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Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization

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Abstract: In order to clarify the taxonomy of species of *Phomopsis* associated with grapevines, 61 isolates were obtained from 58 different vineyards in the grapevine growing areas of the Western Cape province of South Africa. Species delimitation was primarily based on alpha conidium and conidiophore morphology, cultural characteristics, pathogenicity to *Vitis vinifera,* and the ability to form the teleomorph in vitro. The identity of each taxon was confirmed by means of phylogenetic analyses of the nuclear ribosomal DNA internal transcribed spacers (5.8S, ITS1 and ITS2) and the 5′ end partial sequence of the mitochondrial small subunit (mtSSU). We also re-examined the four taxa that previously had been associated with grapevines in Australia. Three of the latter taxa, and a *Phomopsis* species commonly associated with shoot blight of peaches in the USA, *P. amygdali,* were identified among the South African isolates. *Phomopsis amygdali* was isolated once only and appeared to be of lesser importance in this disease complex. Furthermore, *Diaporthe perjuncta* and *Phomopsis* sp. 1 were also rarely encountered and proved to be nonpathogenic, indicating their nonfunctional role in Phomopsis cane and leaf spot disease. *Phomopsis viticola* was common and widely distributed throughout diseased vineyards. This taxon was associated with the typical cane and leaf spot disease symptoms and proved to be highly virulent. Morphologically collections designated in previous studies as taxon 2 corresponded best with *P. viticola,* which was also neotypified in this study. Examination of the Australian culture designated as taxon 4 revealed it to be a species of *Libertella,* thus excluding it from the *P. viticola* complex. An Italian isolate was found to represent a species of *Phomopsis* not previously known from grapevines, and this was subsequently designated as *Phomopsis* sp. 2. A key to taxa of *Phomopsis* from grapevine is also provided.

Key Words: *Diaporthe,* Phomopsis cane and leaf spot, *Phomopsis viticola,* systematics

INTRODUCTION

Phomopsis cane and leaf spot disease of grapevine (*Vitis vinifera* L.), caused by *Phomopsis viticola* (Sacc.) Sacc., can lead to losses of up to 50% of the normal yield (Pine 1958, Berrysmith 1962, Pscheidt and Pearson 1989). Losses occur from shoots breaking off near their base, stunting of vines, loss of vigor, reduced bunch set and fruit rot (Punithalingam 1979, Chairman et al 1982, Nicholas et al 1994, Pearson and Goheen 1994).

This disease occurs in most countries where grapevines are grown (Punithalingam 1979). In South Africa it was first noticed in 1935 (Du Plessis 1938) and was reported to cause serious problems (Synnott 1958). It since has occurred sporadically in the Helderberg, Firgrove, Somerset West, Rawsonville, and Slanghoek areas of the Western Cape province (Marais 1981).

Phomopsis viticola was originally described as *Phoma viticola* Sacc. from canes of *V. vinifera* collected in France (Saccardo 1880). Cooke (1885) described a later synonym from Britain as *Phoma viniferae* Cooke. *Phomopsis cordifolia* Brunaud, described from grapevines in Italy, resembled *P. viticola* in having similar alpha conidium dimensions and therefore was considered synonymous (Uecker and Johnson 1991). Saccardo (1915) established a new combination for *Phoma viticola* as *Phomopsis viticola* (Sacc.) Sacc., and for the first time cited a specimen. This specimen was not the French material on which the name *Phoma viticola* was originally based, but was from *Vitis aestivalis* Michx. in Albany, New York, USA, collected by H.D. House (No. 149) (Saccardo 1915). In addition to these names, several other *Phomopsis* species have been described from grapevines (TABLE I). In these descriptions the dimensions of alpha conidia were longer and narrower than those described by Saccardo (1915).

Reddick (1909) collected canes of *Vitis labrusca* L.

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in the USA and found a fungus similar to *P. viticola,* which he named *Fusicoccum viticolum* Reddick. Even though symptoms caused by *P. viticola* are similar to those caused by *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & de Not. (anamorph: *Fusicoccum aesculi* Corda) (Phillips 1998), it is evident from the illustrations that *F. viticolum* was indeed a species of *Phomopsis,* which consequently led to the new combination and later homonym, *Phomopsis viticola* (Reddick) Goid. (Goidànich 1937). Goidànich never examined Reddick's material, which we also have been unable to locate (BPI, NY, CUP, K, IMI, B, PAD). *Phomopsis viticola* (Reddick) Goid. was cited as synonym of *P. viticola* (Sacc.) Sacc. by Punithalingam (1979). To further confuse the matter, the culture lodged by Goidànich in 1938 (CBS 252.38) was found to be representative of *Coniella granati* (Sacc.) Petrak & Syd. (Merrin et al 1995).

A comparative study by Pine (1958) of *Phomopsis* isolates from cane and leaf spot symptoms from Canada, South Africa, Italy and New York showed no difference in their cultural or morphological characteristics. In his subsequent treatment of the pathogen, Punithalingam (1979) established a revised concept of *P. viticola* and also placed several of the former names in synonymy. However, a recent study in Australia distinguished four taxa from grapevines based on alpha conidium morphology, pycnidium formation, color and structure, cirrhus color and mycelial growth rate (Merrin et al 1995). These groupings were also supported by host inoculation, conidium germination and growth data, as well as pectic enzyme profiles. On the basis of conidium dimensions, Merrin et al (1995) suggested that their taxon 1 correlated with Saccardo's description of *P. viticola,* and that taxon 2 could resemble *F. viticolum* (Merrin et al 1995). In a re-examination of these species, however, Phillips (1999) concluded that the name *Diaporthe perjuncta* Niessl was available for taxon 1, while taxon 2 resembled *P. viticola* (Sacc.) Sacc. In addition to the four taxa already associated with the *P. viticola* complex of grapevines, two further species were recently described from this host. *Phomopsis longiparaphysata* Uecker & Kuo (1992) was distinguished primarily on its prominent paraphyses, while *P. vitimegaspora* Kuo & Leu (1998) was characterized by large alpha conidia and different disease symptoms.

The research reported in the present study was aimed at clarifying the taxonomy of the various species of *Phomopsis* associated with grapevines, and circumscribing *Phomopsis viticola,* the causal organism of Phomopsis cane and leaf spot disease.

MATERIALS AND METHODS

Isolates and morphology.—Symptomatic shoots and leaves from wine and table grape cultivars were collected from 58 vineyards in the Western Cape and Orange River production areas of South Africa. Most of the collections were made from autumn (Apr) to spring (Sep) of 1997. Two isolates collected during an earlier study of endophytes (Mostert et al 2000) were also included. Additional isolates were those identified as *P. viticola* from grapevines in Australia, Italy, Portugal, Turkey and the USA, together with an ex-type culture of *P. vitimegaspora* from grapevines in Taiwan (Kuo and Leu 1998). Isolates of *Phomopsis* spp. from diverse hosts such as rose (*Rosa* sp.), plum (*Prunus* sp.), protea (*Protea* sp.) and pear (*Pyrus* sp.) were also included (TABLE II).

Vine shoots and leaves were surface sterilized in 70% ethanol for 20 s, 1% sodium hypochlorite for 2 min, and 70% ethanol for 20 s. Dissected shoot and leaf pieces were placed in Petri dishes lined with sterilized, moist tissue paper, and incubated at 25 C on the laboratory bench for 1–2 wk to encourage pycnidial formation. Isolations were also made from lens-shaped lesions that appeared on shoots, as well as the dark brown leaf spots with yellow halos. Pycnidia and tissue pieces were plated directly onto water agar [Biolab Diagnostics (Pty) Ltd, Midrand, South Africa], amended with 1 mL/L streptomycin (WAS). Isolates were incubated at 22 C under near-ultraviolet light. Cultures sporulated after approx 14 d of incubation, and single conidium isolates were obtained by making dilution plates of exuding spore masses on WAS plates. Cultures from four single germinating conidia were retained from each collection. Three stock cultures (STE-U) of each were maintained at 5 C in McCartney bottles containing 2% malt extract agar (MEA, Biolab) sterile water, or sterile paraffin oil.

All 61 South African and other representative isolates were compared morphologically by growing them on 4-cm-long pieces of double autoclaved vine shoots on water agar (WAV), and potato dextrose agar (PDA, Biolab) in divided plates. The plates were incubated at 25 C under near-ultraviolet light. Colors of the colony surface and reverse were rated according to color charts of Rayner (1970). Colony texture, margin, elevation and zonal growth were recorded for isolates growing on PDA, while the pycnidial density and color of the exuding conidial cirrhi were recorded on WAV (Wechtl 1990). Plates were monitored daily to record the time of pycnidial formation on WAV. Pycnidia were characterized according to their distribution, shape, color, presence or absence of aerial hyphae, position in agar medium, color of the conidial cirrhus, number of ostioles, pycnidial dimensions and wall anatomy (Wechtl 1990). Sporulating pycnidia produced on WAV were mounted on slides in lactophenol and examined at ×1000. Several characters were noted, including the presence or absence and general morphology of alpha, beta and gamma conidia; conidiophore aggregation, shape, size, septation and branching; conidiogenous cell morphology, the presence of collarettes, periclinal thickening; and paraphyses. Sporulating perithecia were mounted in water and lactophenol, and the morphology of asci, paraphyses and ascospores noted. Vertical sections through fruiting bodies were cut with a Leica CM1100 freezing microtome. Sections (10 μm) were mounted in lactic acid for examination. Thirty measurements were taken of all morphological structures and averages were deter-

TABLE I. Chronological description of *Phomopsis* and related taxa from grapevines

Species	Alpha conidium morphology			Reference
	Length × width (μm)	Shape	Guttulation	
Phoma viticola Sacc.	7 × 4 μm	Ellipsoid	Eguttulate	Saccardo (1880)
Phoma viniferae Cooke	7 × 4 μm	—	Eguttulate	Cooke (1885)
Fusicoccum viticolum Reddick	6–11 × 2–3 μm	Fusoid	Multi- to biguttulate	Reddick (1909)
Phomopsis cordifolia Brunaud	7–9 × 2.5–3 μm			Brunaud (1912)
Phomopsis viticola (Sacc.) Sacc.	7 × 4 μm			Saccardo (1915)
Phomopsis ampelopsidis Petrak	6–11 × 2–3 μm	Bacillar to fusoid	Bi- to triguttulate	Petrak (1916)
Phomopsis viticola (Sacc.) Grove	7–10 × 2–2.5 μm	Ellipsoid-fusoid	—	Grove (1917)
Phomopsis viticola (Sacc.) Sacc. var. *ampelopsidis* Grove	8–9 × 2 μm	Ellipsoid-fusoid	Biguttulate	Grove (1919)
Phomopsis ampelina (Berk. & Curt.) Grove	8–10 × 2–2.5 μm	Ellipsoid-fusiod	Biguttulate	Grove (1919)
Phomopsis viticola (Sacc.) Sacc.	8–9 × 2 μm	Ellipsoid-fusoid	Biguttulate	Saccardo (1931)
Phomopsis longiparaphysata Uecker & Kuo	(5–)6–7(–11) × 2–2.5 (–3.5) μm	Fusoid-ellipsoid	Biguttulate to multiguttulate	Uecker and Kuo (1992)
Diaporthe perjuncta	(4–)5–7(–9) × (1–)1.5–2(–3) μm	Ellipsoid to oblong	Biguttulate	Merrin et al (1995)
Phomopsis viticola	(7–)8–12(–14) × (1.5–)2–3(–4) μm	Fusoid-ellipsoid	Multiguttulate	Merrin et al (1995)
Phomopsis sp. 1 (Taxon 3)	(5.5–)6–9(–9) × (1.5–)1.5–2(–2.5) μm			Merrin et al (1995)
Phomopsis vitimegaspora Kuo & Leu	(10–)13-8(–22) × (3–)4–5(–6) μm	Fusoid-ellipsoid	Multiguttulate	Kuo and Leu (1998)

mined. The minimum and maximum measurements are in parentheses.

Sexual compatibility.—Isolates were grouped based on similar morphological and cultural characteristics. A subset of 26 isolates was chosen representing the various groups, and plated onto WAV. Since scant information was available on the mating behavior of the *Phomopsis* spp. on grapevines, the technique described by Schoch et al (1999) was used to pair isolates in all possible combinations in an attempt to induce teleomorph formation. Plates were incubated in the dark for 3 wk at 25 C, after which they were exposed to continuous near-ultraviolet light at 10 C. Plates were examined weekly over a 5-mo period for the presence of perithecia. When perithecia were observed, squash mounts in water, as well as lactophenol were made to observe the morphology of asci, ascospores and paraphyses.

Cardinal temperatures for growth.—Recent studies have shown that specific temperature requirements for growth can be used to allocate isolates to the various taxa recognized in the *P. viticola* complex (Merrin et al 1995). The subset of isolates used for the mating studies were plated on MEA in 90-mm diam plastic Petri dishes, and incubated for 7 d in the dark at 8 temperatures ranging from 5–40 C in 5 C intervals. Three plates were used for each isolate at each temperature and the experiment was repeated once. Linear mycelial growth was determined from two perpendicular measurements for each plate, and then by calculating the mean of the six measurements for each isolate at each temperature.

Stem inoculations.—Host response was determined in wound-inoculated green shoots. Seventeen South African grapevine *Phomopsis* isolates were chosen: 14 of *P. viticola,* and one each of *Diaporthe perjuncta, Phomopsis* sp. 1, and *P. amygdali.* Three *Phomopsis* isolates obtained from other hosts such as protea, plum, and pear were also tested for their ability to infect grapevine. Green shoots (1–2 cm diam, 30 cm long) were pruned from healthy Chenin blanc grapevines and placed in 500-mL flasks (1 shoot/flask), each containing 300 ml of a nutrient solution (Hewitt 1952). Shoots were swabbed with 70% ethanol and wounded (at 1-cm diam) by removing the cortex with a 3-mm-diam cork borer. Colonized mycelial plugs from 2-wk-old cultures grown on MEA were inserted into the wounds and the wound sealed with Parafilm. Uncolonized MEA plugs were used for control inoculations. Each isolate, including the control, was replicated three times on separate shoots, with one inoculation per shoot. Flasks with shoots were maintained in the laboratory at 22 C with a 12-h fluorescent white light/dark regime. Flasks were supplemented with fresh nutrient solution every second day. Lesion lengths were measured 7 d after inoculation and reisolations were made from the lesion margins to confirm Koch's postulates. Lesion lengths were calculated by subtracting the size of the wound formed on the control shoot from the lesion formed on the inoculated shoots. Analysis of variance was used to establish the significance of differences between lesion lengths caused by the different taxa.

Additional pathogenicity tests were conducted to test the cross pathogenicity of *P. viticola* and the grapevine *P. amyg-*

TABLE II. *Diaporthe* and *Phomopsis* species included in the sequence analysis

Accession no.	Host	Origin	Taxon	Area sequenced
STE-U 2655	*Vitis vinifera*	South Africa	*D. perjuncta*	I[a], M[b]
STE-U 2676	*Vitis vinifera*	Australia	*D. perjuncta*	I, M
STE-U 2677	*Vitis vinifera*	Portugal	*D. perjuncta*	I, M
STE-U 2638	*Vitis vinifera*	South Africa	*P. viticola*	I
STE-U 2641	*Vitis vinifera*	South Africa	*P. viticola*	I
STE-U 2642	*Vitis vinifera*	South Africa	*P. viticola*	I, M
STE-U 2646	*Vitis vinifera*	South Africa	*P. viticola*	I
STE-U 2648	*Vitis vinifera*	South Africa	*P. viticola*	I
STE-U 2660	*Vitis vinifera*	France	*P. viticola*	I
STE-U 2662	*Vitis vinifera*	Australia	*P. viticola*	I, M
STE-U 2666	*Vitis vinifera*	Portugal	*P. viticola*	I, M
STE-U 2669	*Vitis vinifera*	Portugal	*P. viticola*	I
STE-U 2671	*Vitis vinifera*	Italy	*P. viticola*	I
STE-U 2672	*Vitis vinifera*	Turkey	*P. viticola*	I
STE-U 2673	*Vitis vinifera*	America	*P. viticola*	I
STE-U 2679	*Vitis vinifera*	America	*P. viticola*	I
STE-U 2654	*Vitis vinifera*	South Africa	*Phomopsis* sp. 1	I, M
STE-U 2656	*Pyrus* sp.	South Africa	*Phomopsis* sp. 1	I, M
STE-U 2659	*Protea* sp.	South Africa	*Phomopsis* sp. 1	I, M
STE-U 2664	*Vitis vinifera*	Australia	*Phomopsis* sp. 1	I, M
STE-U 2661	*Vitis vinifera*	Australia	*Phomopsis* sp. 1	I, M
STE-U 2668	*Vitis vinifera*	Portugal	*Phomopsis* sp. 1	I, M
STE-U 2632	*Vitis vinifera*	South Africa	*P. amygdali*	I, M
STE-U 2675	*Vitis vinifera*	Taiwan	*P. vitimegaspora*	I
STE-U 2657	*Prunus* sp.	South Africa	*D. ambigua*	I
STE-U 3390	*Prunus* sp.	South Africa	*D. ambigua*	I
STE-U 2674	*Vitis vinifera*	Italy	*Phomopsis* sp. 2	I
STE-U 2680	*Rosa* sp.	South Africa	*Phomopsis* sp.	I, M

[a] I = 5.8S and flanking ITS1 and ITS2 rDNA.
[b] M = Mitochondrial small subunit rDNA.

dali isolate to peach and grapevines. Colonized mycelial plugs of one isolate of each of the two species were used to inoculate 4-mo-old pot-grown *Prunus persica* L. cv. Kakemas and *Vitis vinifera* cv. Riesling. Each isolate was inoculated as previously described on three plants of each host. Uncolonized MEA plugs were used for control inoculations. Disease severity was evaluated 12 d after inoculation by measuring lesion lengths, and re-isolations made as described above.

Sequence comparisons.—A total of 29 isolates representing the different taxa were selected for sequencing (TABLE II). Sequences of additional *Phomopsis* species from plum, pear and peach (Uddin and Stevenson 1998) were retrieved from GenBank (U94898, U91617 and U86406, respectively) and included in the analysis. DNA was extracted as described by Lee and Taylor (1990). The ITS1 and ITS2 including the 5.8S ribosomal RNA gene were amplified using primers ITS1 and ITS4. The small subunit of the mitochondrial ribosomal RNA genes (mtSSU) was amplified using primers MS1 and MS2. Genomic locations and primer sequences are presented in White et al (1990). The PCR products were purified using Wizard PCR Preps (Promega Corporation, Madison, Wisconsin). Both strands of the PCR product were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). A Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase (Perkin-Elmer) was used for sequencing reactions. Fragments of sequencing reactions were finally purified using Centri-Sep Spin columns (Princeton Separations, Adelphia, New Jersey). Sequences were aligned using Sequence Navigator, from which a consensus sequence was created. Sequences obtained from this study and GenBank retrievals were aligned with Clustal W (Thompson et al 1994). The final alignment was optimized manually. Alignment gaps were coded as missing data in the analysis. *Cryphonectria parasitica* (Murrill) Barr (GenBank AF 172658 and AF 029891) was used as the outgroup both in the analysis of ITS data, and the data composed of ITS and mtSSU. Phylogenetic analyses were performed with PAUP* version 4.0b2a (Swofford 1999). Maximum parsimony analysis was performed using heuristic search option with 1000 random sequence input orders for exact solution. The unconstrained topologies of the equally parsimonious trees were compared using the Kishino-Hasegawa test. The best topology was selected as the most parsimonious tree topology and evaluated with 1000 bootstrap replications to

test the clade stability of the tree. The decay indices were also calculated using AutoDecay (Eriksson 1998) to further test the robustness of the branches of the tree. Other measures including tree length, consistency index, retention index, rescaled consistency index and homoplasy index (CI, RI, RC and HI) were also calculated. The ITS data included 32 sequences. Because of ambiguities in alignment (data not shown, but available from the corresponding author), short sequence segments in ITS1 (49–81) and ITS2 (362–379) were excluded from the analysis. A partition homogeneity test in PAUP* (Swofford 1999) was conducted for the ITS and mtSSU sequences of 14 taxa to examine the possibility of a joint analysis of the two data sets. Sequence data has been lodged in GenBank (AF230743–AF230773), and the alignment in TreeBase (S515, M747, 748).

RESULTS

Isolates and morphology.—Sixty-one isolates of *Phomopsis* were obtained from grapevines in the area from Lutzville to Swellendam in the Western Cape Province. No isolates were obtained in the hot, dry climate of the Oudtshoorn and Orange River vineyard growing areas. *Phomopsis viticola* was isolated mostly from buds and nodes, indicating that these probably are important sites in which the fungus survives during winter.

Analyses of the morphological characteristics of all the isolates studied indicated that the most commonly isolated species was *P. viticola.* Only one isolate of *Diaporthe perjuncta, Phomopsis* sp. 1, and *P. amygdali* were obtained, and none of the *Libertella* sp. or *Phomopsis* sp. 2. The cultural and morphological characteristics that could be used to distinguish the different taxa included colony growth patterns and alpha conidial dimensions and shape. Secondary characters included pycnidium shape and distribution, alpha conidium guttulation, beta conidium formation, conidiophore branching and septation. The presence of collarettes on conidiogenous cells was not useful for distinguishing taxa.

Sexual compatibility.—Of the 26 grapevine isolates included in the mating study, only three isolates (STE-U 2655, 2676 and 2677) formed the teleomorph after 51 d. Only the Australian isolate (STE-U 2676) was of ascospore origin. The teleomorph was self-fertile, confirming this fungus to be homothallic (Phillips 1999). None of the other pairings produced any perithecia.

Stem inoculations.—Phomopsis viticola, P. amygdali and an isolate from pear formed similar, distinct dark brown lesions (16–20 mm in length). *Diaporthe perjuncta* and the grapevine isolate of *Phomopsis* sp. 1 formed a dark brown rind around the wound (less than 2 mm in length). The protea and plum isolates of *Phomopsis* sp. 1 formed no lesions on the grapevine shoots.

Peach trees inoculated with *P. viticola* and the control formed no lesions. However, the *P. amygdali* isolate produced prominent brown lesions on the inoculated peach trees, as well as on grapevines. In each case where lesions were formed, the fungus was re-isolated to confirm Koch's postulates. Control inoculations remained healthy.

Sequence comparisons.—Maximum parsimony analysis of the ITS data gave 39 equally most parsimonious trees (MPT) with 54 parsimony informative characters in the alignment. In accordance with the result of the Kishino-Hasegawa likelihood test (data not shown), the best tree topology of the 39 MPT was selected as the phylogenetic tree topology and evaluated with 1000 bootstrap replications and decay indices for clade stability. The result of the partition homogeneity test ($P = 0.32$, where $P \geq 0.05$ was significantly incongruent) indicated that the two data sets could be combined. Maximum parsimony analysis of the combined data sets resulted in 24 MPT with 49 parsimony informative characters in the alignment. The best tree topology of the 24 MPT which was indicated by the Kishino-Hasegawa likelihood test (data not shown), was selected as the phylogenetic tree topology and evaluated with 1000 bootstrap replications and decay indices for the clade stability. The final phylogenetic trees (FIGS. 1, 2) were compatible with accepted morphological delimitation of taxa in the *P. viticola* complex. Isolates of *P. viticola* clustered together with a strong bootstrap. The clade representing *Phomopsis* sp. 1 was not well supported by bootstrap and contained isolates from grapevine, pear and protea. The clade consisting of ascospore (STE-U 2676) and conidial (STE-U 2677 and STE-U 2655) isolates of *Diaporthe perjuncta* was strongly supported by bootstrap. The South African grapevine isolate (STE-U 2632) showed a 100% sequence similarity with the peach shoot blight pathogen, *Phomopsis amygdali.* South African plum and pear isolates grouped distant from isolates from the same hosts (GenBank U94898 and U91617), suggesting that they represent another species. The *Libertella* sp. (cited as taxon 4 by Merrin et al 1995) grouped distant from the genus *Phomopsis,* and was therefore excluded from the analyses. An Italian grapevine isolate (STE-U 2674) grouped separately, and was subsequently designated as *Phomopsis* sp. 2.

Diaporthe perjuncta Niessl, Hedwigia 17: 44. 1878.
FIGS. 3–12

Anamorph. Phomopsis sp.

Perithecia globose, solitary, scattered to aggregated,

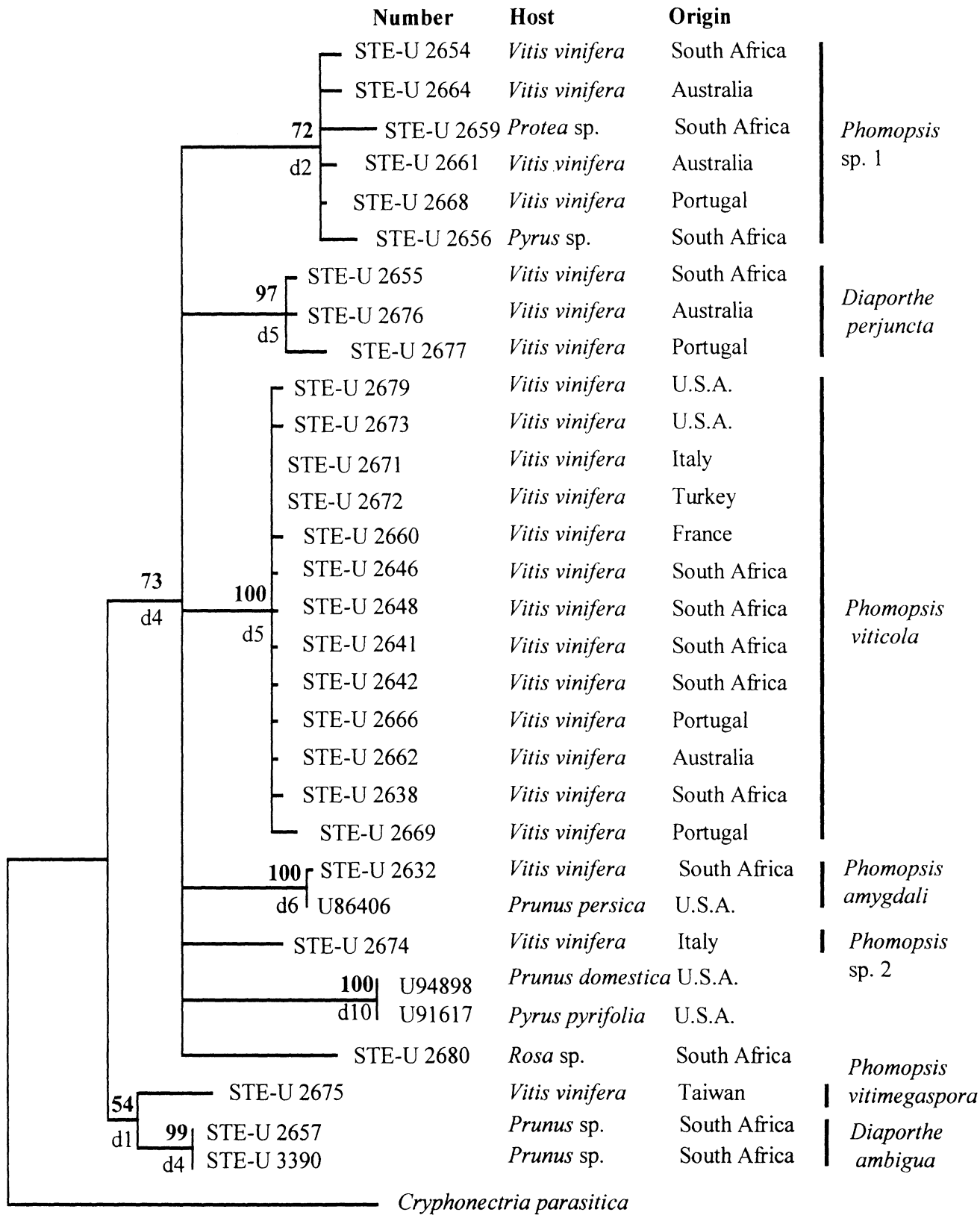


FIG. 1. One of 39 MPTs resulting from maximum parsimony analysis (heuristic search option) of aligned sequences of the 5.8S rRNA gene and flanking ITS1 and ITS2 regions (length = 356 steps, CI = 0.629, RI = 0.749, RC = 0.471 and HI = 0.371). Bootstrap values above 52% are shown as well as decay indices. *Cryphonectria parasitica* was used as the outgroup.

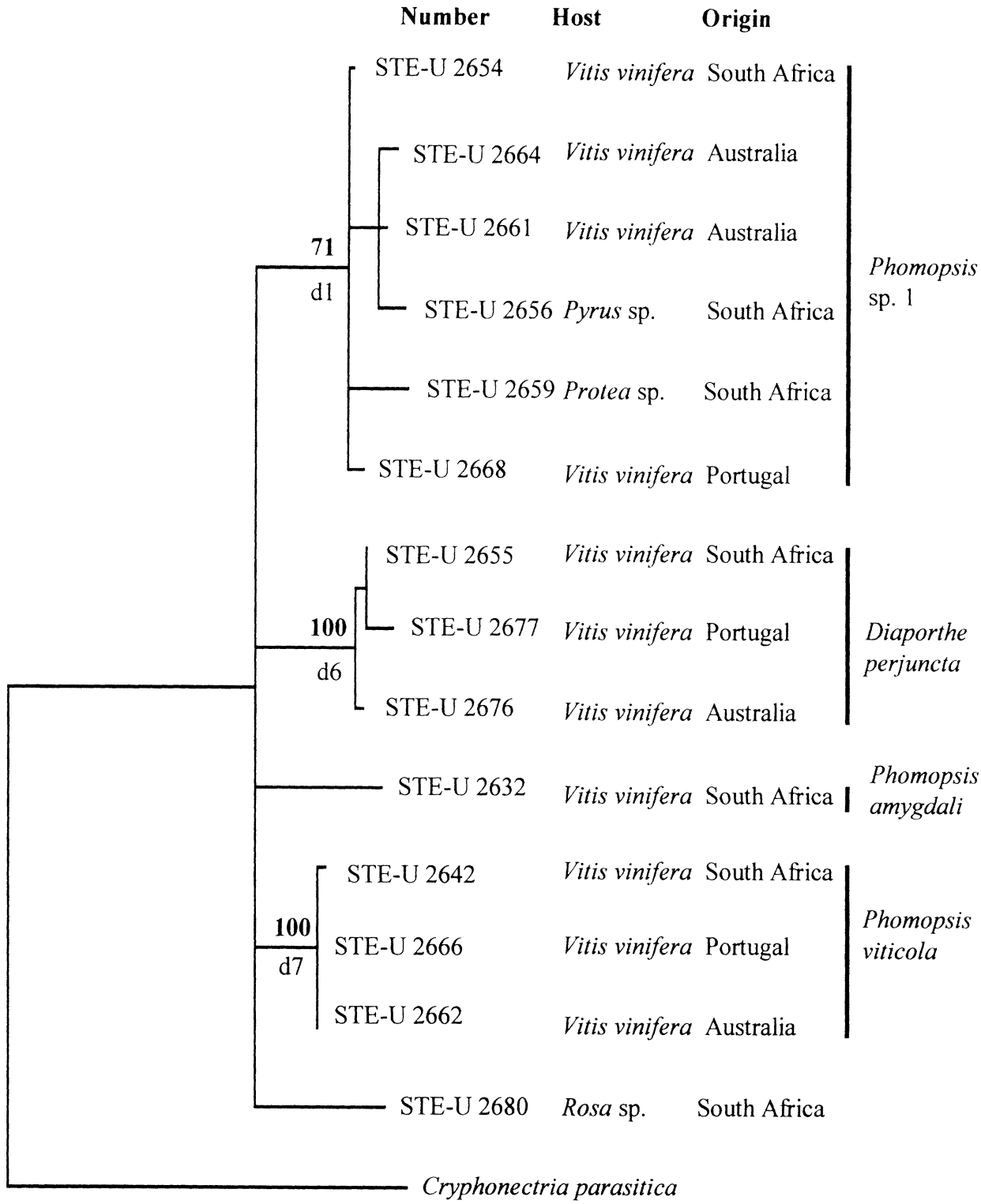
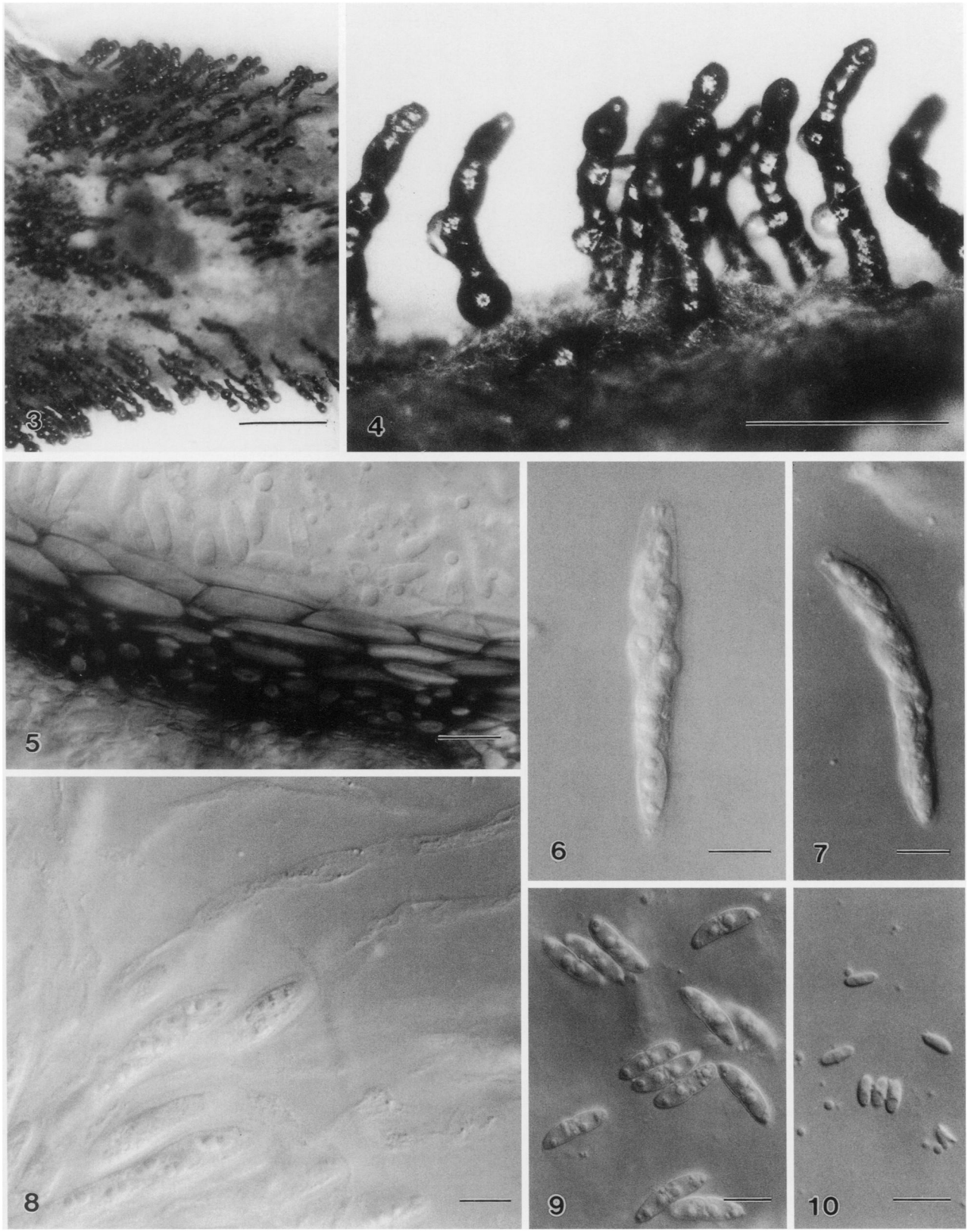
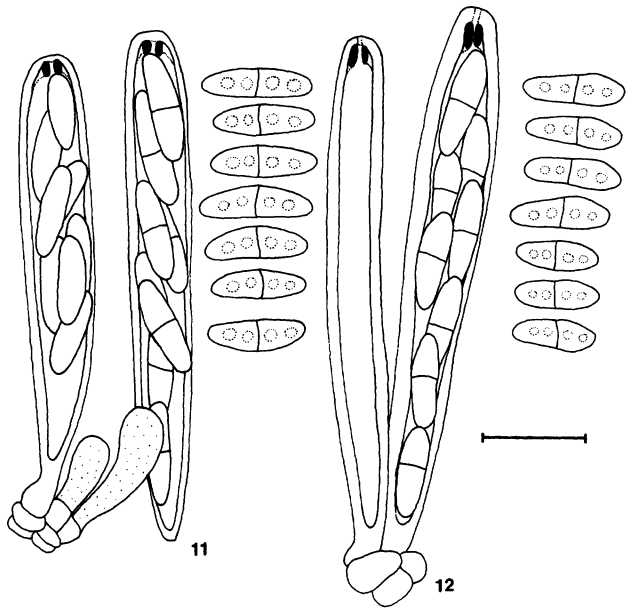


FIG. 2. One of 24 MPTs resulting from maximum parsimony analysis (heuristic search option) of aligned sequences of the 5.8S rRNA gene and flanking ITS1 and ITS2, and the mtSSU regions (length = 347 steps, CI = 0.790, RI = 0.723, RC = 0.571 and HI = 0.210). Bootstrap values above 52% are shown as well as decay indices. *Cryphonectria parasitica* was used as the outgroup.



FIGS. 3–10. *Diaporthe perjuncta* with *Phomopsis* anamorph (STE-U 2655). 3, 4. Perithecia on grapevine canes. Bars = 1000 μm. 5. Longitudinal section through perithecial wall. 6, 7. Asci. 8. Asci with paraphyses. 9. Ascospores. 10. Alpha conidia. Bars = 10 μm.



FIGS. 11, 12. Asci and ascospores of *Diaporthe perjuncta* from a South African (STE-U 2655) and Portuguese (STE-U 2677) collection, respectively. Bar = 10 μm.

subepidermal, 210–500 μm wide, 250–350 μm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, 3–4 cells thick, 15–35 μm wide; inner region pale brown, 3–4 cells thick, 12–32 μm wide. Perithecial necks elongate, without external hyphae, red-brown at the apex, becoming dark brown near the base, centrally constricted, 870–1500 × 70–100 μm ($\bar{x}$ = 1128 × 83 μm), of *textura prismatica,* with outer region dark brown, 10–20 μm wide, inner region hyaline, 5–10 μm wide. Ostiole red-brown, widening following exudation of spores, 90–150 μm wide. *Asci* unitunicate, cylindrical-clavate, with refractive apical ring, 8-spored, 55–61 × 5–8.5 μm ($\bar{x}$ = 57 × 6.5 μm). *Paraphyses* septate, unbranched, tapering towards apex with a rounded tip, extending above the asci, 48–109 μm long, 4–7 μm wide at bottom, 2–3 μm at the apex. *Ascospores* biseriate, hyaline, smooth, medially 1-septate, slightly constricted at the septum, 1–2 guttules per cell, fusoid, widest at the septum, tapering towards both ends, straight or slightly curved, (8–)11.5–13(–15) × (2.5–)3–3.5(–4.0) μm ($\bar{x}$ = 12 × 3.5 μm); hyaline appendages terminal, punctiform, 1–2 μm long. *Pycnidia* formed after 17 d at 25 C in the dark. *Alpha conidial mass* globose, white. *Beta conidial mass* in cirrhi, yellow. *Alpha and beta conidiophores* identical, septate, branched, 17–34 × 1.5–2.5 μm ($\bar{x}$ = 18 × 2 μm), tapering towards the apex with branches arising from immediately below the septa. *Alpha and beta conidiogenous cells* identical, filiform, tapering towards the apex, collarette and periclinal thickening present, 10–14 × 1.5–2.5 μm ($\bar{x}$ = 14 × 2 μm). *Alpha conidia* biguttulate, rarely eguttulate, fusoid or oblong to ellipsoid with obtuse ends, 5–7 × 2–2.5 μm ($\bar{x}$ = 6.5 × 2.5). *Beta conidia* straight or curved, 12–20 × 0.5–1 μm ($\bar{x}$ = 18 × 0.5 μm) (description based on STE-U 2655 and 2677).

Cultures. Colonies woolly, predominantly white, with grayish sepia tufts (15‴i) and rosy buff (13″d) undertone. Reverse, buff (19″d) to rosy buff (15″d).

Cardinal temperatures. Colonies attained maximum growth of 67–93 mm diam at 20 C after 7 d in the dark. No growth occurred below 10 C or above 35 C.

Hosts. Vitis vinifera, but presumed wide host range (Phillips 1999).

Distribution. Australia, Portugal and South Africa.

Cultures examined. AUSTRALIA. SOUTH AUSTRALIA: Coonawarra, Mildara, on Shiraz grapevine, Jul 1995, *R.W.A. Schepers* (RS85 = STE-U 2676). PORTUGAL. Oeiras, on Galego Dourado grapevine, Jan 1998, *A.J.L. Phillips* (P/CA/15/20/1 = STE-U 2677). SOUTH AFRICA. WESTERN CAPE: Stellenbosch, on Riesling grapevine, Nov 1997, *L. Mostert,* (STE-U 2655) (specimen PREM 56458).

Notes. The morphology of the *Diaporthe* isolate from South Africa (STE-U 2655) corresponded well with that of the Australian isolate (STE-U 2676). Differences in the morphology of the perithecial necks and ascopores were, however, observed between these isolates and one from Portugal (STE-U 2677). STE-U 2655 and STE-U 2676 had single, solitary perithecia, red-brown at the tip with sparse external hyphae covering perithecial necks, and fusoid ascospores. In contrast, STE-U 2677 had aggregated perithecia, yellow-brown at the apex, with extensive hyphae covering perithecial necks, and fusoid-ellipsoidal ascospores with obtuse ends. In spite of these morphological differences, these isolates could not be distinguished based on their DNA sequence data. Phillips (1999) also concluded that even though some isolates lacked mucous ascospore appendages, they still represented the same taxon.

Recently, the teleomorph of the Australian *Phomopsis* taxon 1 (STE-U 2676) (Merrin et al 1995) was collected in Australia and attributed to *Diaporthe viticola* (Scheper et al 2000). A *Diaporthe* sp. was also recently found on grapevines in Portugal (Phillips 1999). Following the concepts of Wehmeyer (1933), Phillips (1999) determined that based on the limited entostroma, ascospores that were wider than 2.5 μm, and perithecia with single, erumpent ostioles, the name *Diaporthe perjuncta* Niessl would be more suitable than *D. viticola* for isolates of taxon 1 collected in Australia and Portugal (Phillips 1999). We concur with his determination and use the name *D. perjuncta* for taxon 1.

Several *Diaporthe* Nitscke teleomorphs have been associated with grapevines. The first *Diaporthe* species described from this host was *D. viticola* Nitscke, re-

ported from Germany and Maine (Nitschke 1870) and *D. silvestris* Sacc. & Berl. on *V. vinifera* in Italy (Saccardo and Berlese 1885). Although known teleomorphs of *Phomopsis* are species of *Diaporthe* (Wehmeyer 1933), Shear (1911) described *Cryptosporella viticola* Shear as the teleomorph of *Fusicoccum viticolum* Reddick. This material could not be located, however, and the identity of this teleomorph thus remains uncertain.

Phomopsis viticola (Sacc.) Sacc., Ann. Mycol. 13: 118. 1915. FIGS. 13–29

≡ *Phoma viticola* Sacc., Michelia 2: 92. 1880.

= *Fusicoccum viticolum* Reddick, Cornell Univ. Agr. Exp. Sta. Bull. 263: 331–332. 1909.

≡ *Phomopsis viticola* (Reddick) Goid., Atti R. Accad. Naz. Lincei 26: 107–112. 1937.

= *Phomopsis viticola* Sacc. var. *ampelopsidis* Grove, Bull. Misc. Inf. (Kew) 4: 183–184 (1919).

= Phomopsis ampelina (Berk. & Curt.) Grove, Bull. Misc. Inf. (Kew) 4: 184. 1919.

Teleomorph. Unknown *Diaporthe* sp.

Pycnidia produced within 4–10 d on WAV. *Conidial mass* globose or in cirrhi, white, pale-yellow to yellow, but predominantly pale yellow; pycnidia eustromatic, subepidermal, brown to black, scattered or aggregated, globose, flask-shaped to conical, outer surface smooth, convoluted to unilocular, ostiolate, up to 430 μm wide, 190–300 μm tall, including short necks which rarely are present. Pycnidial wall consisting out of two regions of *textura angularis*; the outer region brown, 2–3 cells thick, 5–7 μm wide, inner region brown, 3–4 cells thick, 7–15 μm wide, with the outside cells compressed. *Alpha conidiophores* cylindrical, some filiform, rarely septate and branched, 5–35 × 1–3 μm ($\bar{x}$ = 25 × 2 μm). *Beta conidiophores* ampulliform to subcylindrical, rarely branched, 10–34 × 1–2 μm ($\bar{x}$ = 26 × 1.5 μm). *Alpha conidiogenous cells* subcylindrical, tapering towards the apex, collarettes and periclinal thickening present, 3–19 × 1–2.0 μm ($\bar{x}$ = 10 × 1.5 μm). *Beta conidiogenous cells* subcylindrical, tapering towards the apex, collarette and periclinal thickening present, 7–14 × 1–2 μm ($\bar{x}$ = 11–1.5 μm). *Alpha conidia* more abundant than beta conidia, fusoid-ellipsoidal, apex acutely rounded, base obtuse to subtruncate, multiguttulate with guttules grouped at the polar ends, rarely biguttulate, (7–)9.5–10.5(–13) × (1.5–)2–3(–3.5) μm ($\bar{x}$ = 10 × 2.5 μm). *Beta conidia* less abundant than alpha conidia, straight, curved or hamate, 20–25 × 0.5–1 μm ($\bar{x}$ = 23–1 μm). *Gamma conidia* rarely found, fusoid to subcylindrical, apex acutely rounded, base subtruncate, multiguttulate, 12–18 × 1.5–2 μm ($\bar{x}$ = 15 × 2 μm) (description based on STE-U 2637).

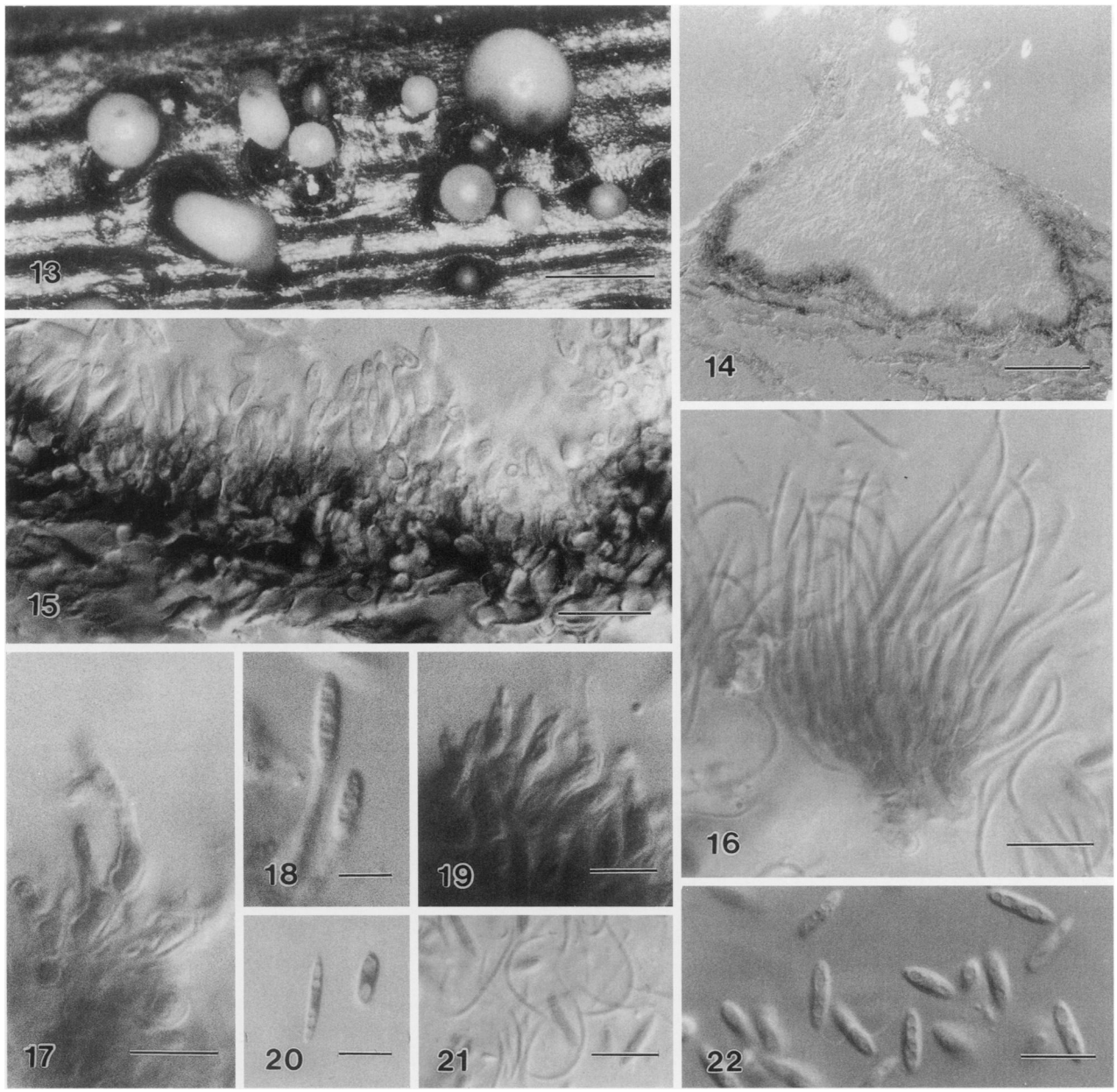
Colonies. Colony color was predominantly buff (19″d) to honey (19″b) with smoke gray (21‴f) and citrine green (23″) patches. Reverse, buff (19″d) to grayish sepia (15‴i) with some darker iron gray patches (23⁗k). Colonies slightly raised, with a felty texture and prominent growth rings.

Cardinal temperatures. Colonies obtained maximum growth of 16–52 mm diam at 25 C after 7 d in the dark. No growth occurred below 10 C or above 35 C. Four isolates reached their maximum growth at 20 C (STE-U 2669, STE-U 2638, STE-U 2642, STE-U 2649) and one at 30 C (STE-U 2666).

Hosts. Vitis aestivalis Michx., *V. vinifera.*

Distribution. Widely distributed with host.

Cultures examined. AUSTRALIA. TASMANIA: Cloverhill Vineyard, on Chardonnay vines, Jul 1996, *R.W.A. Schepers* (RS91 = STE-U 2662). Marion's Vineyard, on Chardonnay vines, Jul 1996, *R.W.A. Schepers* (RS 110 = STE-U 2663). SOUTH AUSTRALIA: Padthaway, on *Vitis vinifera, N.G. Nair* (VRU 0083 = STE-U 2665). ITALY. On *V. vinifera, A. Zazzerini* (CBS 267.8 = STE-U 2671). FRANCE. BORDEAUX: Naujan-et-Postiac, on Cabernet Sauvignon grapevine, May 1998, *P. Larignon* (PV F98–1 = STE-U 2660) (specimen PREM 56460). PORTUGAL. Oeiras, on Galego Dourado grapevine, Dec 1997, *A.J.L. Phillips* (P/CA/20/28/1/1 = STE-U 2666); Jan 1998, *A.J.L. Phillips* (P/CA/51/34/1 = STE-U 2667); Santo Tirso, on Loureiro grapevine, Feb 1998, *A.J.L. Phillips* (P/BU/2/2 = STE-U 2669, P/BU/5/4 = STE-U 2670). SOUTH AFRICA. WESTERN CAPE: Rawsonville, Excelsior, on Chenin blanc grapevine, Mar 1997, *L. Mostert* (STE-U 2633); Vredendal, Uitsig, on Muscat d'Alexandrie grapevine, Apr 1997, *L. Mostert* (STE-U 2634); Bonnievale, Middelvlei, on *V. vinifera,* Apr 1997, *L. Mostert* (STE-U 2635); Slanghoek, Twee heuwels, on Colombar grapevine, Apr 1997, *L. Mostert* (STE-U 2636); Philadelphia, Joostfontein, on Chenin blanc grapevine, May 1997, *L. Mostert* (STE-U 2637); Darling, The Granneries, on Chenin blanc grapevine, May 1997, *L. Mostert* (STE-U 2638); Somerset West, Eendrag, on Carignan grapevine, May 1997, *L. Mostert* (STE-U 2639); Stellenbosch, Groenerivier, on Weiser Riesling grapevine, May 1997, *L. Mostert* (STE-U 2640); Paarl, St. Peter's Roches, on Red Gobe grapevine, Jun 1997, *L. Mostert* (STE-U 2641) (specimen PREM 56462); Stellenbosch, Vorentoe, on Cinsaut grapevine, Jul 1997, *L. Mostert* (STE-U 2642) (specimen PREM 56461); Hermanus, Old Lands Stud & Vineyard, on Sauvignon blanc grapevine, Oct 1997, *L. Mostert* (STE-U 2643); Franschhoek, Deu Donne, on Chenin blanc grapevine, Oct 1997, *L. Mostert* (STE-U 2644); Lutzville, Omega, on Emerald Riesling grapevine, Oct 1997, *L. Mostert* (STE-U 2645); Riebeek-Kasteel, Dagbreek, on Red Globe grapevine, Oct 1997, *L. Mostert* (STE-U 2646); Riebeek-Kasteel, Dagbreek, on Dan Ben Hannah grapevine, Oct 1997, *L. Mostert* (STE-U 2647); Porterville, De Tuine, on Red Globe grapevine, Oct 1997, *L. Mostert* (STE-U 2648); Worcester, Diepkloof, on Raisno blanc grapevine, Oct 1997, *L. Mostert* (STE-U 2649); So Verby, on Colombar grapevine, Oct 1997, *L. Mostert* (STE-U 2650); Malmesbury, Elsana, on Chenin blanc grapevine, Nov 1997, *L. Mostert* (STE-U 2651).



FIGS. 13–22. *Phomopsis viticola* (STE-U 2660). 13. Pycnidia on grapevine cane. Bar = 1000 µm. 14. Longitudinal section through pycnidium. Bar = 100 µm. 15. Section through pycnidium wall. 16. Beta conidiophores and conidiogenous cells. 17. Branched alpha conidiophore. 18, 19. Alpha conidiogenous cells with collarettes. 20. Alpha and gamma conidia. 21. Alpha and beta conidia. 22. Multiguttulate alpha conidia. Bars = 10 µm.

SOUTHERN CAPE: Swellendam, Olivedale, on Frans grapevine, Nov 1997, *L. Mostert* (STE-U 2652). WESTERN CAPE: Botriver, Beaumont, on Chenin blanc grapevine, Nov 1997, *L. Mostert* (STE-U 2653). TURKEY. Unknown, on *V. vinifera, Ali Anbaroglu* (CBS 323.77 = STE-U 2672). USA. CALIFORNIA: On Tokay vines, 1979, *J.D. Cucuzza* (ATCC 48153 = STE-U 2673).

Specimens examined. FRANCE. BORDEAUX: Naujan-et-Postiac, on Cabernet Sauvignon grapevine, May 1998, *P. Larignon* (NEOTYPE designated here, PREM 56460), culture ex-type PV F98–1 = STE-U 2660. GERMANY. Mähr-Weisskurchenand, on *Ampelopsidis quinquefolia,* 3 Aug 1920, *F. Petrak* No. 1439, (PR 7579, BPI 358265) (*P. ampelopsidis*). USA. NEW YORK: On *Vitis aestivalis,* Mar 1914, *H.D. House* No. 149, (PAD 1268) (*P. viticola*). PENNSYLVANIA: On *Vitis* sp., Herb. Berk No. 4094, (K 58408) [*P. ampelina* (Berk & Curt.) Grove].

Notes. The alpha conidium shape was uniform for most isolates of *P. viticola.* Three isolates of *P. viticola* (STE-U 2641, STE-U 2649 and STE-U 2642) had

densely aggregated, small pycnidia, which were mainly depressed. STE-U 2641 and STE-U 2649 were also culturally different from most isolates in producing numerous dark brown pycnidia scattered across the surface, and olivaceous (21″m) mycelium. These isolates also grew slower at 25 C and caused smaller lesions on inoculated stems. These differences did not prove to be significant, however, since isolates clustered with strong bootstrap support with other isolates of *P. viticola* (FIGS. 1, 2).

As mentioned earlier, *P. viticola* was originally described from grapevines collected in France. In the original description of *Phoma viticola,* no type specimen was designated (Saccardo 1880). When the latter was redisposed to *Phomopsis,* Saccardo (1915) cited an American specimen (H.D. House No. 149, PAD). This specimen was examined and is characterized as follows: pycnidia on stems, immersed, solitary, black, depressed, uniloculate. Pycnidial wall stromatic, composed of several layers of pseudoparenchymatic cells, with several dark brown, thick-walled, chlamydospore-like cells around the pycnidium on the host surface. *Conidiogenous cells* hyaline, simple, ampulliform, with minute periclinal thickening, without visible collarettes, 5–10 × 5–8 μm. Very few conidia were observed. *Alpha conidia* ellipsoidal, widest in the middle or slightly above, apex rounded to acute, base flat, mono or biguttulate, 6–10 × 2.5–4 μm (FIG. 28). This specimen is depauperate, however, and should therefore not be selected as type. Because no other type material could be located, a neotype had to be chosen. The French material designated as neotype (PREM 56460) here corresponds with the House specimen in morphology, and also clustered with other isolates of *P. viticola* in the phylogenetic analyses.

Grove (1919) distinguished *P. ampelina* (Berk. & Curt.) Grove (K 58408) from *P. viticola* by its external appearance on the host. However, conidia are ellipsoid-fusoid, 8–12 × 2.5–3.5 μm, thus similar to that of *P. viticola* (FIG. 29), and we consider them synonymous. No material could be located of *P. viticola* Sacc. var. *ampelopsidis* Grove, and although the synonymy proposed by Punithalingam (1979) could not be confirmed, we accept that synonymy here.

Punithalingam (1979) regarded *P. ampelopsidis* Petrak to be synonymous with *P. viticola.* An examination of material of this fungus (PR 7579, BPI 358265) suggests, however, that it is distinct. Conidiophores were long and slender, tightly aggregated, 0–3-septate, 15–25 × 2.5–3.5 μm, with conidiogenous cells that were 8–15 × 2.5–3 μm. Alpha conidia were generally smaller than those of *P. viticola,* namely (6–)7–9(–12) × 2–1.5(–3) μm (FIG. 30).

Phomopsis sp. 1 FIGS. 31–41

Pycnidia formed after 17–27 d with only a few remaining sterile. *Conidial mass* mostly formed cirrhi or globose, yellow-white to white droplets; pycnidia eustromatic, dark brown to black, almost superficial, separate, sparse, ampulliform, conical to finger-like, covered with hyphae, convoluted, up to 400 μm wide and 570–810 μm tall, including prominent neck. Pycnidial wall consists of two regions; outer region thick-walled, dark brown, 3–4 cells thick, 10–30 μm wide, becoming pale brown inwardly with inner region of *textura globulosa* to *angularis,* 3–4 cells thick, 20–40 μm wide. *Alpha conidiophores* branched, septate, 10–38 × 1–2 μm ($\bar{x}$ = 22 × 1.5 μm). *Beta conidiophores* septate, branched, 13–38 × 1–2.5 μm ($\bar{x}$ = 25 × 1.5 μm). *Alpha conidiogenous cells* filiform, tapering towards the apex, collarette and periclinal thickening present, 5–18 × 1–2 μm ($\bar{x}$ = 12 × 1.5 μm). *Beta conidiogenous cells* subcylindrical, tapering towards the apex, collarette and periclinal thickening present 10–25 × 1 μm ($\bar{x}$ = 14 × 1 μm). Alpha and beta conidiogenous cells occurred in the same pycnidium, but with beta conidia and conidiophores being more prominent. *Alpha conidia* fusoid with obtuse apices, mostly biguttulate, (4–)6.5–7(–10) × (1.5–)2(–2.5) μm ($\bar{x}$ = 7 × 2 μm). *Beta conidia* straight or curved, 19–25 × 0.5–1 μm ($\bar{x}$ = 21 × 1μm), more abundant than alpha conidia. *Gamma conidia* rarely observed, fusoid to subcylindrical with acute apices, multiguttulate 10–17 × 1–2 ($\bar{x}$ = 13 × 1 μm) (description based on STE-U 2654).

Cultures. Colonies were woolly, predominantly white on the surface with citrine green (23″), hazel brown (17‴) and grayish sepia (15⁗i) patches. Reverse, olivaceous buff (17‴d) to grayish sepia (15⁗i). Mycelium raised, mostly without growth zones.

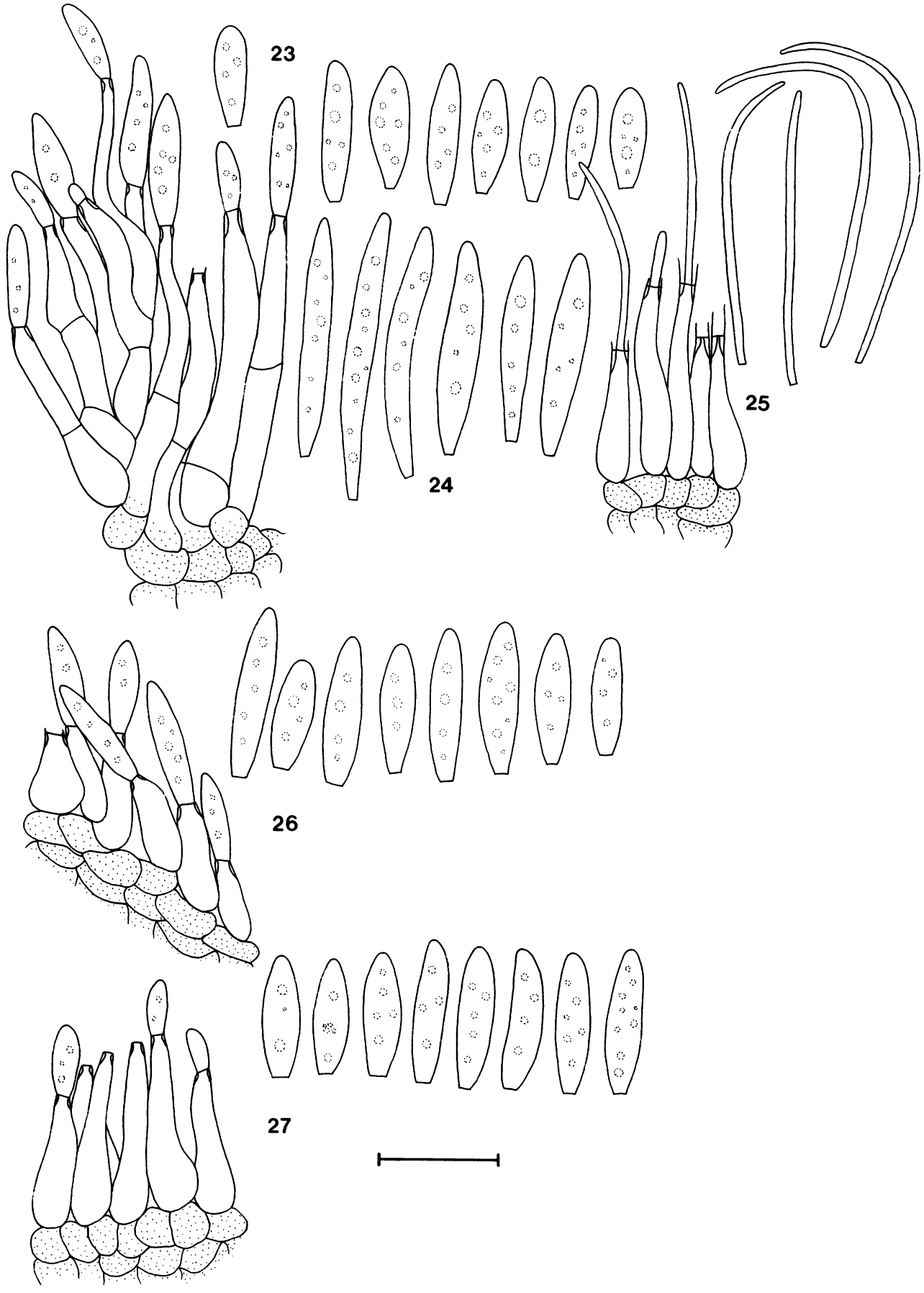
Cardinal temperatures. Colonies attained maximum diameter of 49–67 mm at 25 C after 7 d in the dark. No growth occurred below 10 C or above 35 C.

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

Distribution. Australia, Portugal, South Africa.

Cultures examined. AUSTRALIA. SOUTH AUSTRALIA: Adelaide Plains, Two Wells, on Grenache vines, Aug 1996, *R.W.A. Schepers* (RS 114 = STE-U 2661). VICTORIA: Yarra Valley, on Chardonnay vines, *D.L. Whisson* (DAR 69458 = STE-U 2664). PORTUGAL. Montemor-o-Novo, on Aragonez 229 grapevine, April 1998, *A.J.L. Phillips* (CAP 78 = STE-U 2668). SOUTH AFRICA. WESTERN CAPE: Stellenbosch, on Riesling grapevine, Nov 1997, *L. Mostert,* (STE-U 2654) (specimen PREM 56457); on *Pyrus* sp., Sep 1997, *L. Basson* (STE-U 2656); on *Protea* sp., Oct 1997, *S. Denman* (STE-U 2659); on *Prunus* sp., Nov 1992, *S. Denman* (STE-U 2657); Grabouw, on *Rosa* sp., Oct 1998, *S. Denman* (STE-U 2658).

Notes. Alpha conidia from pear (STE-U 2656) had


23
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acute apices, whereas alpha conidia of STE-U 2655 had oblong apices. STE-U 2668 and STE-U 2654 also formed multiguttulate alpha conidia while the other isolates formed biguttulate alpha conidia. STE-U 2676 formed beta conidia only. STE-U 2664 had distinctly long beta conidiophores, (15–)25.5(–38) μm. The isolate from Portugal, STE-U 2668, had paraphyses and its spore mass was crystalline. STE-U 2661 and STE-U 2668 had pycnidia that were small and densely distributed with white spore masses. Given the low bootstrap obtained for this clade (FIGS. 1, 2), *Phomopsis* sp. 1 appears to represent more than one species. More isolates are required, and additional areas need to be sequenced, however, to suitably address this issue. *Phomopsis ampelopsidis* Petrak (FIG. 30) closely resembles *Phomopsis* sp. 1. However, we suspect that *Phomopsis* sp. 1 may contain several species. When the species occurring in this complex are fully resolved, the name *P. ampelopsidis* would be available for some of these isolates.

Libertella sp. FIGS. 42–45

Pycnidia formed after 11 d. *Conidial mass* mostly in cirrhi on grapevines, pale yellow to orange-pink; pycnidia stromatic, sparse, subepidermal, inconspicuous, multilocular, convoluted, up to 700 μm wide. Pycnidial wall consisting out of two regions of *textura angularis*; outer region of pycnidial wall pale brown, 4–5 cells thick, 3–15 μm wide, inner region 3–4 cells thick, 5–15 μm wide; hyphal growth present around pycnidia. *Conidiophores* subcylindrical, branched, 14–40 × 1.5–2 μm ($\bar{x}$ = 21 × 1 μm). *Conidiogenous cells* subcylindrical, tapering to a minute apex, proliferating sympodially, 10–20 × 1–2.0 μm ($\bar{x}$ = 13 × 1.5 μm). *Paraphyses* prominent, subcylindrical, tapering slightly towards an obtuse apex, branched, septate, arising from the hymenium, 21–45 × 1–2 μm ($\bar{x}$ = 31.5 × 1.5 μm). *Conidia* curved or hamate, nonseptate, (14–)16–18(–20.0) × 1 μm ($\bar{x}$ = 17 × 1μm).

Cultures. Colonies were woolly, white with buff (19″f) undertones. Reverse buff (19″f) with a few grayish sepia (19‴i) patches. After 20 d abundant dull green (27″m) mycelial growth patches were observed. Colonies were erumpent without growth zones.

Cardinal temperatures. Colonies obtained maximum growth of 97 mm diam at 25 C after 7 d in the dark. No growth occurred below 10 C or above 35 C.

Host. Vitis vinifera.

Distribution. Australia.

Culture examined. AUSTRALIA. NEW SOUTH WALES: Hunters Valley, on Chardonnay grapevine, 1994, *S.J. Merrin* (DAR 69484 = STE-U 3313).

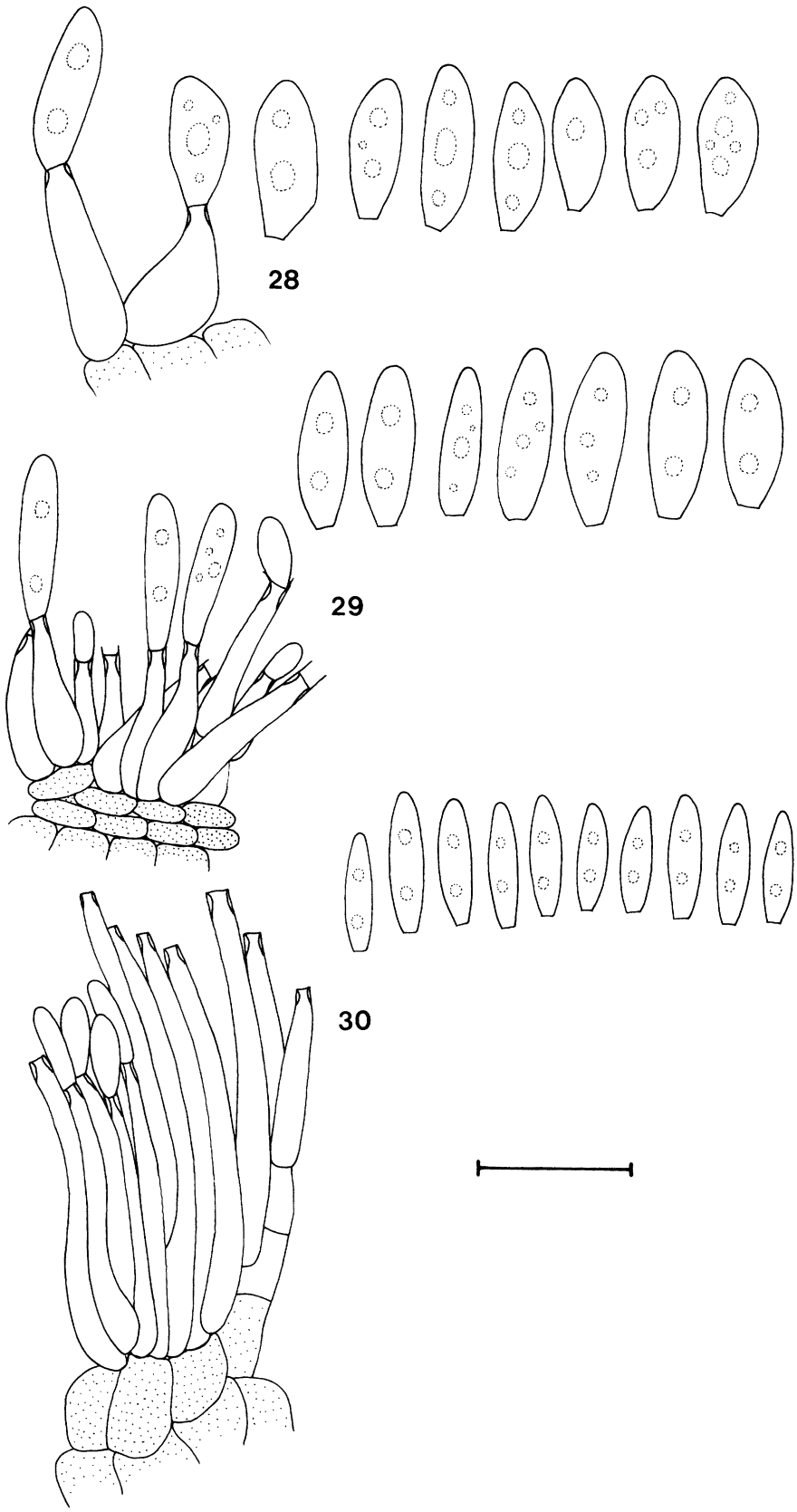
Notes. Taxon 4 of the *Phomopsis viticola* complex is characterized by isolates that form beta conidia only (Merrin et al 1995). An examination of the strain typifying this taxon showed, however, that it would be better accommodated in *Libertella* Desm. than *Phomopsis*. The main difference resides in the mode of conidiogenesis. The sympodial proliferation observed here is characteristic of *Libertella* (Glawe and Rogers 1985), and the nongerminating scolecospores and orange cirrhi also resemble species of *Libertella*, many of which have *Eutypa* Tul. & C. Tul. teleomorphs. The presence of paraphyses and the much shorter conidia distinguish this fungus from *Eutypa lata* (Pers.: Fr.) Tul. & C. Tul. (anamorph *Libertella blepharis* A.L. Smith), which causes Eutypa dieback on grapevines. However, differences in conidium size and cultural characteristics have been found among *Eutypa* isolates from grapevine (Glawe and Rogers 1982), suggesting that more than one species is present on this host.

Phomopsis sp. 2 FIG. 46

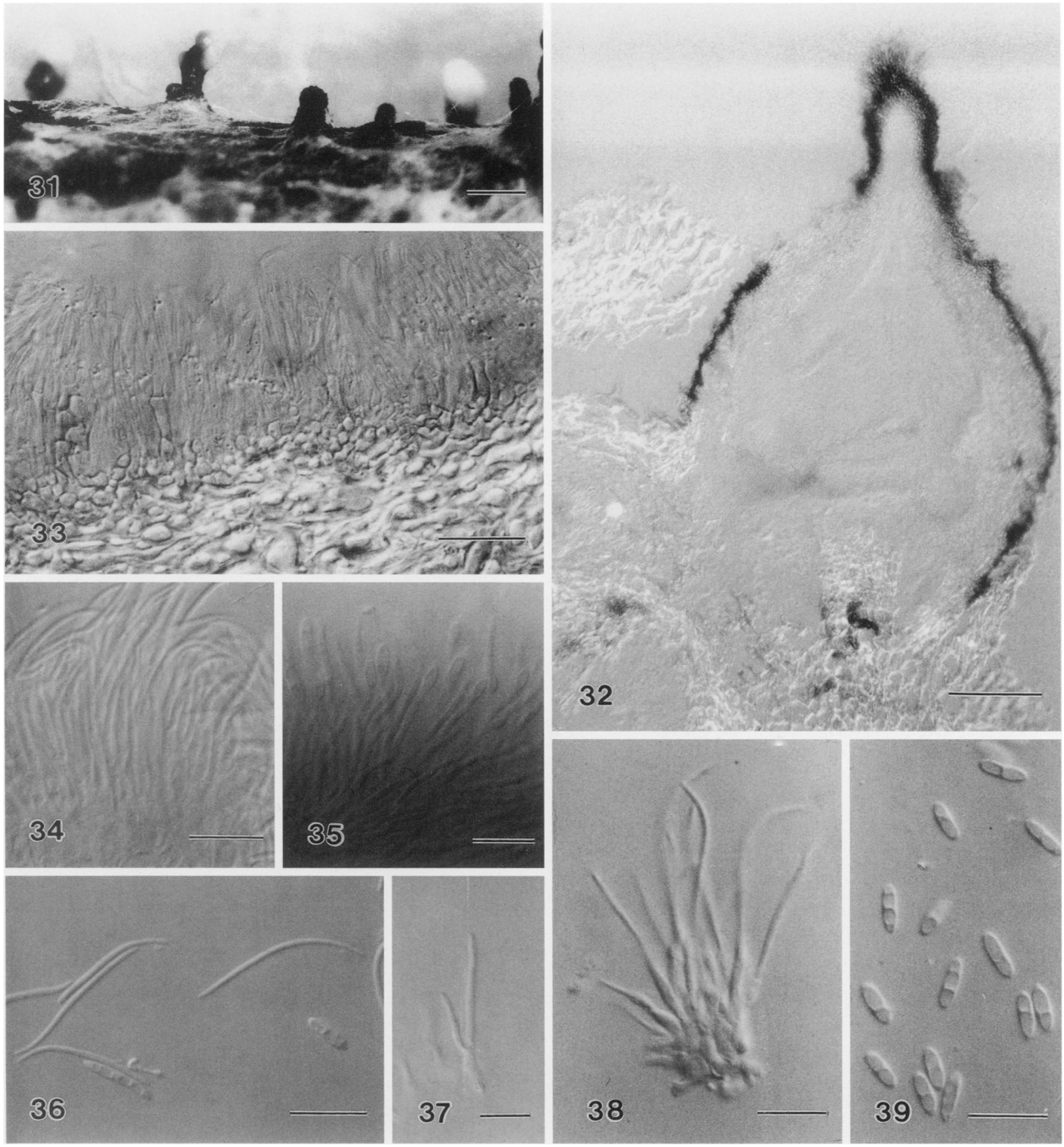
Pycnidia sporulated within 17 d on WAV. *Conidial mass* globose or in exuding cirrhi, pale yellow to white; pycnidia eustromatic, dark brown to black, almost superficial, separate, sparse, circular, covered with hyphae, convoluted up to 550 μm wide and 370 μm tall, including short necks. Pycnidial wall consisting of two regions of *textura angularis*; outer region brown, 4–5 cells thick, 30–40 μm wide; inner region light brown to hyaline, 3–4 cells thick, 10–15 μm wide. *Alpha conidiophores* cylindrical, septate, 15–46 × 1–3 μm ($\bar{x}$ = 31 × 2 μm). *Beta conidiophores* branched, septate, subcylindrical 15–32 × 1.5–2 μm ($\bar{x}$ = 22 × 2 μm). *Alpha conidiogenous cells* cylindrical, filliform, collarette and periclinal thickening present, 10–31 × 1–2 μm ($\bar{x}$ = 21 × 1.5 μm). *Beta conidiogenous cells* subcylindrical tapering towards the apex, collarette and periclinal thickening present, 8–12 × 1 μm ($\bar{x}$ = 10 μm). *Alpha conidia* fusoid, apices narrowly acute, biguttulate, (8–)10–11(–13) × (1.5–)2(–2.5) μm ($\bar{x}$ = 10 × 2 μm). *Beta conidia*

←

FIGS. 23–27. *Phomopsis viticola.* 23. Alpha conidiophores, conidiogenous cells and conidia. 24. Gamma conidia. 25. Beta conidiogenous cells and conidia (STE-U 2660). 26. Alpha conidiogenous cells and conidia (STE-U 2673). 27. Alpha conidiogenous cells and conidia (STE-U 2642). Bar = 10 μm.



FIGS. 28–30. Alpha conidia and conidiogenous cells of *Phomopsis* spp. 28. *Phomopsis viticola* (H.D. House No. 149, PAD). 29. *Phomopsis viticola* (type of *P. ampelina,* K 58408). 30. *Phomopsis ampelopsidis* (BPI 358265). Bar = 10 μm.

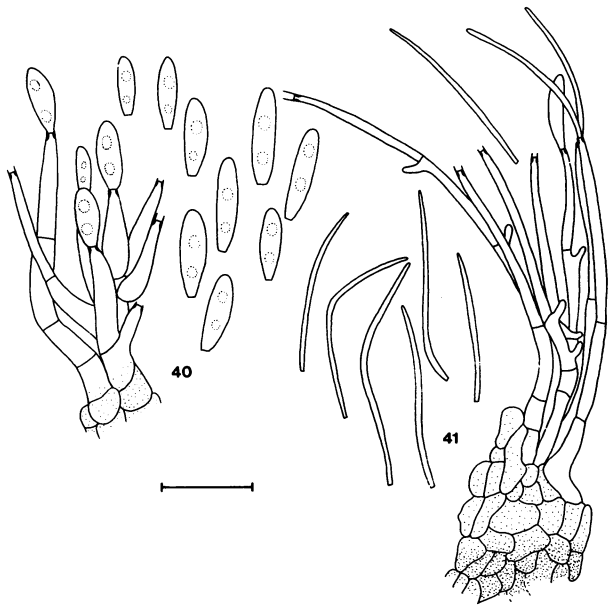


FIGS. 31–39. *Phomopsis* sp. 1 (STE-U 2654). 31. Pycnidia on vine cane. Bar = 1000 µm. 32. Longitudinal section through pycnidium. Bar = 100 µm. 33. Section through pycnidial wall. 34. Branched beta conidiophores. 35. Alpha conidiophores. 36. Alpha, beta and gamma conidia. 37. Branched alpha conidiophore. 38. Beta conidiogenous cells. 39. Alpha conidia. Bars = 10 µm.

curved, hamate, 13–21 × 1 µm ($\bar{x}$ = 15 × 1 µm). No gamma conidia were observed.

Cultures. Colony growth felty with woolly tufts, buff (19″f) with grayish sepia (15‴i) patches. Reverse buff (19″f) with grayish sepia (15‴i) and fuscous black (7⁗k) patches. Colony erumpent without growth zones.

Cardinal temperatures. Colonies attained maximum growth of 60–69 mm diam at 25 C after 7 d in the dark. No growth occurred below 10 C or above 35 C.



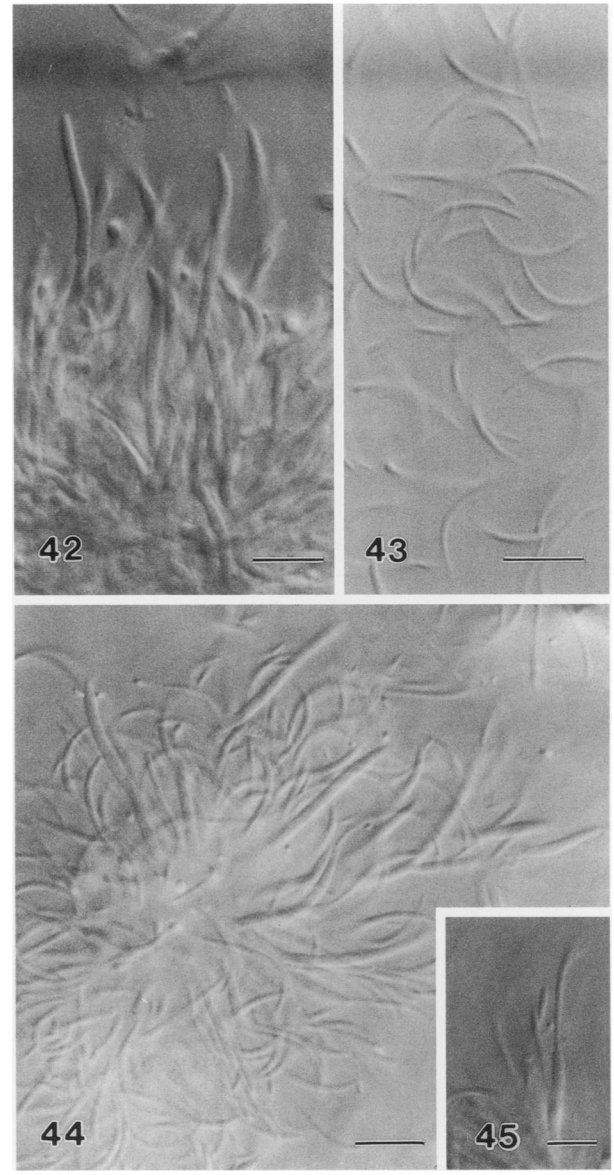
FIGS. 40, 41. *Phomopsis* sp. 1 (STE-U 2654). 40. Alpha conidiophores and conidia. 41. Alpha and beta conidiophores and beta conidia. Bar = 10 μm.

Host. Vitis vinifera.
Distribution. Italy.
Culture examined. ITALY. On *Vitis* sp., 1992, *F. Gobbo* (IMI 352882 = STE-U 2674).

Phomopsis amygdali (Del.) Tuset & Portilla, Can. J. Bot. 67: 1280. 1989. FIGS. 47–53
≡ *Fusicoccum amygdali* Del., Bull. Trimest. Soc. Mycol. Fr. XXI, 3: 184. 1905.
= *Phomopsis amygdalina* Canonaco, Riv. Patol. Veg. XXVI: 157. 1936.

Pycnidia sporulate within 10 d on WAV. *Conidial mass* globose, mostly white, rarely yellow; pycnidia eustromatic, subepidermal, dark brown to black, separate, sparsely distributed, ampulliform to finger-like, hairy, multiloculate and convoluted, 700–800 μm wide and 400–600 μm tall, including necks, which were seldom observed. Pycnidial wall consisting of two regions of *textura angularis*; outer region prominent, dark brown, 3–4 cells thick, 10–40 μm wide; inner region brown, 4–5 cells thick, 20–40 μm. Extensive hyphal growth occurred around conidiomata. *Alpha conidiophores* subcylindrical, 6–25 × 1–2 μm ($\bar{x}$ = 15 × 1.5 μm). *Beta conidiophores* filiform, septate, branched, 6–75 × 1–2 μm ($\bar{x}$ = 33 × 1.5 μm). *Alpha conidiogenous cells* seldom branched, collarette and periclinal thickening present, 3–15 × 1–2 μm ($\bar{x}$ = 10 × 1.5 μm). *Beta conidiogenous cells* filiform, tapering towards the apex, collarette present, periclinal thickening prominent, 3–15 × 1–2 μm ($\bar{x}$ = 11 × 1.5 μm). *Alpha conidia* fusoid, apices acute, eguttulate, (4.5–)5–6(–8) × 1–2 μm ($\bar{x}$ = 5.5 × 1.5 μm). *Beta conidia* straight, slightly curved, 12–20 × 0.5–1 μm ($\bar{x}$ = 16 × 1 μm). Alpha and beta conidia can occur in equal proportions in the same pycnidium, or with either dominating. *Gamma conidia* rarely found, fusoid to subcylindrical, eguttulate, 8–11 × 1 μm ($\bar{x}$ = 9 × 1 μm).

Cultures. Colony growth woolly, predominantly pale olivaceous gray (21′′′′d) on surface, with lighter shades to white tufts and a few patches of grayish sepia (15′′′′i) and olivaceous buff (21′′′b). Reverse olivaceous gray (21′′′′i) with patches of iron gray (24′′′′k). Felty to woolly texture, colony raised with no growth zones.



FIGS. 42–45. *Libertella* sp. (STE-U 3313). 42. Paraphyses. 43. Conidia. 44. Paraphyses and conidia. Bars = 10 μm. 45. Conidiogenous cells. Bar = 5 μm.

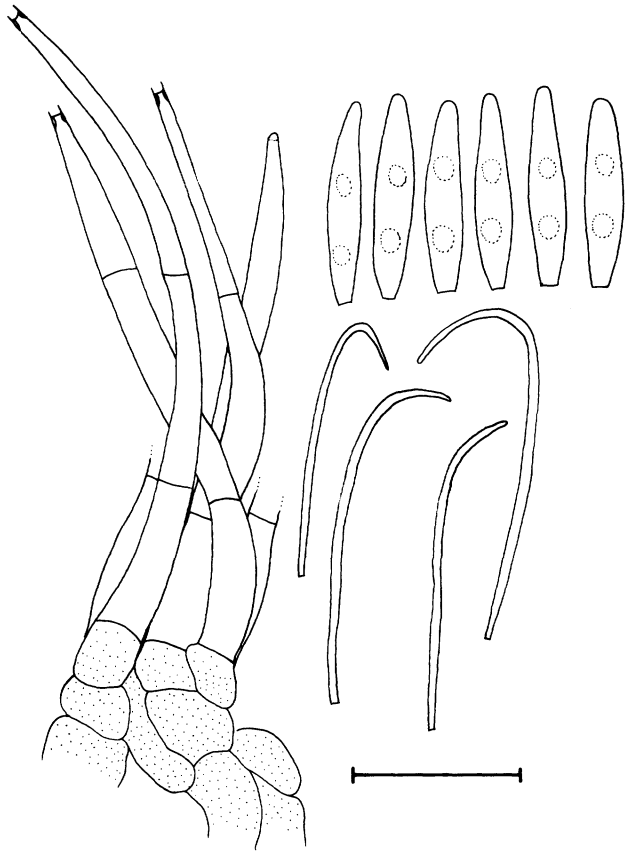


FIG. 46. Conidiophores, alpha and beta conidia of *Phomopsis* sp. 2 (STE-U 2674). Bar = 10 μm.

Cardinal temperatures. Colonies obtained maximum growth of 105 mm diam at 25 C after 7 d in the dark. No growth occurred below 10 C or above 35 C.

Hosts. Prunus armeniaca L., *P. dulcis* (Mill.) A.A. Webb., *P. persica* (L.) Batsch, *Vitis vinifera.*

Distribution. Italy, South Africa, USA.

Culture examined. SOUTH AFRICA. WESTERN CAPE: Constantia, on Pinotage grapevine, Mar 1997, *L. Mostert,* (STE-U 2632) (specimen PREM 56460).

Notes. Alpha conidial and colony morphology of the *Vitis* isolate match that reported by Farr et al (1999) for fruit tree isolates of *P. amygdali.* Tuset and Portilla's (1989) description differed in that the alpha conidia were biguttulate, whereas our isolate lacked guttules. However, Delacroix (1905) noted in his description of *F. amygdali* that guttulation varied from none to two. Apart form these morphological differences the phylogenetic analyses showed that the South African isolate clustered with 100% bootstrap support with *P. amygdali* from peach trees. Results from the cross pathogenicity studies suggest that this isolate is pathogenic to peach, but the disease has not yet been recorded on this host in South Africa.

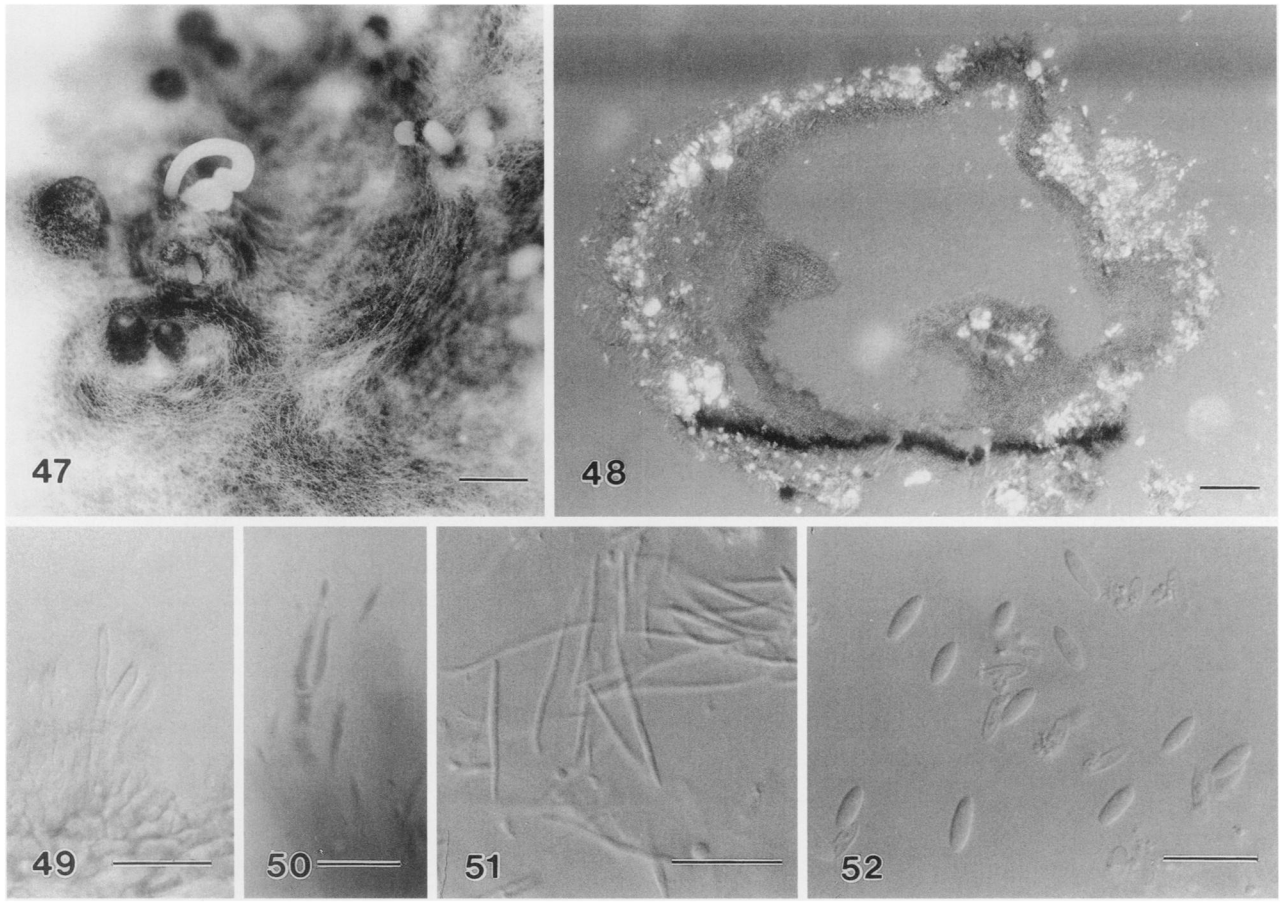
DISCUSSION

Six taxa associated with Phomopsis cane and leaf spot disease of grapevines were characterized in the present study. Previous studies have revealed that the delimitation of species of *Phomopsis* on the basis morphological criteria is unreliable because of character plasticity (Hahn 1930, Van der Aa et al 1990, Rehner and Uecker 1994, Uddin et al 1997). Varying emphasis has subsequently been placed on different morphological structures.

Phillips (1999) considered conidiophore size, septation and branching to be important additional characters. Conidiophore structure, however, has been neglected in most descriptions of *Phomopsis* species and also was not included in Wechtl's (1990) synoptic key of *Phomopsis* species. Farr et al (1999) also reported conidiophores of *Phomopsis* to be variable. Although some variation in conidiophore structure was observed in vitro in the present study, general trends in size and aggregation could be observed that did indicate differences between taxa.

The production of beta conidia was variable as reported by Uecker (1988), and influenced by factors such as dextrose and nitrogen concentration, temperatures above 30 C, carbon dioxide-saturated atmosphere and the presence of *Bacillus subtilis* (Nitimargi 1935, Pine 1957, Punithalingam 1979). Alpha conidium shape, guttulation and dimensions were used as distinguishing features for the delimitation of *D. perjuncta* and *P. viticola* on grapevines (Merrin et al 1995). However, shape and guttulation have been observed to change in culture (Wechtl 1990). Using standardized media and growth conditions as described in the present study, these characters proved to be stable for different isolates, but still varied among isolates of the same species. Based on these findings, it became apparent that it would be more dependable to delimit taxa by combining morphological data with cultural, pathogenicity, physiological and molecular characteristics (Rehner and Uecker 1994, Merrin et al 1995, Farr et al 1999).

The nucleotide sequences of the 5.8S rRNA gene and ITS1 and ITS2 flanking regions have been successfully used to determine the phylogenetic relationships of species in many diverse genera (Schoch et al 1999, Stewart et al 1999, Yao et al 1999). Rehner and Uecker (1994) used this genomic area to elucidate the host specificity of several different *Phomopsis* species. The genetic uniqueness of three pathogenic *Phomopsis* isolates from peach, plum and pear was also confirmed by comparing their ITS1 and ITS2 regions (Uddin and Stevenson 1998). However, based on similar data, Farr et al (1999) were unable to determine the broader relationships among dif-



FIGS. 47–52. *Phomopsis amygdali* (STE-U 2632). 47. Pycnidia on grapevine cane. Bar = 1000 μm. 48. Longitudinal section through pycnidium. Bar = 100 μm. 49, 50. Branched alpha conidiophores. 51. Beta conidia. 52. Alpha conidia. Bars = 10 μm.

ferent *Phomopsis* species, and suggested that a more conserved rDNA or protein coding gene may prove to be more informative. In the present study sequence data from this region was combined with that obtained from the 5′ end partial sequence of the mtSSU. Although these data could be used to address the questions posed in the present study, it is possible that other, more informative sites will be able to provide a finer division of morphologically variable isolates presently treated under one epithet.

The cultural, morphological and pathological differences between *P. viticola* and *D. perjuncta, P. amygdali* and *Phomopsis* sp. 1 were confirmed by the separate groupings obtained in the phylogenetic analyses. *Phomopsis* sp. 2 (STE-U 2674) did not cluster with any other species from grapevines, suggesting it to be distinct. The differences observed in the alpha conidium morphology and cultural growth pattern of this isolate further supported its distinct DNA phylogeny.

Although host plants have been used in the past as a key feature in the identification of species of *Phomopsis* (Brayford 1990, Wechtl 1990), results obtained in recent studies (Rehner and Uecker 1994, Farr et al 1999) suggested that one species of *Phomopsis* could infect more than one host, or that host switching may have occurred frequently during speciation. These observations were confirmed by results obtained in the present study. It is, therefore, necessary to establish whether *Phomopsis* spp. have a wide host range, thereby allowing other agricultural crops and plants grown nearby to serve as a source of inoculum. Host specificity data would also reduce the estimated number of distinct *Phomopsis* species that exist (Uecker 1988).

Species of *Phomopsis* from pear and protea included in the present study clustered with grapevine isolates of *Phomopsis* sp. 1. Cross pathogenicity studies with the pear (STE-U 2656) and *Protea* (STE-U 2659) isolates onto grapevines were, however, inconclusive. The other *Phomopsis* sequence from pear (U91717) included in the phylogenetic analysis grouped with a plum isolate, and both isolates were found by Uddin et al (1997) to cause cankers when inoculated onto

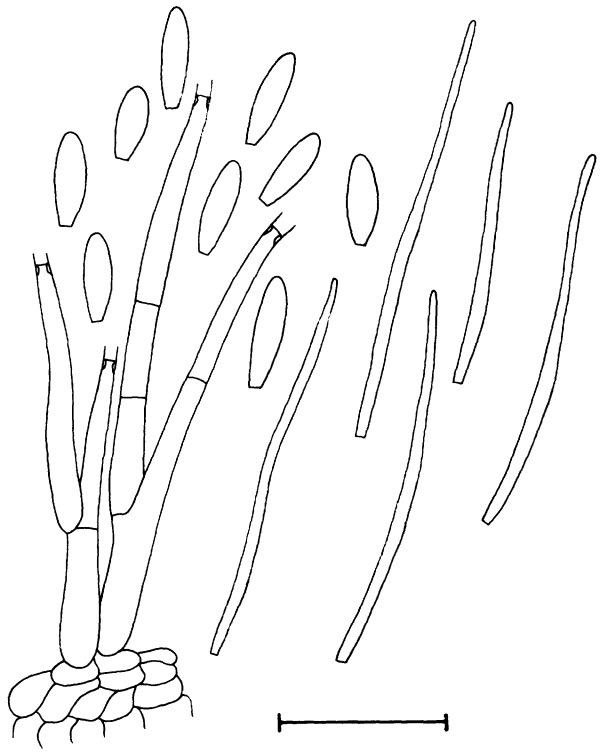


FIG. 53. Branched alpha conidiophores, alpha and beta conidia of *Phomopsis amygdali* (STE-U 2632). Bar = 10 μm.

peach shoots. Stem inoculations conducted in the present study confirmed that the grapevine *P. amygdali* isolate could be a serious pathogen of peach. Uddin and Stevenson (1998) showed that the *Phomopsis* species from peach had a wide host range, being able to infect plum, pear and apple shoots. These trees could thus also act as hosts for inoculum of *P. amygdali*. Pathogenicity studies on plum and pear trees would, however, still be required to confirm this. Some species of *Phomopsis* do appear to be host specific, as reported by Rehner and Uecker (1994) for the *Phomopsis* species isolated from blueberry (*Vaccinium*). A similar scenario could be true for *P. viticola* (FIG. 1) on grapevines.

In conclusion, based on material collected in Australia, Merrin et al (1995) characterized four taxa in the *Phomopsis viticola* complex of grapevines. *Phomopsis viticola* was found to be the cause of Phomopsis cane and leaf spot disease, and was neotypified in this study. Three additional species, *D. perjuncta, P. amygdali* and *Phomopsis* sp. 1 were also found to be present in South Africa. Furthermore, a distinct isolate from Italy was described as *Phomopsis* sp. 2 in the *P. viticola* complex. *Phomopsis amygdali,* which is associated with Phomopsis shoot blight of peach in the USA was also newly reported from South Africa, where it occurs on grapevines. Other than adding valuable information to the *P. viticola* complex on grapevines, this study has once again reiterated the importance of integrating molecular and morphological techniques in the identification of species of *Phomopsis.*

KEY TO THE *PHOMOPSIS VITICOLA* COMPLEX ON GRAPEVINES

1. Colonies felty on MEA; conidiophores seldom branched; alpha conidia fusoid-ellipsoidal, (7–)9.5–10.5(–13) ($\bar{x}$ = 10 μm) long ..... *P. viticola*
1. Colonies woolly on MEA; conidiophores prominently branched; alpha conidia fusoid or oblong-ellipsoidal 2
   2. Alpha conidia with narrowly acute apices, (8–)10–11(–13) ($\bar{x}$ = 10 μm) long ..... *Phomopsis* sp. 2
   2. Alpha conidia shorter than 10 μm, tapering to broadly acute or rounded apices 3
3. Alpha conidia (4.5–)5–6(–8) ($\bar{x}$ = 5.5 μm) long; pathogenic to peach and grapevine ..... *P. amygdali*
3. Alpha conidia similar in length, but not pathogenic to hosts above 4
   4. Teleomorph produced in culture; alpha conidia 5–7 × 2–2.5 μm ($\bar{x}$ = 6.5 μm) long .. *D. perjuncta*
   4. Teleomorph not produced in culture; alpha conidia (4–)6.5–7(–10) × (1.5–)2(–2.5) μm ($\bar{x}$ = 7 μm) long ..... *Phomopsis* sp. 1

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