* 6, 650–660.
Santos J.M. and A.J.L. Phillips, 2009. Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. *Fungal Diversity* 34, 111–125.
Smit W.A., C.D. Viljoen, B.D. Wingfield, M.J. Wingfield and F.J. Calitz, 1996. A new canker disease of apple, pear, and plum rootstocks caused by *Diaporthe ambigua* in South Africa. *Plant Disease* 80, 1331–1335.
Sofia J., T. Gonçalves and H. Oliveira, 2006. Spatial distribution of esca symptomatic plants in Dão vineyards (Centre Portugal) and isolation of associated fungi. *Phytopathologia Mediterranea* 45, S87–S92.
Swofford D.L., 2003. Phylogenetic analysis using parsimony. Version 4.0b10. Sinauer Associates: Sunderland, MA, USA.
Taylor A., G.E.St.J. Hardy, P. Wood and T. Burgess, 2005. Identification and pathogenicity of *Botryosphaeria* species associated with grapevine decline in Western Australia. *Australasian Plant Pathology* 34, 187–195.
Trouillas F.P., J.R. Úrbez-Torrez and W.D. Gubler, 2010. Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. *Mycologia* 102, 319–336.
Úrbez-Torrez J.R., G.M. Leavitt, T.M. Voegel and W.D. Gubler, 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. *Plant Disease* 90, 1490–1503.
van Niekerk J.M., P.W. Crous, J.Z. Groenewald, P.H. Fourie and F. Halleen, 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96, 781–798.
van Niekerk J.M., P.H. Fourie, F. Halleen and P.W. Crous, 2006. *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 45, S43–S54.
van Niekerk J.M., J.Z. Groenewald, D.F. Farr, P.H. Fourie, F. Halleen and P.W. Crous, 2005. Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology* 34, 27–39.
van Niekerk J.M., W. Bester, F. Halleen, P.W. Crous and P.H. Fourie, 2010. First record of *Lasiodiplodia crassispora* as a pathogen of grapevine trunks in South Africa. *Plant Disease* 94, 1063.
White T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols, a Guide to Methods and Applications* (Innis M.A., Gelfand D.H., Sninsky J.J., ed.), Academic Press Inc., New York, NY, USA, 315–322.
White C., F. Halleen and L. Mostert, 2011. Symptoms and fungi associated with esca in South African vineyards. *Phytopathologia Mediterranea* 50 (Supplement), 236–246.

Accepted for publication: November 15, 2011