CSIRO PUBLISHING

www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2005, **34**, 27–39

Reassessment of *Phomopsis* species on grapevines

J. M. van Niekerk[A], *J. Z. Groenewald*[A,B], *D. F. Farr*[C], *P. H. Fourie*[A], *F. Halleen*[D] *and P. W. Crous*[A,B,E]

[A]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.
[B]Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.
[C]USDA-ARS, Systematic Botany & Mycology Laboratory, Rm 304, Bldg 011A, Beltsville, MD 20705, USA.
[D]Disease Management, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.
[E]Corresponding author. Email: crous@cbs.knaw.nl

Abstract. Ten species of *Phomopsis* have previously been identified from grapevines. Of these, *P. viticola*, the causal agent of Phomopsis cane and leaf spot, and *P. vitimegaspora*, the causal agent of swelling arm of grapevines, have been confirmed as severe pathogens of this host. Earlier taxonomic treatments of *Phomopsis* species chiefly distinguished taxa based on host specificity, cultural characteristics and morphology. More recent studies have indicated, however, that these characteristics can no longer be used to distinguish species of *Phomopsis* due to the wide host ranges of some species, and the morphological plasticity of others. Using morphology, DNA sequences (ITS-1, 5.8S, ITS-2) and pathogenicity data, 15 *Phomopsis* spp. were distinguished from grapevines in the present study. *Diaporthe helianthi*, a known pathogen of sunflowers, is for the first time reported from grapevines. A further six, presently unknown species of *Phomopsis*, are also distinguished from grapevines. A phylogenetic analysis of ITS data generated in this study distinguished three clades containing isolates previously identified as *D. perjuncta*. Based on type studies, the name *D. viticola* can be applied to collections from Portugal and Germany. A new species, *D. australafricana,* is proposed for South African and Australian isolates formerly treated as *D. perjuncta* or *D. viticola.* A description for *D. perjuncta* is provided based on newly designated lectotype and epitype specimens. *D. perjuncta* is distinguished from *D. viticola* and *D. australafricana* based on morphology and DNA phylogeny. Artificial inoculations of green grapevine shoots indicated that, of the species tested, *P. amygdali*, a known pathogen of peaches in the USA, and *P. viticola* were the most virulent.

Additional keywords: *Phomopsis*, grapevines, *Diaporthe perjuncta*, taxonomy.

Introduction

The genus *Phomopsis* (Sacc.) Bubák contains more than 800 species that are recorded as being either plant pathogenic or saprobic (Uecker 1988). Numerous species are routinely isolated from stems, roots, leaves and fruit of a wide variety of hosts (Rehner and Uecker 1994). Species concepts in *Phomopsis* and its teleomorph *Diaporthe* Nitschke were initially based on morphological and cultural characteristics. However, due to the plasticity of these characteristics (Rehner and Uecker 1994), *Phomopsis* species concepts have in recent years mainly been based on host affiliation. Recent studies have shown, however, that various species of *Phomopsis* are able to infect a wide variety of hosts (Rehner and Uecker 1994; Uddin *et al*. 1997, 1998; Mostert *et al*. 2001), and that host association is no longer sufficient for identification purposes. The use of anamorph/teleomorph relationships in identifying *Phomopis* species is also untenable, since *Diaporthe* teleomorphs have been described for only approximately 20% of the *Phomopsis* species known to date (Uecker 1988).

Phomopsis cane and leaf spot is an important disease of grapevines, causing serious losses due to shoots breaking off at the base, stunting, dieback, loss of vigour, reduced bunch set and fruit rot (Pine 1958, 1959; Pscheidt and Pearson 1989; Pearson and Goheen 1994). Ten species of *Phomopsis* are currently known to occur on grapevines (Mostert *et al*. 2001). Among these, only four have been confirmed as being pathogenic to this host; *P. viticola* (Sacc.) Sacc., *P. vitimegaspora* Kuo & Leu (teleomorph *Diaporthe kyushuensis* Kajitani & Kanem.), *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla and a species previously referred to as *D. perjuncta* Niessl (Kuo and Leu 1998; Kajitani and Kanematsu 2000; Mostert *et al*. 2001; Rawnsley *et al*. 2001).

In an earlier study aimed at characterising the *Phomopsis* species occurring on grapevines, Merrin *et al*. (1995) employed morphological and cultural characteristics to distinguish four taxa within the *P. viticola* complex. Phillips (1999) concluded that the name *D. perjuncta* could be applied to isolates identified as taxon 1, whereas those treated as taxon 2 by Merrin *et al*. (1995) were representative of

© Australasian Plant Pathology Society 2005 10.1071/AP04072 0815-3191/05/010027

P. viticola. Confusion pertaining to the taxonomy of taxon 1 still remains, however, as Scheper *et al*. (2000) described taxon 1 isolates from Australia as *Diaporthe viticola* Nitschke. Although this species was initially regarded as the causal organism of grapevine bud mortality, it was later shown only to cause a bleaching of grapevine canes (Scheper *et al*. 2000; Rawnsley *et al*. 2001; Rawnsley and Wicks 2002).

During the past 2 years, a number of *Phomopsis* isolates have been obtained from grapevines in the Western Cape province of South Africa. Isolations were made from severe *Phomopsis*-like symptoms occurring on shoots, internally in the wood, on pruning wounds and asymptomatic nursery plants. Subsequent isolates obtained from this material varied greatly in morphology and cultural characteristics. Given the wide host ranges and plasticity of morphological characteristics observed, it was difficult to identify species based on these characteristics. The aims of this study were, therefore, to reassess the different *Phomopsis* species occurring on grapevines using a combination of molecular, morphological, cultural and pathological data, and to clarify the taxonomy of isolates previously identified as *D. perjuncta.*

Methods

Isolates and morphology

Symptomatic and asymptomatic grapevine material and pruning debris were collected over a 2 year period to obtain isolates of *Phomopsis* spp. Plant tissues were surface sterilised in 70% ethanol for 30 s, 1% NaOCl for 1 min, and again in 70% ethanol for 30 s before drying under a laminar flow hood. Small pieces of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto 2% potato-dextrose agar (PDA; Biolab, Midrand, South Africa). The PDA plates were incubated at 25°C in the dark to promote mycelial growth. Single-conidial isolations were made onto fresh PDA plates from sporulating colonies. In the case of the pruning debris, shoot pieces (5–7 cm long) were incubated in moist chambers in the laboratory for 1 week, and single-spore isolations were made from sporulating pycnidia or perithecia. In order to induce sporulation, isolates were plated on PDA, as well as water agar plates containing 4-cm-long pieces of double autoclaved vine shoots (WAV). Inoculated plates were incubated at 25°C under near ultraviolet (nuv) light in a 12 h light-darkness regime for 2–3 weeks to enhance sporulation. To study the morphology of the teleomorph, isolates were plated on WAV, incubated for 3 weeks at 25°C, and thereafter placed under nuv light at 10°C for a further 2 months. Fruiting structures were mounted in 70% lactic acid. Thirty measurements were taken of morphological structures. Spores were measured for length and width and the minimum and maximum ranges of spore dimensions were recorded and the averages determined. Cardinal temperature requirements for growth and cultural characteristics were determined for isolates plated onto PDA in 90 mm Petri dishes and incubated in the dark for 7 days at seven different temperatures, ranging from 5 to 35°C in 5° intervals. Three plates were used for each isolate of a specific species, at each temperature. Radial mycelial growth was measured perpendicularly (from the edge of the colony to the centre of the plate, four measurements per plate) on each plate, and the mean calculated to determine the cardinal temperature requirement for each isolate. Cultural colours were described from isolates of each species incubated at 25°C for 7 days, using the colour scheme of Rayner (1970). Cultures are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), and the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS) (Table 1).

Deoxyribonucleic acid isolation, amplification and sequence analysis

The isolation protocol of Lee and Taylor (1990) was used to extract genomic DNA from fungal mycelia grown on PDA. The primers ITS1 and ITS4 (White *et al.* 1990) were used to amplify part of the nuclear rRNA operon spanning the 3′ end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5′ end of the 28S (large subunit) of the rRNA gene. The PCR reaction mixture consisted of 1.5 units

Table 1. *Phomopsis* and *Diaporthe* isolates studied

Accession number[A]	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 2657	*D. ambigua*	*Prunus* sp.	Unknown	SA	S. Denman	AF 230767
STE-U 3390	*D. ambigua*	*Prunus* sp.	Unknown	SA	A. Smit	AF 230768
STE-U 5497[B]	*D. ambigua*	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485744
STE-U 2655[C]	*D. australafricana*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230744
STE-U 2676	*D. australafricana*	*V. vinifera*	Unknown	Australia	R.W.A. Scheper	AF 230760
	D. helianthi	*H. annuus*	Unknown	Italy	Unknown	AJ 312348
	D. helianthi	*H. annuus*	Unknown	Italy	Unknown	AJ 312366
STE-U 5355	*D. helianthi*	*V. vinifera*	Pruning debris	SA	J.M. van Niekerk	AY 485745
STE-U 5353[B]	*D. helianthi*	*V. vinifera*	Pruning debris	SA	J.M. van Niekerk	AY 485746
STE-U 5344	*D. helianthi*	*V.vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485747
STE-U 5356	*D. helianthi*	*V. vinifera*	Pruning debris	SA	J.M. van Niekerk	AY 485748
STE-U 5354	*D. helianthi*	*V. vinifera*	Pruning debris	SA	J.M. van Niekerk	AY 485749
STE-U 5683 CBS 113201[B,C]	*D. viticola*	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AY 485750
AR 3461 = CBS 109745[C]	*D. perjuncta*	*U. glabra*	Unknown	Austria	Unknown	AY 485785

(*Continued next page*)

Table 1. Continued

Accession number[A]	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5685	*D. viticola*	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AY 485751
STE-U 2677	*D. viticola*	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AF 230765
STE-U 2632	*P. amygdali*	*V. vinifera*	Bleached cane	SA	L. Mostert	AF 230755
—	*P. amygdali*	*P. persica*	Unknown	USA	W. Miller	AF102996
STE-U 5151[B]	*P. amygdali*	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485752
STE-U 5578	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485753
STE-U 5605	*P. viticola*	*V. vinifera*	Lesions on green shoots	SA	F. Halleen	AY 485754
STE-U 5157	*P. viticola*	*V. vinifera*	Lens-shaped lesions on dormant canes	SA	F. Halleen	AY 485755
STE-U 5576	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485756
STE-U 5604	*P. viticola*	*V. vinifera*	Lesions on green shoots	SA	F. Halleen	AY 485757
STE-U 5577	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485758
STE-U 5513	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485759
STE-U 2671	*P. viticola*	*V. vinifera*	Unknown	Italy	Unknown	AF 230747
STE-U 2672	*P. viticola*	*V. vinifera*	Unknown	Turkey	A. Anbavoylu	AF 230748
STE-U 5514	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485760
STE-U 5602	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485761
STE-U 2679	*P. viticola*	*V. vinifera*	Unknown	USA	Unknown	AF 230745
STE-U 2673	*P. viticola*	*V. vinifera*	Unknown	USA	Unknown	AF 230746
STE-U 2666	*P. viticola*	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AF 230758
STE-U 2662	*P. viticola*	*V. vinifera*	Unknown	Australia	R.W.A. Scheper	AF 230763
STE-U 5570	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485762
STE-U 5639	*P. viticola*	*V. vinifera*	Lesions on green shoots	SA	F. Halleen	AY 485763
STE-U 5511	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485764
STE-U 2648	*P. viticola*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230753
STE-U 2638	*P. viticola*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230764
STE-U 5509	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485765
STE-U 2646	*P. viticola*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230752
STE-U 5637	*P. viticola*	*V. vinifera*	Lens-shaped lesions on bleached canes	SA	F. Halleen	AY 485766
STE-U 2642	*P. viticola*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230756
STE-U 2669	*P. viticola*	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AF 230770
STE-U 5155	*P. viticola*	*V. vinifera*	Lens-shaped lesions on dormant canes	SA	F. Halleen	AY 485767
STE-U 2660[C]	*P. viticola*	*V. vinifera*	Unknown	France	P. Larignon	AF 230751
STE-U 2641	*P. viticola*	*V. vinifera*	Unknown	SA	L. Mostert	AF 230754
STE-U 5572[B]	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485768
STE-U 5510	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485769
STE-U 5512	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485770
STE-U 5638	*P. viticola*	*V. vinifera*	Bleached canes	SA	F. Halleen	AY 4857771
STE-U 5579	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 4857772
STE-U 5568	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 4857773
STE-U 5571	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 4857774
STE-U 5603	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 4857775
STE-U 5599	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485776
STE-U 5569	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485777
STE-U 5565	*P. viticola*	*V. vinifera*	Lens-shaped lesion on dormant spur	SA	F. Halleen	AY 485778
STE-U 5636	*P. viticola*	*V. vinifera*	Internal browning in bleached cane	SA	F. Halleen	AY 485779
STE-U 5575	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485780
STE-U 5600	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485781

(*Continued next page*)

Table 1. Continued

Accession number[A]	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5566	*P. viticola*	*V. vinifera*	Lens-shaped lesion on dormant spur	SA	F. Halleen	AY 485782
STE-U 5574	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485783
STE-U 5601	*P. viticola*	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485784
STE-U 2675[C]	*P. vitimegaspora*	*V. vinifera*	Unknown	Taiwan	Unknown	AF 230749
STE-U 2656	*Phomopsis* sp. 1	*Pyrus* sp.	Unknown	SA	L. Basson	AF 230769
STE-U 5567	*Phomopsis* sp. 1	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485723
STE-U 5573[B]	*Phomopsis* sp. 1	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485724
STE-U 2661	*Phomopsis* sp. 1	*V. vinifera*	Unknown	Australia	R.W.A. Scheper	AF 230759
STE-U 2664	*Phomopsis* sp. 1	*V. vinifera*	Unknown	Australia	D.L. Whisson	AF 230750
STE-U 2668	*Phomopsis* sp. 1	*V. vinifera*	Unknown	Portugal	A.J.L. Phillips	AF 230762
STE-U 2654	*Phomopsis* sp. 1	*V. vinifera*	Unknown	SA	L. Mostert	AF 230743
STE-U 2659	*Phomopsis* sp. 1	*Protea*.sp.	Unknown	SA	S. Denman	AF 230757
STE-U 2674	*Phomopsis* sp. 2	*V. vinifera*	Unknown	Italy	Unknown	AF 230761
STE-U 4407[B]	*Phomopsis* sp. 3	*V. vinifera*	Asymptomatic nursery plant	SA	G.J. van Coller	AY 485725
STE-U 5464[B]	*Phomopsis* sp. 4	*V. vinifera*	Pruning wound	SA	F. Halleen	AY 485726
STE-U 5345	*Phomopsis* sp. 5	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485727
STE-U 5346[B]	*Phomopsis* sp. 5	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485728
STE-U 5463	*Phomopsis* sp. 5	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485729
STE-U 5465	*Phomopsis* sp. 5	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485730
STE-U 5496	*Phomopsis* sp. 5	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485731
STE-U 5461	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485732
STE-U 5467	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485733
STE-U 5466	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485734
STE-U 2680	*Phomopsis* sp. 6	*Rosa* sp.	Unknown	SA	S. Denman	AF 230766
ATCC 56789	*Phomopsis* sp. 6	*V. macrocarpon*	Unknown	USA	D.M. Boone	AF 317580
STE-U 5135[B]	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485735
STE-U 5158	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485736
STE-U 5160	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485737
STE-U 5134	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485738
STE-U 5347	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485739
STE-U 5348	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485740
STE-U 5133	*Phomopsis* sp. 6	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485741
STE-U 5495[B]	*Phomopsis* sp. 7	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485742
STE-U 5462[B]	*Phomopsis* sp. 8	*V. vinifera*	Asymptomatic nursery plant	SA	F. Halleen	AY 485743

[A]STE-U = University of Stellenbosch culture collection, Stellenbosch, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[B]Isolates used in the pathogenicity trial.
[C]Ex-type cultures.

Biotaq (Bioline, London, UK), 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 4 pmol of each primer, approximately 10 to 30 ng of fungal genomic DNA, and was made up to a total volume of 25 µL with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions comprised denaturation for 5 min at 96°C, followed by 30 cycles at 96°C (30 s), 55°C (30 s), 72°C (90 s) and a final 7 min extension step at 72°C to complete the reaction. PCR products were separated by electrophoresis in a 0.8% (w/v) agarose gel and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining. Amplification products were purified using NucleoSpin Extract 2 in 1 kit (Macherey-Nagel, Germany).

Purified PCR products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI Prism Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase. The reaction was set up as follows: denaturing at 94°C for 5 min, followed by 25 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 min, with a final incubation of 30 s at 60°C. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

The ITS nucleotide sequences generated in this study were added to other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov). *Valsa mali* Miyabe & G. Yamada (AF191186) and *Valsa japonica* Miyabe & Hemmi (AF191185) were included as outgroups. The alignments were assembled using Sequence Alignment Editor v2.0a11 (Rambaut 2002) and manual adjustments for improvement were made where necessary. Phylogenetic analyses with neighbour joining (using uncorrected ('p') and Jukes-Cantor substitution models) were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2000). Alignment gaps were treated as missing data, all characters were unordered and of equal weight and any ties encountered were broken randomly. The robustness of the neighbour-joining tree was evaluated by 1000 bootstrap replications (Hillis and Bull 1993).

Pathogenicity

Thirteen isolates, representing 12 different species of *Phomopsis* from the phylogenetic analysis and an isolate of *Clonostachys rosea* (Link: Fr.) Schroers, Samuels, Seifert & W. Gams as the non-pathogen control, were selected as treatments (see Table 3). Other control treatments included were wounded only and inoculation with a non-colonised agar plug. The selected species were plated on PDA and incubated at 25°C for 1 week. Inoculations were made on green shoots of the grapevine cvv. 'Chenin Blanc' and 'Pinotage'. Shoots were cut from vines ~2 months after budburst, and internodes 2–4 were used for inoculations. Five shoots were used for each species. Shoots were wounded on internode 3, between internode 2 and 4, (2 mm deep) with a 3 mm cork-borer. A colonised agar plug, cut from a 1-week-old culture, was placed in the wound, which was subsequently covered with Parafilm. Inoculated shoots were incubated in the dark under moist conditions in the laboratory for 10 days at 22°C. Following the incubation period, the external lesions on the shoots were measured. The layout of the trial was a completely randomised design and the data were statistically analysed using SAS (SAS 1999). An analysis of variance and Student's t-tests for least significant differences were undertaken. The experiment was repeated twice. After the experiment, all plant material was destroyed by autoclaving twice for 15 min.

Results

Isolates and morphology

Deoxyribonucleic acid was extracted from 63 isolates, which were isolated from symptomatic and asymptomatic material, and subjected to sequencing (Table 1). All isolates sporulated on PDA and WAV after being incubated at 25°C under nuv light in a 12 h light-darkness regime for 2–3 weeks. Teleomorphs were successfully induced for isolates of *D. ambigua* and *D. viticola* after 2 months incubation at 10°C under nuv light. Morphological descriptions and measurements are reported in the taxonomic component of this paper.

Sequence analysis

Approximately 510–530 bases were determined for each isolate and added to the alignment. The manually adjusted alignment (spanning ITS1, 5.8S rRNA gene, ITS2) of the nucleotide sequences contained 99 taxa and 510 characters including alignment gaps (data not shown). Due to the inclusion of sequences from GenBank that were shorter on the 5′ and 3′ ends, the complete sequences determined in this study were not used in the phylogenetic analysis. Sequences were deposited in GenBank (Table 1), and the alignment in TreeBASE (SN 1693). The neighbour-joining analysis resulted in a phylogenetic tree delimiting six main clades and several sub-groups (Fig. 1). The topology of this tree was the same irrespective of which substitution model was used. The first main clade (85% bootstrap support) consists of *P. vitimegaspora* AF230749, an unknown species designated as *Phomopsis* sp. 3 (STE-U 4407), and two sub-groups. The first sub-group (bootstrap support of 99%) contains grapevine isolates that cluster together with two GenBank sequences of *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. The second sub-group (bootstrap support of 100%) contains a single grapevine isolate, STE-U 5497, which groups together with sequences of *Diaporthe ambigua* Nitschke. The second major clade (68% bootstrap support) consists of four sub-groups. The first sub-group (97% bootstrap support) contains three grapevine isolates from Portugal, of which one (AF230765) was previously identified as *Diaporthe perjuncta* Niessl. The second sub-group contains additional isolates previously identified as *D. perjuncta* from South Africa (AF230744) and Australia (AF230760). These two sub-groups cluster with a bootstrap support value of 100%. The third sub-group in this clade (bootstrap support value of 100%) consists of three isolates of *Phomopsis amygdali* (Delacr.) J.J. Tuset & M.T. Portilla, two from grapevines in South Africa (STE-U 5151 and AF230755) and a GenBank sequence (AF102996). The fourth sub-group (76% bootstrap support) in this clade consists of five grapevine isolates (STE-U 5346, 5463, 5345, 5465, 5496), and represents an unknown taxon referred to as *Phomopsis* sp. 5. Another grapevine isolate, *Phomopsis* sp. 4 (STE-U 5464), clusters without any bootstrap support with the second main clade. The third main clade, *Phomopsis* sp. 6 (100% bootstrap support) contains several South African grapevine isolates that cluster with a South African isolate from roses (AF230766) and another from cranberries in the USA (AF317580). The fourth main clade (68% bootstrap support) contains an isolate of

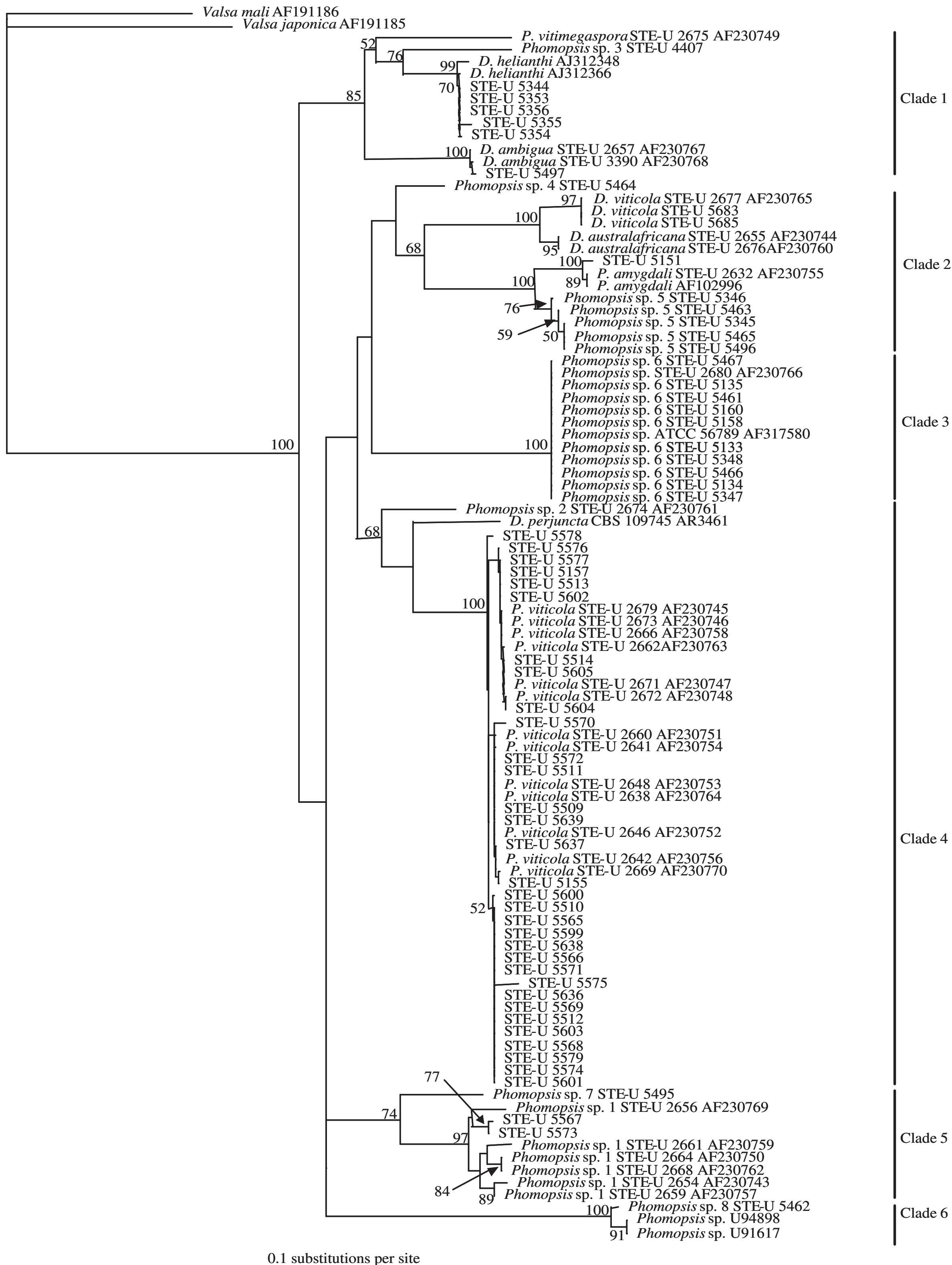


Fig. 1. Neighbour-joining tree of *Diaporthe* and *Phomopsis* spp. obtained from a phylogenetic analysis of aligned ITS sequence data. Bootstrap support values from 1000 replicates are shown at the nodes. *Valsa mali* and *V. japonica* were used as outgroups. The scale bar shows the number of substitutions per site.

Phomopsis sp. 2 (AF230761) and the ex-epitype strain of *D. perjuncta* (CBS 109745 = AR 3461). Included in this clade is a sub-group containing isolates of *P. viticola* (100% bootstrap support). The fifth main clade (74% bootstrap support) contains *Phomopsis* sp. 7 (STE-U 5495) and a sub-group containing isolates of *Phomopsis* sp. 1. The *Phomopsis* sp. 1 subgroup (97% bootstrap support) consists of grapevine isolates from South Africa (STE-U 5567 and 5573, AF230743), Australia (AF230759, AF230750) and Portugal (AF230762). Additional South African isolates from *Protea* L. (AF230757) and *Pyrus* L. (AF230769) also cluster in this subgroup. The sixth main clade (100% bootstrap support) contains *Phomopsis* sp. 8 (STE-U 5462), which clustered with two unnamed *Phomopsis* species occurring on plum and pear trees in the USA.

Taxonomy

Diaporthe viticola Nitschke, *Pyrenomycetes Germanici* **2**: 264 (1870).

Description and illustration. Phillips (1999); Mostert *et al*. (2001).

Cultures. Colonies convex with undulating, smooth margins, buff (19''f) on the surface, and rosy buff (13''f) underneath, obtaining a radius of 30 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 30°C, opt. 25°C.

Host. Vitis vinifera.

Distribution. Germany, Portugal.

Specimens examined. Germany, Westfalen, Münster, bei der Wienburg, on *Vitis vinifera*, Feb. 1866, Nitschke, holotype in B. Portugal, Santo Tirso, Burgaes, on *Vitis vinifera*, 16 Feb. 1998, A.J.L. Phillips, EPITYPE specimen Herb. CBS 7950, ex-epitype culture STE-U 5683, CBS 113201. Italy, Sardiniae, on *V. vinifera*, holotype of *Diaporthe silvestris* Sacc. & Berl., PAD 228.

Notes. The fungus that Merrin *et al*. (1995) referred to as *Phomopsis* taxon 1 on grapevines was argued to be the same as *D. perjuncta* by Phillips (1999). Later, Scheper *et al*. (2000) again referred to it as *D. viticola.* In a subsequent study, Mostert *et al*. (2001) chose to follow the arguments of Phillips (1999), and used the name *D. perjuncta* for taxon 1; however, at this stage Mostert *et al*. (2001) also noted that minor morphological differences existed in perithecia and ascospores between Portuguese and South African and Australian material. Molecular analysis did not clearly separate these taxa. Since then, a specimen from the original location and host of *D. perjuncta* has been collected that was morphologically similar, but phylogenetically distinct. Furthermore, additional grapevine isolates have also been studied, which resolved the fact that the South African and Australian material represent a distinct taxon from the Portuguese material. A re-examination of the type of *D. viticola* revealed that this name could indeed be used for the European collections, having ascospores which were (9–)12–15 × 3.5–4(–4.5) µm, being widest in the middle of the apical cell, and frequently having terminal mucous caps, as observed in the Portuguese material. The South African and Australian material has ascospores that are widest at the median septum, and these collections are described as new below. The type specimen of *D. silvestris* Sacc. & Berl. in PAD was also examined (A.J.L. Phillips, personal communication). In this collection, ascospores also proved to be widest in the middle of the apical cell and were 11–16 × 3.5–5 µm in size, thus being very similar to *D. viticola*, but still distinct from the South African and Australian material. If future studies reveal *D. silvestris* to be synonymous with *D. viticola*, the latter name, which is older, would retain priority. An epitype specimen (herb. CBS 7950) and ex-epitype culture (CBS 113201) is herewith selected to be used as future reference for the name *D. viticola*.

Diaporthe australafricana Crous & J.M. van Niekerk, sp. nov.

Diaporthe viticolae similis, sed peritheciis solitariis, ostiolis sursum rubro-brunneis, sparse hyphis obtectis, ascosporis fusoideis, (8–)11.5–13(–15) × (2–) 3.5–3.5(–4) µm distincta.

Etymology. Referring to its known distribution in Australia and Africa.

Illustration. Mostert *et al*. (2001).

Perithecia globose, solitary, scattered to aggregated, subepidermal, 210–500 µm wide, 250–350 µm tall, red-brown at the tip with sparse external hyphae covering perithecial necks; wall consisting of two regions of textura angularis: outer region dark brown, 3–4 cells thick, 15–65 µm wide; inner region pale brown, 3–4 cells thick, 12–32 µm wide; perithecial neck of textura prismatica, with outer region dark brown, 10–20 µm wide, inner region hyaline, 5–10 µm wide; ostiole red-brown, widening when spores start exuding, 90–150 µm wide; necks long, with external hyphae, red-brown at the tip, becoming dark brown towards the perithecium, constricted in the middle of the neck, 870–1500 × 70–100 µm (av. = 1128 × 83 µm, $n = 30$); asci unitunicate, cylindrical-clavate, with refractive apical ring, 8-spored, biseriate, 55–61 × 5–8.5 µm (av. = 57 × 6.5 µm, $n = 33$); paraphyses septate, unbranched, tapering towards apex with a rounded tip, extending above the asci, 48–109 µm long, 4–7 µm wide at bottom and 2–3 µm at the apex; ascospores hyaline, smooth, fusoid, widest at the septum, tapering towards both ends, slightly curved or straight, medianly septate, slightly constricted at the septum with 1 to 2 large guttules, (8–)11.5–13(–15) × (2.5–)3–3.5(–4.0) µm (av. = 12 × 3.5 µm, $n = 33$); hyaline appendages sometimes present, punctiform, restricted to the tips of ascospores, 1–2 µm long; sterile pycnidia formed after 17 days at 25°C in the dark; conidia: alpha conidia biguttulate to eguttulate, fusoid with obtuse ends, 5–5.5(–6) × 1.5–2 µm (*in vivo*). Beta

conidia and gamma conidia absent. Description based on ex-type culture, CBS 113487.

Host. *Vitis vinifera.*

Distribution. Australia, South Africa.

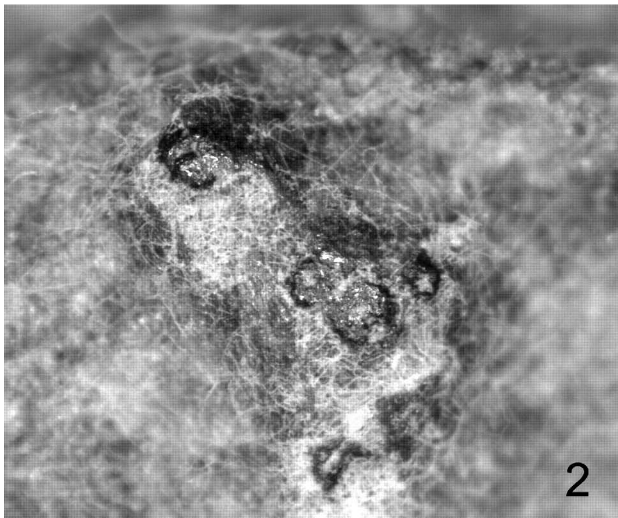
Specimen examined. South Africa, Western Cape Province, Stellenbosch, on Riesling grapevines, Nov. 1997, L. Mostert, HOLOTYPE specimen PREM 56458, ex-type culture STE-U 2655, CBS 113487.

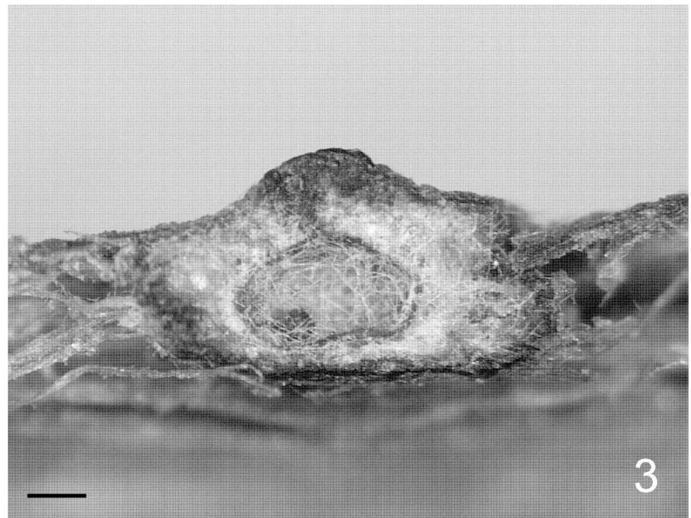
Diaporthe perjuncta Niessl, *Hedwigia* **17**: 44 (1878). Figs 2–9

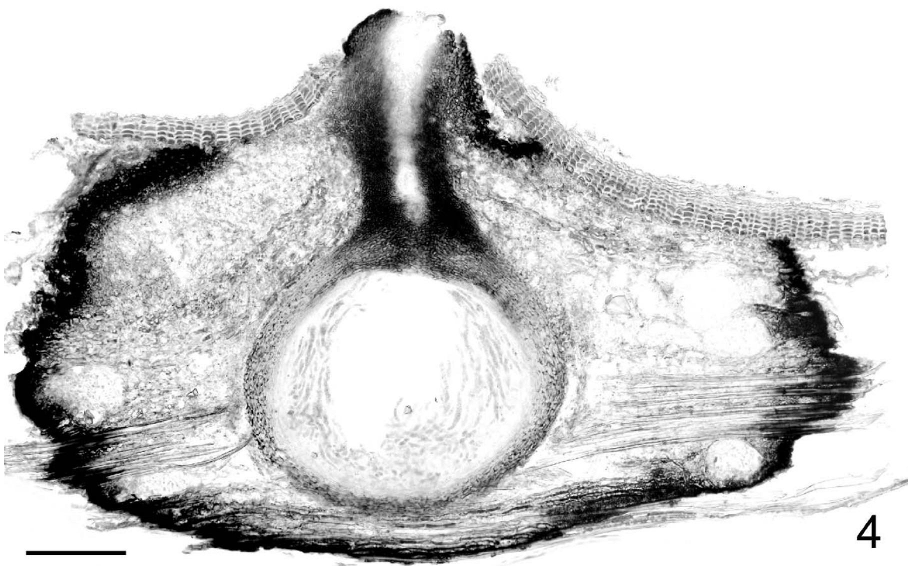
[≡ Diaporthe conjuncta Niessl, *Hedwigia* **15**: 153 (1876) non (Nees) Fuckel 1869].

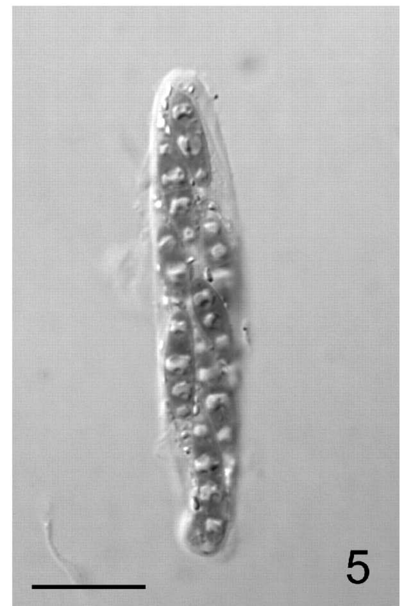
= *Diaporthe saccardiana* Kunze ex Sacc., *Sylloge Fungorum* **1**: 628 (1882).

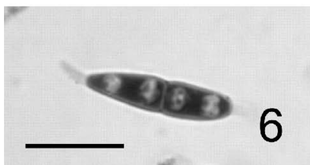
≡ Chorostate saccardiana (Sacc.) Trav., *Flora Italica Cryptogama* **2**: 206 (1906).

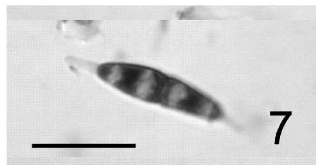


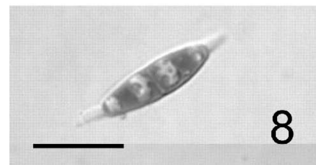


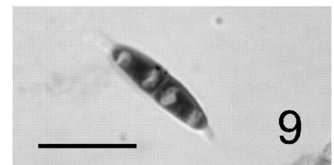












Figs 2–9. Morphological structures of *Diaporthe perjuncta* (BPI 748437 = CBS 109745). **Fig. 2.** Stroma on host tissue. **Fig. 3.** Vertical section through stroma. Bar = 200 µm. **Fig. 4.** Vertical section through a perithecium. Bar = 100 µm. **Fig. 5.** Ascus with ascospores. Bar = 10 µm. **Fig. 6.** Ascospores with gelatinous appendages. Bar = 10 µm. **Fig. 7.** Ascospores with gelatinous appendages. Bar = 10 µm. **Fig. 8.** Ascospores with gelatinous appendages. Bar = 10 µm. **Fig. 9.** Ascospores with gelatinous appendages. Bar = 10 µm.

Stroma on *surface* as papillate pustules, 0.5–2 mm in longest dimension, to 0.7 mm deep, penetrating into wood, stroma prosenchymatous with embedded host tissue from both periderm and wood, little differentiation between entostroma and ectostroma, strongly delimited to sides and bottom by a dark brown to black layer of thick walled compact hyphae, layer 25–50 µm thick, dorsal zone absent, ostioles generally singly erumpent or in small groups, never united, often surrounded by a whitish disk, 3–10 perithecia per stroma; perithecia globose to somewhat flattened, 360–450 × 260–400 µm, wall composed of several layers of compact, rectangular cells, somewhat thickened, brown in outer layers becoming hyaline in inner layers, 30–50 µm thick, necks 200–400 µm long, 100–170 µm wide at apex, wall pseudoparenchymatous, cells dark brown to black, thick-walled, ostiole periphysate; asci unitunicate, cylindric-clavate, with a refractive ring apparatus, mostly biseriate 48–74(–78) × 7.5–10 (av. 62 × 8.5, $n = 60$); ascospores hyaline, smooth, fusiform, ends obtuse, one-septate, often constricted at septum, guttulate with two large oil globules per cell, 11–15 × 3–4.5 (av. 12.8 × 3.8, $n = 76$), gelatinous appendages present on both ends, in KOH appendages appear as short, cylindrical to flaring projections with an irregular end, 2–4 × 1.5 µm, in lactic acid appendages cylindrical with rounded ends 1.5–5 × 1–1.5 µm. Anamorph not observed.

Cultures. Colonies flat with smooth, undulating margins, white on the surface and straw coloured (21'd) underneath, obtaining a radius of 29 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 15°C, max. 30°C, opt. 25°C.

Hosts. On fallen branches of *Ulmus campestris* and *U. glabra*.

Distribution. Austria, Germany.

Specimens examined. Austria, St Margareten i. Ros., Schwarzupt-Ostseite, Grid square 9452/4, on *Ulmus glabra*, 1 Jun 2000, *Walter Jaklitsch 1480* (BPI 748437, ex-epitype culture CBS 109745 (= AR 3461), herein designated EPITYPE of *Diaporthe perjuncta*). Germany, Stralsund, on *Ulmus campestris*, *Fischer* (Rabenhorst, Fungi europaei exsiccati, Series nov., no. 2325 issued in 1876. The specimen of this number was examined from BPI in the bound exsiccati and is herein designated LECTOTYPE of *Diaporthe perjuncta*); Germany, Saxony, Neckendorfer Thal near Islebiam, on *Ulmus campestris*, May, 1875, *W. Krieger* (BPI 616993 Johs. Kunze, Fungi selecti exsiccati 123, herein designated LECTOTYPE of *Diaporthe saccardianum*; BPI 619992; Germany, Saxony, Nossen, Dec. 1885, Jul 1886, Apr 1887, *W. Krieger* (BPI 616992 Krieger, Fungi Saxonici 632).

Notes. *Diaporthe perjuncta* is a distinctive species that is apparently restricted to species of *Ulmus* (Ulmaceae) in Austria and Germany. This species is unusual within *Diaporthe* in having scattered, solitary or clustered beaks emerging through bark with relatively small stroma evident as raised areas and relatively large, appendaged ascospores. The entostroma is well delimited with a black line that goes around the entire stroma. None of the specimens examined nor the culture showed evidence of an asexual state.

The name *Diaporthe perjuncta* Niessl was published to replace the later homonym *D. conjuncta* Niessl 1876 non (Nees) Fuckel 1869; both names are based on the same type specimen. The lectotype and epitype specimens agree in all aspects except for the size of the asci and ascospores. In the lectotype specimen the asci and ascospores were difficult to examine, possibly due to previous handling of the specimen. The asci and ascospores in the lectotype were slightly larger than given above. In the lectotype specimen, the asci are 57–75 × 8.5–12.5 µm (avg. 66.9 × 10.2, $n = 12$) and the ascospores 12.5–18 × 3.5–5 µm (avg. 15.5 × 4.3, $n = 29$). The asci and ascospores of the epitype agree with the protologue in Niessl (1876) and Wehmeyer (1933) and are considered correct for this species. Based on an examination of type and authentic specimens, the name *Diaporthe saccardiana* is confirmed as a synonym of *D. perjuncta* as previously noted by Wehmeyer (1933). This name was originally proposed on a herbarium label but lacked a description and thus the name dates from the publication of a description in Saccardo (1882).

***Phomopsis* sp. 1**

Description and illustration. Mostert *et al*. (2001).

Hosts. *Protea* sp., *Pyrus* sp., *Vitis vinifera*.

Distribution. Australia, Portugal, South Africa.

Notes. This species was treated in detail by Mostert *et al*. (2001). It is known from grapevines in Australia, Portugal and South Africa, and also occurs on proteas and pears in the latter country (Mostert *et al*. 2001; Figs 40, 41). Presently this species appears to have a wide host range, and it is probable that future studies will be able to link these cultures (Table 1) to an established name.

***Phomopsis* sp. 2**

Description and illustration. Mostert *et al*. (2001).

Host. *Vitis vinifera*.

Distribution. Italy.

Notes. This species was treated in detail by Mostert *et al*. (2001). Although it is morphologically distinct given the shape of its alpha conidia (Mostert *et al*. 2001; Fig. 46), we decline to name it as new as it is presently known from only one collection (Table 1).

***Phomopsis* sp. 3**

Cultures. Colonies flat with undulating, smooth margins, white on the surface, and straw coloured (21'd) underneath, obtaining a radius of 18 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 30°C, opt. 25°C.

Host. *Vitis vinifera*.

Distribution. South Africa.

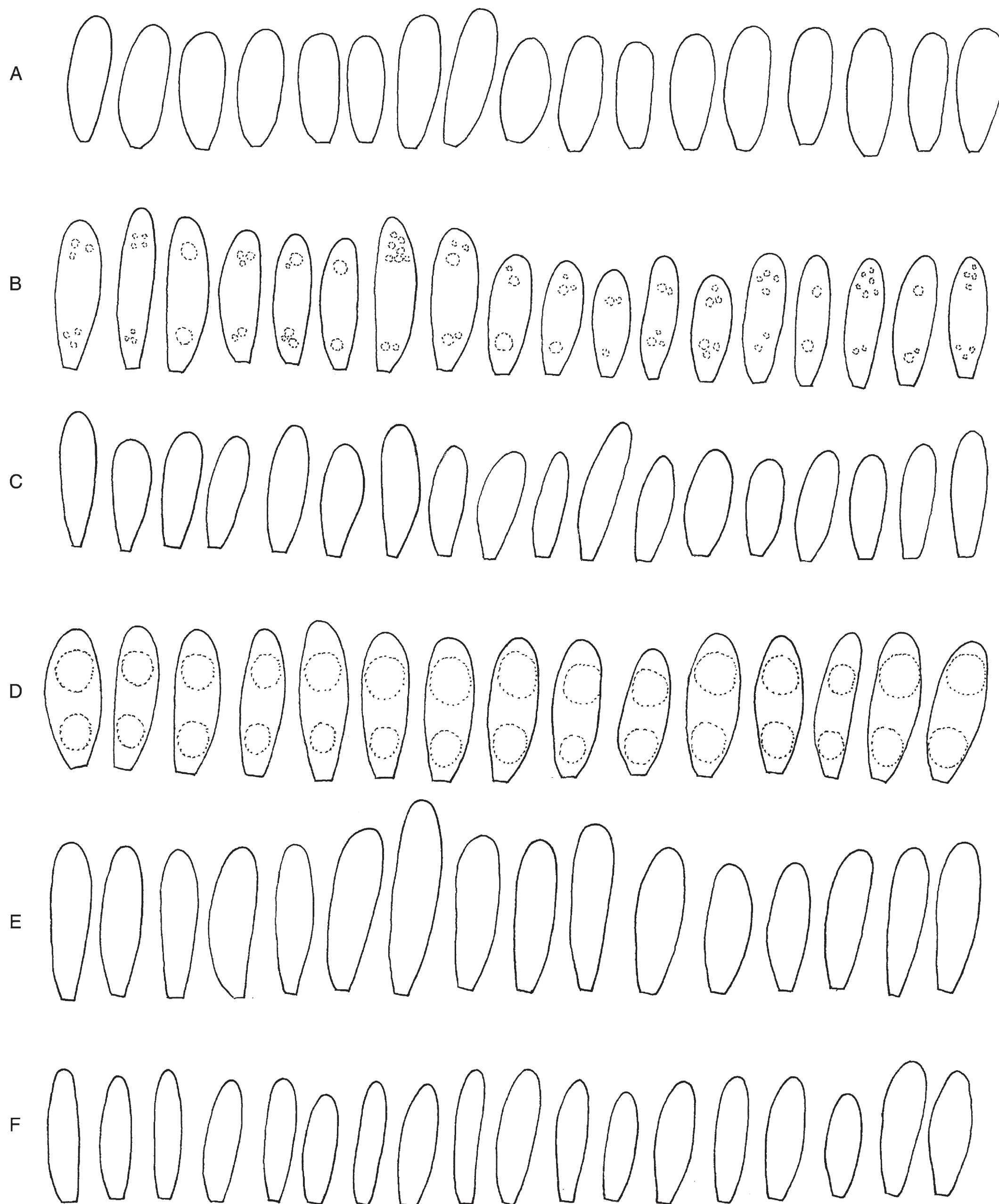


Fig. 10. Alpha conidia of various *Phomopsis* spp. as produced on grapevine canes *in vitro*. (*A*) *Phomopsis* sp. 3 (STE-U 4407), (*B*) *Phomopsis* sp. 4 (STE-U 5464), (*C*) *Phomopsis* sp. 5 (STE-U 5496), (*D*) *Phomopsis* sp. 6 (STE-U 5348), (*E*) *Phomopsis* sp. 7 (STE-U 5495), (*F*) *Phomopsis* sp. 8 (STE-U 5462).

Notes. This species is presently known from a single collection (STE-U 4407) (Table 1), and has ellipsoidal, non-guttulate alpha conidia, (5–)6–8(–9) × (2–)2.5–3 µm (Fig. 10*A*).

Phomopsis sp. 4

Cultures. Colonies umbonate with smooth, even margins, hazel (17'''i) on the surface, and isabelline (17''i) underneath, obtaining a radius of 18 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Host. *Vitis vinifera.*

Distribution. South Africa.

Notes. This species is known from a single collection from vines in South Africa (STE-U 5464) (Table 1). It is characterised by having fusoid–ellipsoidal alpha conidia that are multiguttulate, and taper from an obtusely rounded apex to a subtruncate base, (6–)7–9(–10) × 2–2.5(–3) µm, and

straight, curved or hamate beta conidia, 25–30 × 1–1.5 µm (Fig. 10*B*).

Phomopsis sp. 5

Cultures. Colonies appressed, convex with smooth, even margins, white on the surface, and white to olivaceous (21''m) underneath, obtaining a radius of 16 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 15°C, max. 35°C, opt. 30°C.

Host. *Vitis vinifera*.

Distribution. *South* Africa.

Notes. This species is known from several collections obtained from grapevines in South Africa (STE-U 5346, 5463, 5345, 5465, 5496) (Table 1). Alpha conidia are 6–8(–9) × 2(–2.5) µm, and have a characteristic taper, being widest in the middle or upper third, and tapering sharply to a truncate base, 0.5 µm wide (Fig. 10*C*).

Phomopsis sp. 6

Cultures. Colonies flat with smooth, even margins, hazel (17'''i) to buff (19''d) on the surface, and brown-vinaceous (5'''m) to honey (19''b) coloured underneath, obtaining a radius of 12 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Hosts. *Rosa* sp., *Vaccinium* sp., *Vitis vinifera.*

Distribution. South Africa, USA.

Notes. *Phomopsis* sp. 6 appears to be common on grapevines in South Africa (STE-U 5348, 5133, 5160, 5467, 5134, 5466, 5347, 5135, 5158, 5461), but also occurs on other hosts such as *Rosa* (South Africa), and *Vaccinium* (USA) (Table 1). This species has relatively large, fusoid–ellipsoidal alpha conidia, (6–)7–9(–10) × 2–2.5 µm (Fig. 10*D*), being slightly shorter than those of *P. viticola* (Mostert *et al*. 2001).

Phomopsis sp. 7

Cultures. Colonies appressed, convex with undulating margins, white on the surface, and buff (19''d) to white underneath, obtaining a radius of 31 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 30°C, opt. 25°C.

Host. *Vitis vinifera*.

Distribution. South *Africa*.

Notes. This *species* is presently known from a single South African collection (STE-U 5495) (Table 1). Morphologically, it is characterised by having slender, fusoid–ellipsoidal alpha conidia, (8–)9–10(–11) × 2–2.5(–3) µm, being slightly narrower than those of *P. viticola* (Mostert *et al.* 2001) (Fig. 10*E*).

Phomopsis sp. 8

Cultures. Colonies convex with smooth margins, white on the surface, and olivaceous-buff (21'''d) underneath, obtaining a radius of 10 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Hosts. *Prunus* sp., *Pyrus* sp., *Vitis vinifera*.

Distribution. South Africa, USA.

Notes. Based on phylogeny obtained here, the grapevine isolate (STE-U 5462) (Table 1) clustered with a *Phomopsis* sp. that is regarded as a serious pathogen of pears and plums in the USA (Uddin *et al*. 1998). Morphologically, it had alpha conidia that were slender, fusoid to narrowly ellipsoidal, with acutely rounded apices and subtruncate bases, (6–)7–9 × 2–2.5(–3) µm (Fig. 10*F*). Uddin *et al*. (1998) described the alpha conidia of the plum and pear isolates as being fusiform, biguttulate to multiguttulate. The conidial measurements of the plum isolate were given as 7.5–15 × 1.25–3 µm, and those of the pear isolate as 6.5–12.5 × 2.5–4.5 µm. Although phylogenetically similar, the USA collections thus seem to be morphologically distinct, and a detailed study would be required to resolve their status.

Other species which occurred on grapevines included *P. vitimegaspora* (STE-U 2675) (Kuo and Leu 1998; Mostert *et al*. 2001) and *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov (anamorph *P. helianthi* M. Muntañola-Cvetkovič & M. Petrov), known previously only from *Helianthus annuus* L. (Muntañola-Cvetković *et al*. 1981). Two species that are commonly associated with fruit trees also occurred on vines, *D. ambigua* Nitschke (STE-U 2657, STE-U 3390, STE-U 5497) (Mostert *et al*. 2001; Moleleki *et al*. 2002) and *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla (STE-U 2632, STE-U 5151) (Mostert *et al*. 2001). Most of the isolates obtained in this study, however, proved to be representative of *P. viticola* (Table 1), the common agent of Phomopsis cane and leaf spot disease (Mostert *et al*. 2001).

Pathogenicity

Analysis of variance for mean lesion lengths caused by selected *Phomopsis* isolates and controls on green shoots of cvv. 'Chenin Blanc' and 'Pinotage' showed significant interaction between cultivar and treatment (Table 2), and data were therefore not pooled in further analyses. This interaction was most probably due to *P. amygdali*, which caused lesions on 'Pinotage' that were significantly larger than those on 'Chenin Blanc' (Table 3). Conversely, *P. viticola*

Table 2. Analysis of variance of lesion length caused by *in vitro* inoculations with selected isolates of *Phomopsis* and controls on green shoots of the grapevine cultivars 'Pinotage' and 'Chenin Blanc'

Source of variation	DF	SS	MS	SL
Model	38	51808.5	1363.4	<0.0001
Repeat	1	92.2	92.2	0.0102
Shoots	8	206.7	25.8	0.0639
Treatment	14	50032.5	3573.8	<0.0001
Cultivar	1	2.0	2.0	0.7037
Cultivar × Treatment	14	1475.1	105.4	<0.0001
Error	255	3510.2	13.8	
Corrected total	293	55318.7		

Table 3. Mean lesion length caused by *in vitro* inoculations with selected isolates of *Diaporthe*, *Phomopsis* and controls on green shoots of the grapevine cultivars 'Pinotage' and 'Chenin Blanc'

Treatment	Mean lesion length (mm)[A]	
	Pinotage	Chenin Blanc
D. ambigua (STE-U 5497)	6.2 h-m	8.1 f-i
D. helianthi (STE-U 5353)	4.1 k-m	5.4 h-m
D. viticola (STE-U 5683)	12.7 e	10.5 ef
P. amygdali (STE-U 5151)	60.6 a	44.0 b
P. viticola (STE-U 5572)	35.1 d	39.9 c
Phomopsis sp. 1 (STE-U 5573)	5.2 i-m	6.8 g-l
Phomopsis sp. 3 (STE-U 4407)	6.3 g-m	6.6 g-m
Phomopsis sp. 4 (STE-U 5464)	8.4 f-i	9.6 e-g
Phomopsis sp. 5 (STE-U 5346)	3.4 m	4.5 j-m
Phomopsis sp. 6 (STE-U 5135)	6.2 h-m	8.6 f-h
Phomopsis sp. 7 (STE-U 5495)	6.4 g-m	7.6 f-j
Phomopsis sp. 8 (STE-U 5462)	7.7 f-j	8.6 f-h
Non-pathogen (*Clonostachys rosea*)	6.2 h-m	7.3 f-k
Wounded only	3.4 m	4.7 j-m
Agar plug	3.4 m	3.9 lm
LSD ($P = 0.05$)		3.304

[A]Means followed by the same letter are not significantly different.

formed significantly larger lesions on 'Chenin Blanc' than on 'Pinotage'. These two species, and to a lesser extent *D. viticola*, were the only species that caused lesions significantly larger than the control treatments on both cultivars. *D. ambigua*, *Phomopsis* sp. 1, *Phomopsis* sp. 3, *Phomopsis* sp. 4, *Phomopsis* sp. 6, *Phomopsis* sp. 7 and *Phomopsis* sp. 8 formed lesions on both grapevine cultivars that were not significantly larger than the *C. rosea* control, and in some cases not significantly larger than the agar treated and wounded-only controls. *D. helianthi* and *Phomopsis* sp. 5 formed lesions similar in size to that of the agar and wounded-only controls. Re-isolations from the inoculated green shoots were successful in all cases and species were identified as being the same as originally used in inoculations, thereby satisfying Koch's postulates.

Discussion

Fifteen species of *Phomopsis* were found to occur on grapevines in the present study. Of these taxa, *P. viticola* and *P. vitimegaspora* are currently known to be pathogens of grapevines, whereas six species of *Phomopsis* and one species of *Diaporthe*, *D. viticola*, are probably minor pathogens of this host. A further two species, previously reported from other hosts, were newly reported from grapevines. These were *P. amygdali*, a known pathogen of peaches and almonds (Farr *et al.* 1999) and *D. helianthi*, a known pathogen of sunflowers. These findings thus provide further support for host switching within *Phomopsis* (Mostert *et al.* 2001). One of the unknown *Phomopsis* species also contains isolates obtained from grapevines, roses and cranberries. These findings further support those of previous studies (Farr *et al.* 1999; Mostert *et al.* 2001), that host specificity cannot be used to distinguish between different *Phomopsis* species. The possibility that a *Phomopsis* species could have more than one host has great implications for the management of diseases caused by *Phomopsis* species, as alternative hosts might act as a source of inoculum. In this study isolates were obtained from typical *Phomopsis* symptoms, pruning wounds, pruning debris and asymptomatic nursery plants.

The *in vitro* pathogenicity trials tested the ability of mycelium of selected *Phomopsis* species to infect wounded green shoots of two grapevine cultivars. All species were successfully re-isolated from the respective lesions and should thus be considered as potential pathogens of grapevines. *P. amygdali* and *P. viticola* caused the most severe lesions on both cultivars tested. In this study, *P. amygdali* was isolated only once from asymptomatic nursery plants and, together with the isolate obtained in the study of Mostert *et al.* (2001), only two isolates from grapevines are known at this time. The results of the pathogenicity trial indicate that this species could potentially be a serious grapevine pathogen, particularly of green shoots. Apart from being isolated from tissue with typical Phomopsis cane and leaf spot symptoms, *P. viticola* was also isolated from pruning wounds. This indicates that *P. viticola* can infect pruning wounds. The other species identified in this study showed variable degrees of lesion formation on the green shoots. Like *P. viticola* and *P. amygdali* that were isolated from pruning wounds and asymptomatic nursery plants, respectively, the remaining species were also isolated from either pruning wounds or asymptomatic nursery plants. However, at this stage it remains unclear whether the pruning wound infection by the various *Phomopsis* species could develop further to ultimately cause internal wood decay symptoms and, also, if the infection occurring in the nursery would develop further when the grapevines are planted in the field.

The present study has also attempted to resolve the identity of isolates previously identified as *D. perjuncta*, *D. viticola* or *Phomopsis* taxon 1 (Merrin *et al.* 1995; Phillips 1999; Scheper *et al.* 2000; Mostert *et al.* 2001). Based on their phylogeny, which supported minute morphological differences, three species could be distinguished. *D. perjuncta* was recollected from *Ulmus glabra* in Germany, and can be distinguished from *D. viticola* by the length-width ratio of the ascospores. In *D. perjuncta*, the length-width ratio of the ascospores is less than 3.6 whereas in *D. viticola*, it is greater than 3.7. Among isolates on *Vitis* in Europe, ascospores were widest in the middle of the apical cell, frequently having polar mucous caps, correlating with the type specimen of *D. viticola*. In contrast, however, South African and Australian material had ascospores that were widest at the median septum, and which were phylogenetically distinct. A new species, *D. australafricana*, is proposed for collections from the Southern Hemisphere.

Distinguishing *D. perjuncta* on *Ulmus* from *D. viticola* and *D. australafricana* has additional implications, as several other pathogenicity tests have been conducted on isolates generally fitting the morphology of *D. viticola* as *Phomopsis* taxon 1 (Rawnsley *et al*. 2001; Melanson *et al*. 2002), which led to certain conclusions about its pathogenicity and potential role as a pathogen in vineyards. It is possible, therefore, that several groups were working with morphologically similar, but different, *Phomopsis* species.

Acknowledgements

The authors acknowledge the South African National Research Foundation (NRF) and Winetech for financial support. Dr Alan J. L. Phillips (Universidade Nova de Lisboa, Portugal) is thanked for providing isolates and giving us access to unpublished material. Dr Walter Jaklitsch is warmly acknowledged for his generosity and knowledge in providing the specimen of *D. perjuncta*. Dr Lisa A. Castlebury (USDA Systematic Botany and Mycology Laboratory, Beltsville, MD, USA) is thanked for the sequence of *D. perjuncta*, and Dr Amy Y. Rossman for commenting on the script. Dr Belinda Rawnsley and R. W. A. Scheper (University of Adelaide, Australia) are thanked for providing isolates, while Frikkie Calitz and Mardé Booyse (ARC-Agrimetrics Institute, Stellenbosch, South Africa) are thanked for the statistical layout and analyses of the pathogenicity trials.

References

Farr DF, Castlebury LA, Pardo-Schultheiss RA (1999) *Phomopsis amygdali* causes peach shoot blight of cultivated peach trees in the southeastern United States. *Mycologia* **91**, 1008–1015.

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**, 182–192.

Kajitani Y, Kanematsu S (2000) *Diaporthe kyushuensis* sp. nov. the teleomorph of the causal fungus of grapevine swelling arm in Japan, and its anamorph *Phomopsis vitimegaspora. Mycoscience* **41**, 111–114.

Kuo K, Leu L-S (1998) *Phomopsis vitimegaspora:* a pathogenic *Phomopsis* from vines. *Mycotaxon* **66**, 497–499.

Lee SB, Taylor JW (1990) Isolation of DNA from fungal mycelia and single spores. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White) pp. 282–287. (Academic Press: San Diego)

Melanson DL, Rawnsley B, Scheper RWA (2002) Molecular detection of *Phomopsis* taxa 1 and 2 in grapevine canes and buds. *Australasian Plant Pathology* **31**, 67–73. doi: 10.1071/AP01075

Merrin SJ, Nair NG, Tarran J (1995) Variation in *Phomopsis* recorded on grapevine in Australia and its taxonomic and biological implications. *Australasian Plant Pathology* **24**, 44–56.

Moleleki N, Preisig O, Wingfield MJ, Crous PW, Wingfield BD (2002) PCR-RFLP and sequence data delineate several *Diaporthe* species associated with stone and pome fruit trees in South Africa. *European Journal of Plant Pathology* **108**, 909–912. doi: 10.1023/A:1021296801350

Mostert L, Crous PW, Kang J-C, Phillips AJL (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.

Muntañola-Cvetković M, Mihaljčević M, Petrov M (1981) On the identity of the causative agent of a serious *Phomopsis-Diaporthe* disease in sunflower plants. *Nova Hedwigia* **34**, 417–435.

Niessl G von (1876) *Hedwigia* **15**, 153.

Pearson RC, Goheen C (1994) Phomopsis cane and leaf spot. In 'Compendium of grape diseases'. (Eds WB Hewitt, RC Pearson) pp. 17–18. (APS Press: St Paul, MI)

Phillips AJL (1999) The relationship between *Diaporthe perjuncta* and *Phomopsis viticola* on grapevines. *Mycologia* **91**, 1001–1007.

Pine TS (1958) Etiology of the dead-arm. *Phytopathology* **48**, 192–197.

Pine TS (1959) Development of the grape dead-arm disease. *Phytopathology* **49**, 738–743.

Pscheidt JW, Pearson RC (1989) Effect of grapevine training systems and pruning practices on occurrence of Phomopsis cane and leaf spot. *Plant Disease* **73**, 825–828.

Rambaut A (2002) 'Sequence Alignment Editor. Version 2.0.' (Department of Zoology, University of Oxford: Oxford)

Rawnsley B, Scott E, Wicks T, Stummer B (2001) *Phomopsis* type 1: a peculiar puzzle to solve. *The Australian Grapegrower & Winemaker* **452**, 41–43.

Rawnsley B, Wicks T (2002) Fungicides for cane and leaf spot control. *The Australian Grapegrower & Winemaker* **465**, 20.

Rayner RW (1970) 'A mycological colour chart.' (Commonwealth Mycological Institute: Kew)

Rehner SA, Uecker FA (1994) Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis. Canadian Journal of Botany* **72**, 1666–1674.

Saccardo PA (1882) Omnium hucusque cognitorum. *Sylloge Fungorum* **1**, 1–766.

SAS (1999) 'SAS/STAT user's guide. Version 8.2, volume 2.' (SAS Institute Inc.: SAS Campus Drive, Cary, NC)

Scheper RWA, Crane DC, Whisson DL, Scott ES (2000) The *Diaporthe* teleomorph of *Phomopsis* Taxon 1 on grapevines. *Mycological Research* **104**, 226–231. doi: 10.1017/S095375629900115X

Swofford DL (2000) 'Phylogenetic analysis using parsimony. Version 4.0b10.' (Sinauer Associates: Sunderland, MA, USA)

Uddin W, Stevenson KL, Pardo-Schultheiss RA (1997) Pathogenicity of a species of *Phomopsis* causing shoot blight on peach in Georgia and evaluation of possible infection courts. *Plant Disease* **81**, 983–989.

Uddin W, Stevenson KL, Pardo-Schultheiss RA, Rehner SA (1998) Pathogenicity and molecular characterization of three *Phomopsis* isolates from peach, plum, and Asian pear. *Plant Disease* **82**, 732–737.

Uecker FA (1988) A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. *Mycologia Memoir* **13**, 1–231.

Wehmeyer LE (1933) 'The genus *Diaporthe* Nitsche and its segregates.' (University of Michigan Press: Ann Arbor)

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White): pp. 315–322. (Academic Press: San Diego)

Received 12 January 2004, accepted 7 July 2004