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Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov.

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Abstract: Botryosphaeriaceae are common dieback and canker pathogens of woody host plants, including stone fruit trees. In the present study the diversity of members of the Botryosphaeriaceae isolated from symptomatic wood of *Prunus* species (plum, peach, nectarine and apricot) was determined in stone fruit-growing areas in South Africa. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS-1, ITS-2 and EF-1α) were used to identify known members and describe novel members of Botryosphaeriaceae. From the total number of wood samples collected (258) 67 isolates of Botryosphaeriaceae were obtained, from which eight species were identified. All species were associated with wood necrosis. *Diplodia seriata* (= ''*Botryosphaeria*'' *obtusa*) was dominant, and present on all four *Prunus* species sampled, followed by *Neofusicoccum vitifusiforme* and *N. australe*. First reports from *Prunus* spp. include *N. vitifusiforme*, *Dothiorella viticola* and *Diplodia pinea*. This is also the first report of *D. mutila* from South Africa. Two species are newly described, namely *Lasiodiplodia plurivora* sp. nov. from *P. salicina* and *Diplodia africana* sp. nov. from *P. persica*. All species, except *Dothiorella viticola*, caused lesions on green nectarine and/or plum shoots in a detached shoot pathogenicity assay.

Key words: *Ascomycetes*, *Botryosphaeria*, *Dothidotthia*, *Dothiorella*, *Fusicoccum*, ITS, *Neofusicoccum*, pathogenicity, *Sphaeropsis*, systematics, translation elongation factor EF1-α

INTRODUCTION

Species of *Botryosphaeriaceae* are cosmopolitan and known to cause canker diseases on a wide range of woody host plants (von Arx and Müller 1954). Although infection commonly occurs via pruning wounds and damaged tissue (Brown and Britton 1986) these pathogens also can infect directly through lenticels (Weaver 1979) and dormant buds (Britton and Hendrix 1989) and frequently occur as endophytes in symptomless tissue (Smith et al 1996), which under stress the onset of disease is stimulated (Pusey 1989). Species of this complex represent important pathogens of fruit trees and are associated with fruit rot, leaf spot, cankers, branch dieback and even tree death (Brown and Britton 1986, Pusey et al 1995).

Taxa such as *Botryosphaeria dothidea* (*Fusicoccum aesculi*), *Diplodia seriata* (''*B.*'' *obtusa*, see Phillips et al 2007) and *Lasiodiplodia theobromae* (''*B.*'' *rhodina*, see Crous et al 2006b for clarification of *Botryosphaeria*-like taxa) are associated commonly with diseases of peach trees (*Prunus persica*) worldwide (Pusey et al 1995). *Botryosphaeria dothidea* is known from several *Prunus* spp. (Pusey et al 1986). Farr et al (1989) list these three species as well as *N. ribis* and *Sphaeropsis peckii* on *Prunus* spp. in USA. Slippers et al (2007) found several strains of *D. seriata* and *Neofusicoccum australe* on stone fruit trees in South Africa. Additional species described from *Prunus* spp. include *Diplodia rosulata* on *P. africana* in Ethiopia (Gure et al 2005) and *Dothiorella sarmentorum* on *P. armeniaca* and other *Prunus* spp. in Europe and/or North America (see synonyms listed by Wollenweber 1941). Wollenweber and Hochapfel (1941) regarded *Diplodia roumegueri* var. *santonensis* as synonymous with *Diplodia phoradendri* that was collected in 1892 on *P. laurocerasus* (cherry laurel) in France. Gure et al (2005) also mentioned *Diplodia persicina* (syn. *Phoma persicina*) on *P. persica*, *Diplodia cerasorum* on *P. avium* and *Diplodia amygdali* on *P. dulcis* and *P. armeniaca*, but these species have not been recollected since their original description and presently are not known from culture.

When consulting the number of Botryosphaeriaceae listed in MycoBank (www.MycoBank.org), a search confined to the names ''*Botryosphaeria*, *Diplodia* and *Fusicoccum*'' results in more than 1500 species names. In a recent compilation of the phytopathogenic fungal species occurring in South

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Africa, Crous et al (2000) listed approximately 20 species of ''Botryosphaeriaceae'' (used throughout this paper to describe this complex excl. *Guignardia*). This rather low number of Botryosphaeriaceae is unexpected because plant diversity in South Africa is high, with an estimated fungal diversity of more than 200 000 species (Crous et al 2006a). From these numbers it is obvious that this group has been poorly studied in this region; Slippers et al (2004a, c) have shown clearly that the approach of von Arx and Müller (1954) to reduce many species to synonymy in fact was unsubstantiated, leading to the conclusion that there should be many more potentially phytopathogenic species than known to date.

Within South Africa vineyards frequently are established near fruit tree orchards, *Eucalyptus* windbreaks and Proteaceae, which grow wild in the mountain fynbos. Presently 11 species of Botryosphaeriaceae are known from *Vitis vinifera* alone, eight of which have been reported from South Africa (van Niekerk et al 2004), while Denman et al (2003) reported five species on Proteaceae in South Africa and Slippers et al (2004b) reported five on *Eucalyptus*, four of them occurring in South Africa. In recent years many new plant pathogenic species of Botryosphaeriaceae have been described (e.g. de Wet et al 2003, Alves et al 2004, Slippers et al 2004b, c, Luque et al 2005, Phillips et al 2005, Burgess et al 2006, Crous et al 2006b), which suggests that if saprobic species also were to be studied this number would increase rapidly.

In an attempt to introduce the ''genus for genus concept'' (Seifert et al 2000) in the Botryosphaeriaceae, a study by Crous et al (2006b) split the genus *Botryosphaeria* into several genera based on different phylogenetic lineages, which correlated with morphology. In a further attempt to reduce the unnecessary introduction of new generic names for separate clades, only one generic name was introduced per clade, either for the anamorph or in some cases for the teleomorph state, as argued by Hawksworth (2006). This resulted in the fact that only the type species, *B. dothidea*, and closely related species remain in *Botryosphaeria*, whereas *Neofusicoccum* was introduced to accommodate the majority of species with fusicoccum-like anamorphs, while anamorph genera such as *Diplodia* (incl. *Sphaeropsis*), *Lasiodiplodia*, *Pseudofusicoccum*, *Macrophomina*, *Neoscytalidium* and *Dothiorella* were used for other prominent clades. According to Crous et al (2006b) ''*Botryosphaeria*'' or ''*B.*'' is used in this paper for species that morpholically are *Botryosphaeria*-like but are distinct phylogenetically and need to be allocated to a different genus.

There are only three reports of Botryosphaeriaceae on stone fruits in South Africa. Doidge and Bottomley (1931) mentioned an uncommon dieback of plum in the Cape region caused by a *Botryosphaeria* sp., while Combrink et al (1984) found *D. seriata* (as ''*B.*'' *obtusa*) as a cause of post-harvest decay of peaches. In addition Slippers et al (2007) recently isolated *Neofusicoccum australe* and *D. seriata* from dieback symptoms of stone fruit trees.

Because *Botryosphaeria* dieback has been well studied on grapevines in South Africa (van Niekerk et al 2004) we decided to survey the fruit trees cultivated near vineyards to determine whether these could act as potential inoculum sinks or alternate hosts for the Botryosphaeriaceae known from grapevines. During a survey in three climatically diverse areas in South Africa we isolated fungal strains from plum, peach, nectarine and apricot wood. In this study we determined the diversity of Botryosphaeriaceae on *Prunus* species in South Africa and describe two new species isolated from wood of *Prunus persica* and *P. salicina*.

MATERIALS AND METHODS

Sampling and fungal isolation.—Branches with symptomatic wood (e.g. dieback, canker, necrosis) and pruning debris were sampled from plum (*P. salicina*), peach (*P. persica*), nectarine (*P. persica* var. *nucipersica*) and apricot (*P. armeniaca*) orchards in high (Stellenbosch, Paarl, Franschhoek) and low (Robertson, Bonnievale, Montagu) winter rainfall areas in Western Cape Province and in summer rainfall areas in Limpopo Province (Mookgopong, Modimolle) of South Africa. Wood pieces (2 × 2 mm) from the margin between necrotic and apparently healthy tissue were surface sterilized (1 min in 3.5% NaOCl and 30 s in 70% ethanol), placed on potato-dextrose agar (2% PDA, Biolab, Midrand, South Africa, supplemented with 100 mg/L streptomycin sulfate and 100 mg ampicillin) and on synthetic nutrient agar medium (SNA, Nirenberg 1976) supplemented with 100 mg penicillin G, 50 mg streptomycin sulphate, 10 mg chlortetracycline hydrochloride, pH 6 and incubated under cool fluorescent white light at 25 C. Additional isolates were derived from pycnidia on the bark of pruning debris. Single conidial isolates were obtained from all strains of *Botryosphaeriaceae* for further study. Reference strains are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), Stellenbosch, South Africa, and Centraalbureau voor Schimmelcultures (CBS) Utrecht, The Netherlands. Isolates used for morphological and sequence analysis are presented (TABLE I).

Morphological analysis.—To enhance sporulation autoclaved filter paper and double-autoclaved pine needles were placed on SNA plates and incubated 2–4 wk at 25 C in the dark or under near-ultraviolet light. A Nikon SMZ800 dissecting microscope and a Nikon Eclipse E600 light microscope with differential interference contrast were used

TABLE I. Isolates of Botryosphaeriaceae studied

Species	Accession number[1]	Host	Location	Collector	Patho. test[2]	GenBank accessions	
						ITS	EF-1α
Diplodia seriata	STE-U 5810/CBS 121425	*Prunus persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445299	EF445365
	STE-U 5811/CBS 121106	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445298	EF445366
	STE-U 5816/CBS 121107	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445296	EF445363
	STE-U 5830/CBS 121108	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445297	EF445364
	STE-U 5903	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445300	
	STE-U 5804	*P. salicina*	Stellenbosch, Western Cape, South Africa	U Damm		EF445301	EF445369
	STE-U 5906/CBS 121109	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U Damm		EF445302	EF445370
	STE-U 5805	*P. salicina*	Stellenbosch, Western Cape, South Africa	U Damm		EF445303	EF445371
	STE-U 5904	*P. armeniaca*	Mookgopong, Limpopo, South Africa	U Damm		EF445304	EF445372
	STE-U 5907	*P. armeniaca*	Mookgopong, Limpopo, South Africa	U Damm		EF445305	EF445373
	STE-U 5905	*P. armeniaca*	Mookgopong, Limpopo, South Africa	U Damm		EF445306	
	STE-U 5806	*P. salicina*	Stellenbosch, Western Cape, South Africa	U Damm		EF445307	
	STE-U 6274/CBS 121110	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445308	EF445374
	STE-U 6275	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445309	
	STE-U 6276	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445310	
	STE-U 6277	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445311	
	STE-U 6278	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445312	
	STE-U 6279	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445313	
	STE-U 6280	*P. armeniaca*	Montagu, Western Cape, South Africa	U Damm		EF445314	EF445375
	STE-U 6281	*P. armeniaca*	Robertson, Western Cape, South Africa	U Damm		EF445315	
	STE-U 6282	*P. armeniaca*	Robertson, Western Cape, South Africa	U Damm		EF445316	

TABLE I. Continued

Species	Accession number[1]	Host	Location	Collector	Patho. test[2]	GenBank accessions	
						ITS	EF-1α
	STE-U 6283	*P. armeniaca*	Robertson, Western Cape, South Africa	U Damm		EF445317	EF445376
	STE-U 6284	*P. armeniaca*	Bonnievale, Western Cape, South Africa	U Damm		EF445318	
	STE-U 6285	*P. armeniaca*	Bonnievale, Western Cape, South Africa	U Damm		EF445319	
	STE-U 6286	*P. persica*	Modimolle, Limpopo, South Africa	U Damm		EF445320	
	STE-U 6287	*P. persica*	Modimolle, Limpopo, South Africa	U Damm		EF445321	
	STE-U 6288	*P. persica*	Modimolle, Limpopo, South Africa	U Damm		EF445322	
	STE-U 5813	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445323	
	STE-U 5814	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445324	
	STE-U 5815	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445325	
	STE-U 5817	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445326	
	STE-U 5818	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445327	
	STE-U 5819	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445328	
	STE-U 5821	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445329	
	STE-U 5822	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445330	
	STE-U 5823	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445331	EF445367
	STE-U 5826	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445332	
	STE-U 5827	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445333	
	STE-U 5828	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445334	
	STE-U 5829	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445335	
	STE-U 5899	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445336	
	STE-U 5900	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445337	EF445368
	STE-U 5902	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445338	
	STE-U 4542/CBS 112876	*V. vinifera*	South Africa	F Halleen		AY343434	AY343353
"Botryosphaeria" tsugae	CBS 418.64*	*Tsuga heterophylla*	Canada	A Funk		DQ458888	DQ458873
Cercospora penzigii	STE-U 4001	*Citrus sinensis*	Swaziland	MC Pretorius		AY343372	AY343335
C. beticola	STE-U 5073/CBS 121.31	*Beta vulgaris*	Austria	unknown, deposited by EW Schmidt		AY34337	AY343334
Diplodia africana	STE-U 5908/CBS 120835*	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445343	EF445382
	STE-U 5946/CBS 121104	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445344	EF445383
	STE-U 6289	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445345	

TABLE I. Continued

Species	Accession number[1]	Host	Location	Collector	Patho. test[2]	GenBank accessions	
						ITS	EF-1α
D. corticola	CBS 112549*	*Quercus suber*	Portugal	A Alves		AY259100	AY573227
	CBS 112545	*Q. suber*	Spain	ME Sánchez, A Trapero		AY259089	AY573226
D. cupressi	CBS 168.87	*Cupressus sempervirens*	Israel	Z Solel		DQ458893	DQ458861
	CBS 261.85	*C. sempervirens*	Israel	Z Solel		DQ458894	DQ458862
D. mutila	STE-U 5824/CBS 120834	*P. salicina*	Paarl, Western Cape, South Africa	U Damm	x	EF445346	EF445381
	CBS 112553	*V. vinifera*	Portugal	AJL Phillips		AY259093	AY573219
D. pinea	STE-U 5808/CBS 121105	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445339	EF445377
	STE-U 5809	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445341	EF445379
	STE-U 5812	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445342	EF445380
	STE-U 5901/CBS 120833	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445340	EF445378
D. pinea A morphotype	CBS 393.84	*Pinus nigra*	Netherlands	HA van der Aa		DQ458895	DQ458880
D. pinea C morphotype	CBS 109725/CMW 4881	*Pinus patula*	Indonesia	MJ Wingfield		DQ458896	DQ458881
D. scrobiculata	CBS 109944	*Pinus greggii*	Mexico	MJ Wingfield		DQ458899	DQ458884
	CBS 113423	*Pinus greggii*	Mexico	MJ Wingfield		DQ458900	DQ458885
Dothiorella viticola	STE-U 5831/CBS 121117	*P. salicina*	Paarl, Western Cape, South Africa	U Damm	x	EF445361	EF445394
	STE-U 6139/CBS 121118	*P. persica* var. *nucipersica*	Modimolle, Limpopo, South Africa	U Damm		EF445360	EF445393
	CBS 117009*	*V. vinifera*	Spain	J Luque, S Martos		AY905554	AY905559
	STE-U 5148/CBS 112870	*V. vinifera*	South Africa	JM van Niekerk		AY343373	AY343336
	CBS 117006	*V. vinifera*	Spain	J Luque, R Maten		AY905555	AY905562
Lasiodiplodia crassispora	CMW13488	*E. urophylla*	Venezuela	S Mohali		DQ103552	DQ103559
L. crassispora	WAC12533*	*Santalum album*	Australia	TI Burgess, B Dell		DQ103550	DQ103557
L. gonubiensis	CMW14077, CBS 115812*	*Syzygium cordatum*	South Africa	D Pavlic		AY639595	DQ103566
	CMW14078	*S. cordatum*	South Africa	D Pavlic		AY639594	DQ103567
L. plurivora	STE-U 5803/CBS 120832*	*P. salicina*	Stellenbosch, Western Cape, South Africa	U Damm	x	EF445362	EF445395
	STE-U 4583/CBS 121103	*V. vinifera*	South Africa	F Halleen		AY343482	EF445396

TABLE I. Continued

Species	Accession number[1]	Host	Location	Collector	Patho. test[2]	GenBank accessions	
						ITS	EF-1α
L. rubropurpurea	WAC12536	*Eucalyptus grandis*	Australia	TI Burgess, G Pegg		DQ103554	DQ103572
	WAC12535*	*E. grandis*	Australia	TI Burgess, G Pegg		DQ103553	DQ103571
L. theobromae	CMW 9074	*Pinus* sp.	Mexico	TI Burgess		AY236952	DQ103565
	STE-U 5051/CBS 110495	*Vitis vinifera*	Argentinia	M Gatika		AY343483	AY343369
L. venezuelensis	WAC12539*	*Acacia mangium*	Venezuela	S Mohali		DQ103547	DQ103568
	WAC12540	*A. mangium*	Venezuela	S Mohali		DQ103549	DQ103569
Neofusicoccum australe	STE-U 5909	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445357	EF445388
	STE-U 6071/CBS 121116	*P. armeniaca*	Bonnievale, Western Cape, South Africa	U Damm		EF445356	EF445387
	STE-U 6072	*P. armeniaca*	Bonnievale, Western Cape, South Africa	U Damm		EF445358	
	STE-U 6073	*P. armeniaca*	Bonnievale, Western Cape, South Africa	U Damm		EF445359	EF445384
	STE-U 5807/CBS 121115	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445355	EF445386
	STE-U 5802/CBS 121114	*P. salicina*	Stellenbosch, Western Cape, South Africa	U Damm	x	EF445354	EF445385
	STE-U 4598/CBS 110864	*V. vinifera*	South Africa	F Halleen		AY343407	AY343348
	CMW6837*	*Acacia* sp.	Australia	MJ Wingfield		AY339262	AY339270
N. vitifusiforme	STE-U 5912/CBS 121112	*P. salicina*	Mookgopong, Limpopo, South Africa	U Damm	x	EF445349	EF445391
	STE-U 5820/CBS 121111	*P. salicina*	Paarl, Western Cape, South Africa	U Damm	x	EF445347	EF445389
	STE-U 5910	*P. persica*	Paarl, Western Cape, South Africa	U Damm	x	EF445351	EF445392
	STE-U 6074/CBS 121113	*P. persica*	Modimolle, Limpopo, South Africa	U Damm		EF445348	EF445390
	STE-U 6075	*P. persica*	Modimolle, Limpopo, South Africa	U Damm		EF445353	
	STE-U 5825	*P. salicina*	Paarl, Western Cape, South Africa	U Damm		EF445350	
	STE-U 5911	*P. persica*	Paarl, Western Cape, South Africa	U Damm		EF445352	
	STE-U 5252/CBS 110887*	*V. vinifera*	South Africa	JM van Niekerk		AY343383	AY343343

[1] STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; WAC: Department of Agriculture Western Australia, Plant Pathogen Collection, Perth, Australia.

[2] Isolates studied in the pathogenicity test.

* Ex-type cultures.

for observations. Measurements and photographs of characteristic structures were made from structures mounted in clear lactic acid. The 95% confidence intervals for conidium dimensions were derived from measurements of 30 conidia, with extremes given in parentheses. Vertical sections through conidiomata were made with a Leica CM1100 cryostat microtome and mounted in lactic acid. Photographic images were captured with a Nikon DXM 1200 digital camera. Radial growth rates, cultural characteristics and cardinal temperatures for growth of selected isolates were determined after 7 d on PDA in the dark at 5–35 C in 5 C intervals. Colony colors were rated according to Rayner (1970) and described for isolates incubated at 25 C in the dark and under near-ultraviolet light for 7 d.

Phylogenetic analysis.—Genomic DNA of all isolates was isolated from fungal mycelium grown on PDA plates following the protocol of Lee and Taylor (1990). The 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS-1 and ITS-2) and the translation elongation factor 1α (EF-1α) were amplified and sequenced respectively with the primer pairs ITS-1F (Gardes and Bruns 1993) + ITS-4 (White et al 1990) and EF1 728F + EF1986R (Carbone and Kohn 1999) according to the conditions and protocols explained in van Niekerk et al (2004). The sequences were added to outgroups (*Cercospora penzigii* STE-U 4001 and *C. beticola* STE-U 5073) and sequences obtained from GenBank (http://www.ncbi.nlm.gov). The alignment was assembled and manually adjusted with Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). Phylogenetic analyses were performed with PAUP v. 4.0b10 (Swofford 2000). The data were analyzed for each region separately as well as with a combined dataset. Ambiguously aligned data positions 102–175 (ITS) and 689 and 762 (EF-1α) were excluded from the analysis. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Maximum parsimony analysis was performed with the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. The robustness of trees was evaluated by 1000 bootstrap replications with 10 random sequence additions (Hillis and Bull 1993). Tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated for the tree. A partition homogeneity test with the same search criteria was conducted in PAUP to examine the possibility of a joint analysis of the ITS and EF-1α datasets. Sequences were lodged at GenBank (TABLE I) and the alignment in TreeBASE (23523).

Pathogenicity tests.—Preliminary pathogenicity tests were conducted with eight taxa on detached nectarine (cv. Alpine) and plum (cv. Ruby Nel) shoots. Depending on strain availability up to three isolates per taxon were used and treated as subsamples in statistical analysis. Vegetative shoots were collected shortly after harvest, cut into 12 cm pieces (5–8 mm diam) and surface sterilized (30 s in 70% ethanol, 2 min in 0.35% NaOCl and 30 s in 70% ethanol). Nectarine and plum cane sections were wounded through the phloem and cortex tissue with a 4 mm diam cork borer and inoculated with a colonized agar plug (4 mm diam) from 1 wk old PDA culture. *Acremonium strictum* (STE-U 6296) and uncolonized PDA plugs were used as negative controls. Inoculated wounds were covered with Parafilm and shoots were incubated at 25 C in moist chambers (>93% RH) for 2 wk, after which surface lesions were measured. Each treatment combination consisted of one shoot, which was replicated four times in each of three blocks (=moist chambers). Re-isolations were made from the leading edges of lesions and the resulting cultures identified. The layout of the trial was a randomized block design with all treatments randomised in three blocks (=three moist chambers). Lesion length data were subjected to analyses of variance with SAS version 8.1 (SAS Institute, Cary, North Carolina) and Student's t-least significant difference was calculated at the 5% significance level to compare treatment means for the different taxa.

RESULTS

Phylogenetic analysis.—The partition homogeneity test (p-value = 0.46) led us to combine the ITS and EF-1α datasets (486 characters in dataset 1, 214 in dataset 2). A selection of 19 isolates was used for the phylogenetic analysis, with further 29 sequences being added from GenBank. The dataset contained 700 characters including gaps, of which 245 were parsimony informative, six were variable and parsimony uninformative and 449 were constant. After a heuristic search 192 most parsimonious trees were retained (Length = 446 steps, CI = 0.738, RI = 0.922, RC = 0.680), of which one is shown (FIG. 1). The topology was similar for the 192 trees obtained. They differed within the *D. seriata/D. pinea/D. scrobiculata*, the *N. vitifusiforme* and the *N. australe* subclades and in the position of ''*B.*'' *tsugae*, *D. corticola*, *D. cupressi* and *D. rosulata* within the *Diplodia* clade.

There are four clades, representing the genera *Lasiodiplodia*, *Diplodia*, *Dothiorella* and *Neofusicoccum*. Within *Lasiodiplodia* isolates STE-U 5803 and STE-U 4583 formed a cluster next to *L. theobromae* (100% bootstrap support), apart from *L. gonubiensis*, *L. venezuelensis*, *L. rubropurpurea* and *L. crassispora*. A large number of isolates grouped in the *Diplodia* clade. One isolate (STE-U 5824) clustered with *D. mutila* strain CBS 112553, whereas isolates STE-U 5908 and 5946 formed a separate subcluster (bootstrap 93%). The majority of the isolates (47 of 66), represented by strains STE-U 5816, 5830, 5811, 5810, 5808 and 5901, grouped with *D seriata*, *D. pinea* and *D. scrobiculata* (bootstrap 98%). While *D. scrobiculata* formed a well supported subgroup (bootstrap 98%), isolates STE-U 5808 and 5901 formed a subgroup with isolates of *D. pinea* (bootstrap 62%). The *Dothiorella* clade contained two strains from *Prunus* (STE-U 5831, STE-U 6139), which clustered with *D. viticola* (bootstrap 100%). Within the *Neofusicoccum* clade

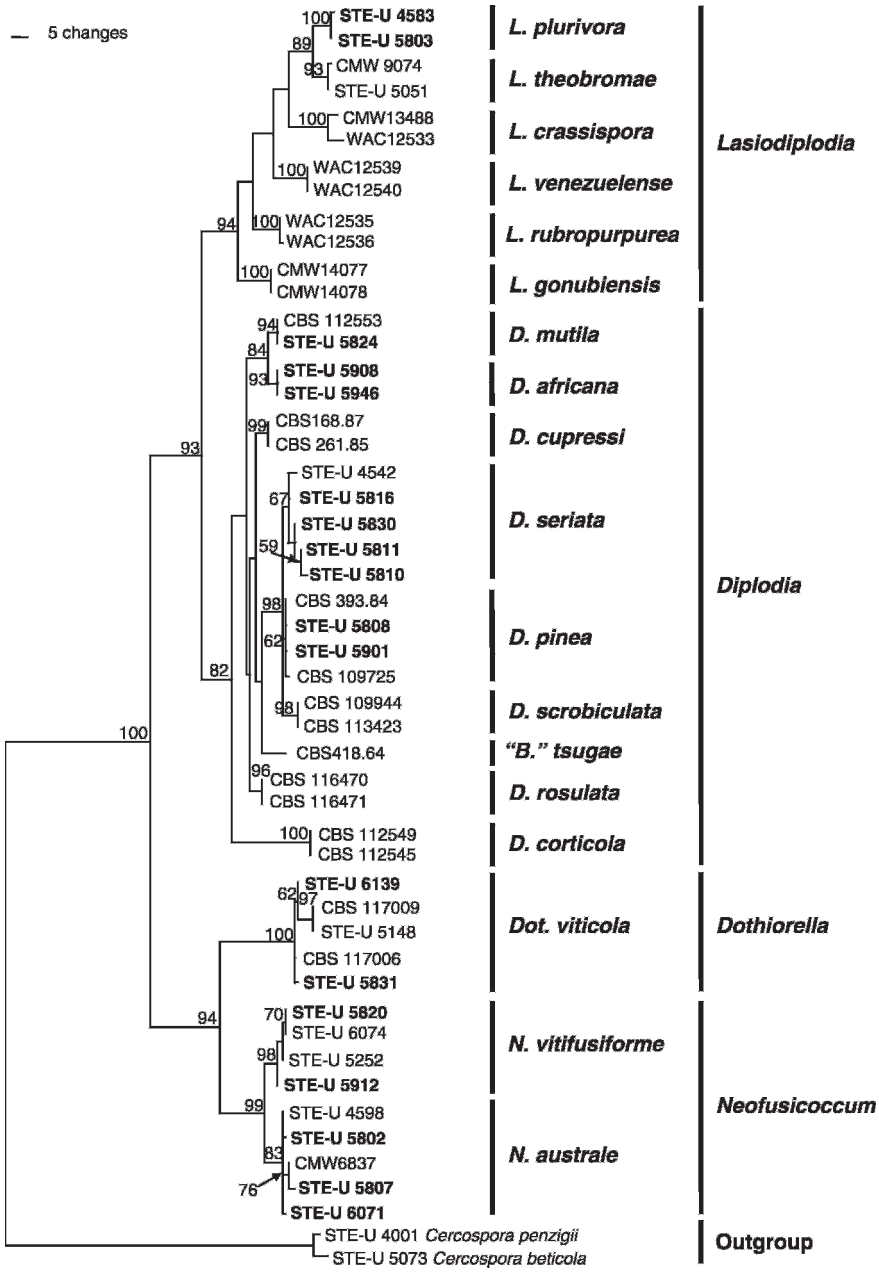


FIG. 1. One of 192 most parsimonious trees obtained from combined ITS and EF-1α sequence data of Botryosphaeriaceae. Bootstrap support values (%) from 1000 replications are shown at the nodes. Numbers of isolates analyzed in this study are emphasized in bold.

isolates STE-U 5820, 6074 and 5912 grouped with *N. vitifusiforme* (bootstrap 98%) and isolates STE-U 5802, 6071 and 5807 with *N. australe* (bootstrap 83%).

TAXONOMY

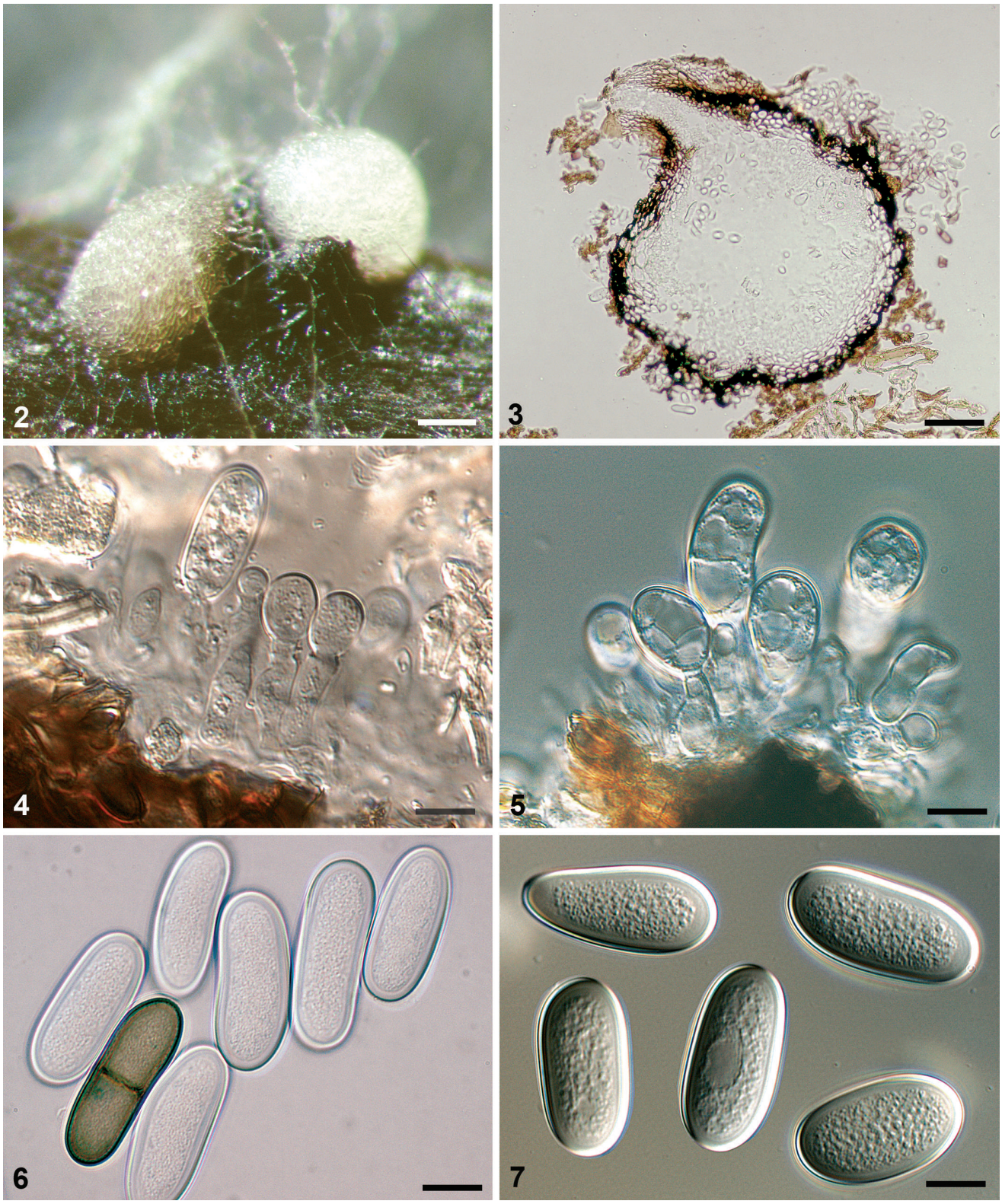
The 67 strains of Botryosphaeriaceae (TABLE I) isolated from stone fruit wood could be assigned to eight species based on the DNA sequence data generated and morphology. Two species proved distinct from known species and are newly described below.

Diplodia africana Damm & Crous sp. nov. MycoBank MB 501323. FIGS. 2–7

Etymology. Named after the continent of origin, Africa.

Diplodiae mutilae similis, sed conidiis longioribus, (17–)25.5–33(–34) × (10–)12–14(–15) μm.

Conidiomata pycnidial, produced on pine needles



FIGS. 2–7. *Diplodia africana* (STE-U 5908). 2. Conidia oozing from pycnidia. 3. Cryosection through a pycnidium. 4, 5. Conidia and conidiogenous cells. 6, 7. Conidia. Bars: 2 = 300 µm, 3 = 100 µm, 4–7 = 10 µm.

on SNA in 2–4 wk, solitary, globose to ovoid, dark brown, up to 500 µm wide, semi-immersed to erumpent, unilocular, sometimes multilocular in vitro, with a short neck and a central ostiole; wall 6–8 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* 1–2 celled, hyaline, 10–25 × 3.5–6 µm. *Conidiogenous cells* holoblastic, hyaline, cylindrical, sometimes ampulliform, proliferating percurrently near the apex, sometimes with periclinical thickening, 3–15 × 3–6 µm. *Conidia* aseptate, hyaline, thick-walled, smooth, subcylindrical to oblong-elliptical, sometimes slightly curved, with rounded ends, hyaline after discharge from pycnidia, a few of them becoming brown, septate and finely verruculose with age, (17–)25.5–33(–34) × (10–)12–14(–15) µm, mean ± SD = 29.2 ± 3.6 × 13 ± 1.1 µm, L/W ratio = 2.2.

Cultural characteristics. Colonies on PDA in the dark: mycelium pale olivaceous-gray, surface pale olivaceous-gray to dark gray-olivaceous, reverse olivaceous-black, umbonate with irregular zonation and lobate edges. Under near ultraviolet: mycelium and surface greenish olivaceous to dark gray-olivaceous; reverse greenish olivaceous to olivaceous-black. Colonies 26.8 mm diam after 2 d, reaching the edge the Petri dish within 5 d; cardinal temperature requirements for growth: minimum 5 C, maximum 35 C, optimum 20 C.

Host. *Prunus persica*.

Distribution. Paarl (South Africa, Western Cape Province).

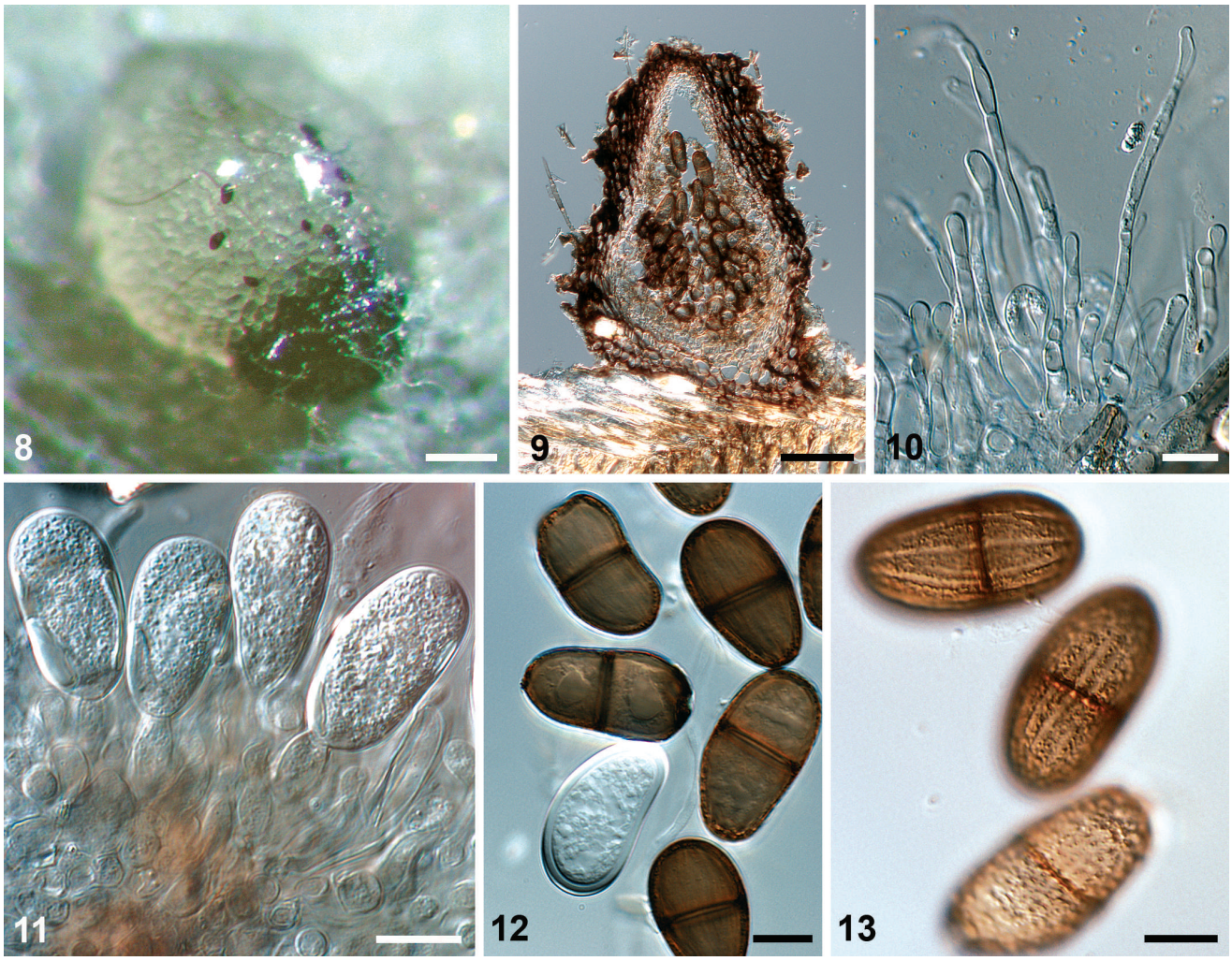
Specimens examined. SOUTH AFRICA. WESTERN CAPE PROVINCE: Paarl, from wood section close to pruning wound of *Prunus persica*, 10 Jun 2004, *U. Damm*, CBS H-19843 HOLOTYPE, culture ex-type CBS 120835 = STE-U 5908; from insect gallery associated with pruning wound canker of *Prunus persica*, 10 Jun 2004, *U. Damm*, CBS 120835 = STE-U 5946, STE-U 6289.

Notes. Unlike *Diplodia seriata*, *D. pinea*, *D. scrobiculata* and *"Sphaeropsis" pyriputrescens* (Shoemaker 1964, de Wet et al 2003, Xiao and Rogers 2004), the conidia of *D. africana* are hyaline and thick-walled even after discharge from pycnidia and only a few conidia become brown and septate with age (FIGS. 2, 6, 7). It shares these features with *D. mutila*, *D. corticola*, *D. cupressi*, *D. quercus*, *D. rosulata*, *D. quercina*, ''*B.*'' *tsugae* and the *Diplodia* anamorph of ''*B.*'' *quercuum*.

No spore dimensions are mentioned in the original description of *Sphaeria mutila* (syn. *D. mutila*) by Fries (1823) and in the description by Montagne (1834), who considered the fungus as the type species of the new genus *Diplodia*. According to the description of the teleomorph *Physalospora mutila* by Stevens (1936) and that by Shoemaker (1964), who proposed the name *Botryosphaeria stevensii*, the conidia are (20–)25–27 × 10–12(–16) µm. These dimensions recently were confirmed by Alves et al (2004), who studied type material of the fungus collected by Montagne. Dimensions provided by Sutton (1980) differ considerably from those, 27–31 × 12–13.5 µm. Conidia of *D. africana* are similar to those of *D. mutila* in being hyaline, aseptate and thick-walled. However this fungus differs from *D. mutila* in having markedly longer conidia, (17–)25.5–33(–34) × (10–)12–14(–15) µm.

Conidia of the *Diplodia* anamorph of ''*B.*'' *quercuum*, *D. rosulata* and *D. cupressi* have a different shape and L/W ratio than those of *D. africana*. While conidia of ''*B.*'' *quercuum* are subglobose, having a L/W ratio of only 1.5 (Shoemaker 1964), conidia of *D. rosulata* are oval to ellipsoid to ovoid, with a L/W ratio of 1.93 (Gure et al 2005) and conidia of *D. cupressi* are ovoid with a L/W ratio of 1.76 (Alves et al 2006), conidia of *D. africana* are subcylindrical to oblong-elliptical and have a L/W ratio 2.2. In addition conidia of *D. rosulata* and *D. cupressi* regularly turn brown and 1-celled after discharge from the pycnidium compared to *D. africana* (Gure et al 2005, Alves et al 2006). Conidia of *D. quercus* (anamorph of ''*B.*'' *quercicola*), measuring (24–)28.8–30.8(–38) × (11–)15.9–17.1(–21.2) (Phillips et al 2005), and *D. quercina*, with a mean of 29.5 × 16 µm (Jacobs and Rehner 1998), are wider than *D. africana*. However the *D. quercina* isolate studied by Jacobs and Rehner (1998) turned out to be *D. corticola* by sequence comparison (Alves et al 2004). Conidia of ''*B.*'' *tsugae* (Funk 1964) are much larger than *D. africana* at 36–41 × 18–22 µm. Conidia of *D. africana* have similar dimensions as *D. corticola*, (27.7–)29.6–30.3(–46.1) × (9.1–)13.4–13.8(–20.5) µm (Alves et al 2004). However the mean size of the conidia of *D. africana* (29.2 × 13 µm) is below the lower 95% confidence limits given for *D. corticola* and the conidia are always less than 40 µm long, unlike those of *D. corticola*. In addition the conidiophores of *D. corticola* are reduced to cylindrical conidiogenous cells (Alves et al 2004) while 1- and 2-celled conidiophores and cylindrical as well as ampulliform conidiogenous cells (FIGS. 4, 5) were observed in *D. africana*. The different shape, smaller size and the absence of pores separate *D. africana* from *Diplodia porosum*, which has ovoid to broadly ellipsoid conidia, (38–)42–45(–47) × (20–)22–25(–30) µm (van Niekerk et al 2004).

Wollenweber and Hochapfel (1941) described *Diplodia mutila* var. *major* (listing several synonyms) from bark of *Pyrus communis* in Karlshof close to former Löwenberg in Schlesia (today Poland) with elongate-ellipsoidal, mostly hyaline, aseptate conidia



FIGS. 8–13. *Lasiodiplodia plurivora* (STE-U 5803). 8. Pycnidium with conidia. 9. Cryosection through a pycnidium. 10. Conidiogenous cells and paraphyses. 11. Conidia and conidiogenous cells. 12, 13. Conidia. Bars: 8 = 300 µm, 9 = 50 µm, 10–13 = 10 µm.

that later became brown and 1(–3) septate, 26–34 × 11.5–16 (23–44 × 10–23) µm, mean = 29 × 13 µm. The description of *Diplodia mutila* var. *major* fits well with that of *D. africana*, except for the conidial shape that is elongate-ellipsoidal compared to *D. africana* that has mainly subcylindrical conidia. No type material could be located in Berlin, and thus this name could not be fully resolved. The ITS and EF-1α sequences of *D. africana* cluster with those of *D. mutila*. However they are markedly different from sequences of strain CBS 112553, which has been linked morphologically to the type material of *D. mutila* (Alves et al 2004) and strain STE-U 5824, confirmed morphologically as *D. mutila* in the present study, and of all other species of Botryosphaeriaceae available on GenBank (FIG. 1). According to its unique DNA sequence data and morphology we regard *D. africana* a distinct species instead of a variety of *Diplodia mutila*.

Lasiodiplodia plurivora Damm & Crous sp. nov. MycoBank MB 501322. FIGS. 8–13

Etymology. Named after its wide host range.

Lasiodiplodiae venezuelensi similis, sed conidiis latioribus, (22–)26.5–32.5(–35) × (13–)14.5–17(–18.5) µm.

Conidiomata pycnidial, produced on pine needles on SNA within 2–4 wk, solitary, globose to ovoid, dark brown, up to 400 µm wide, embedded in needle tissue, semi-immersed, unilocular, with a central ostiole; wall 4–7 cell layers thick, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, discrete, hyaline, cylin-

drical, proliferating percurrently several times near the apex, 8–13 × 4–7 μm. *Paraphyses* hyaline, cylindrical, 2–7 celled, the 1–3 basal cells often broader than the apical cells, apical cell with rounded tip, sometimes branched, up to 130 μm long, 2–5 μm broad at the upper part and up to 10 μm broad at the lower part (basal cells). *Conidia* initially aseptate, thick-walled (wall <3 μm), hyaline, ellipsoidal to obovate, sometimes somewhat irregular, with granular content, becoming 1-septate after release, brown, obovate, verruculose and with longitudinal striations, brittle, with outer wall easily breaking during slide preparations, (22–)26.5–32.5(–35) × (13–)14.5–17(–18.5), mean ± SD = 29.6 ± 2.9 × 15.6 ± 1.2 μm, L/W ratio = 1.9.

Cultural characteristics. *Colonies* on PDA in the dark: mycelium and surface white to pale olivaceous-gray, reverse pale olivaceous-buff to pale gray-olivaceous, flat with undulate margins. Under near-ultraviolet light: mycelium and surface white to pale mouse-gray, reverse pale olivaceous-buff to smoke-gray. Colonies 76 mm diam after 2 d, reaching the edge the Petri dish after 3 d; cardinal temperature requirements for growth: minimum 10 C, maximum ≥35 C, optimum 30 C.

Hosts. *Prunus salicina* and *Vitis vinifera*.

Distribution. Stellenbosch (South Africa, Western Cape Province).

Specimens examined. SOUTH AFRICA. WESTERN CAPE PROVINCE: Stellenbosch, from V-shaped necrotic lesion of *P. salicina*, 28 May 2004, *U. Damm*, CBS H-19844 HOLOTYPE, culture ex-type CBS 120832 = STE-U 5803; Western Cape Province, from *Vitis vinifera*, STE-U 4583.

Notes. Septate, brown, mature conidia with longitudinal striations and the presence of paraphyses characterize this fungus as belonging to the anamorph genus *Lasiodiplodia* within the Botryosphaeriaceae (FIGS. 10, 12, 13). Conidia of *Lasiodiplodia plurivora*, (22–)26.5–32.5(–35) × (13–)14.5–17(–18.5) μm, are markedly longer and wider than *L. theobromae* (20–30 × 10–15 μm) and shorter and narrower than *L. gonubiensis* (28–)32–36(–39) × (14–)16–18.5(–21) (Griffon and Maublanc 1909, Pavlic et al 2004). They are wider than *L. venezuelensis* (26–33 × 12–15) but do not differ much in size from *L. crassispora* ([27–]30[–33] × 14–17) and *L. rubropurpurea* (24–33 × 13–17) (Burgess et al 2006). However an important character of *Lasiodiplodia plurivora* is the paraphyses. They are septate as in *L. venezuelensis* and *L. crassispora*, but in contrast paraphyses of *Lasiodiplodia plurivora* are up to 130 μm long and up to 10 μm wide at the base and frequently branched, whereas paraphyses of the other two species are unbranched, <70 μm long and ≤4 μm wide (Burgess et al 2006). Furthermore ITS and EF1-α sequence data of *L. plurivora* are different from *L. theobromae* and from the four *Lasiodiplodia* species described by Pavlic et al (2004) and Burgess et al (2006) (FIG. 1).

Pathogenicity. The majority of the 67 isolates had been obtained from wood necrosis within living branches of *Prunus* species, which were in cross-section either V-shaped, roundish or irregularly shaped, brown necrotic areas and sometimes situated next to cankers or resin excretion. One isolate of *Lasiodiplodia plurivora* and one isolate of *Dothiorella viticola* had been obtained respectively from internal wood necrosis and bark of small dead trees. Only nine isolates of *Diplodia seriata*, three isolates of *D. pinea* and one isolate of *Neofusicoccum australe* originated from pycnidia from the bark of pruning material found on the orchard floor. Each of the eight species of Botryosphaeriaceae was associated with necrosis symptoms in at least one sample, which indicated a possible pathogenic relationship.

Analyses of variance of the lesion length data on nectarine and plum cane sections indicated a significant treatment effect ($P < 0.0001$, ANOVA tables not shown). All species except *Dothiorella viticola* caused lesions on nectarine shoots, and all species except *D. viticola* and *D. pinea* caused lesions on plum shoots that were significantly longer than the controls (TABLE II). Except for *D. pinea* lesion lengths caused by the species were similar in plum and nectarine shoots. The fungi were re-isolated from these lesions with frequencies of 68.75–100% (TABLE II). No species of Botryosphaeriaceae were isolated from the negative controls.

DISCUSSION

This study revealed eight species of Botryosphaeriaceae to be associated with disease symptoms on stone fruit trees in South Africa. These included *Diplodia seriata*, *D. pinea*, *D. mutila*, *Dothiorella viticola*, *Neofusicoccum australe*, *N. vitifusiforme*, and two new species, *Lasiodiplodia plurivora* and *Diplodia africana*. These species could be distinguished based on their DNA sequence data and unique morphological characteristics.

Based on literature reports *B. dothidea* seems to be the most important species of Botryosphaeriaceae occurring on peach in other countries (Pusey et al 1995). However, because *B. dothidea*, *N. ribis* (*B. ribis*) and *N. parvum* (*B. parva*) often were considered synonymous until Slippers et al (2004a) clarified the concept of *B. dothidea* and distinguished the three species, earlier reports could include all three species (*B. dothidea sensu lato*). In South Africa *B. dothidea s. l.* has been reported from plants such as *Eucalyptus*, *Pistacio* and Proteaceae, *N. ribis* from *Persea*, *Melia*,

TABLE II. Means of lesion lengths caused by different species of Botryosphaeriaceae on detached green nectarine and plum shoots and mean re-isolation frequencies of these species from observed lesions

	Mean of lesion length (mm)[a]		Mean of re-isolation frequency (%)	
Fungal species	Nectarine	Plum	Nectarine	Plum
Diplodia mutila	107.5 a	121.3 a	87.5	100
Lasiodiplodia plurivora	101.5 ab	121.5 a	100	100
Neofusicoccum australe	91.9 ab	108.8 ab	100	91.7
D. africana	89.4 ab	102.9 ab	68.75	100
D. seriata	86.4 ab	89.5 bc	87.5	91.7
N. vitifusiforme	79.5 b	63.8 c	79.2	100
D. pinea	56.7 c	21.8 d	87.5	91.7
Dothiorella viticola	10.0 d	7.2 d	75	75
Acremonium strictum	5.5 d	5.9 d	37.5	50
Agar plug	4.8 d	6.0 d	—	—
LSD ($P < 0.05$)	22.66	31.65		

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

Eucalyptus, *Macadamia*, *Malus*, *Citrus* and Proteaceae (Crous et al 2000), and *N. parvum* from *Vitis* (van Niekerk et al 2004). In spite of these reports however we did not isolate any strains of *B. dothidea*, *N. ribis* or *N. parvum* from *Prunus* wood. In a study on stone and pome fruit trees in South Africa these species also were not found (Slippers et al 2007). In contrast *D. seriata* proved to be the most frequently isolated (43 of 67 isolates) and occurred on all host plants in all three areas studied. It was also the dominant species in the study by Slippers et al (2007). This species also is common on grapevines in South Africa (van Niekerk et al 2004) and Australia (Taylor et al 2005). *Diplodia seriata* has a worldwide distribution and a wide host range, of which 128 host records presently are known from USA alone (Farr et al 1989). Although it is common on peach worldwide (Dingley and Brien 1956, Britton and Hendrix 1982) and has been found on apricot in Wisconsin, USA, (Smith and Stanosz 2006) and on peach fruits and plum and peach wood in South Africa (Combrink et al 1984, Slippers et al 2007), this is the first report of *B. obtusa* on apricot and nectarine in South Africa. The fungus was shown to cause lesions on both nectarine and plum shoots. In a greenhouse experiment by Britton and Hendrix (1982) *D. seriata* caused gummosis canker on peach trees.

Four isolates (STE-U 5808, 5809, 5812, 5901) originating from pycnidia on bark or symptomatic tissue from a peach orchard in the Paarl area were morphologically similar to *D. seriata* but proved to be identical to the *Diplodia pinea* A morphotype (CBS 393.84) based on the ITS and EF-1α dataset and varied from the *D. pinea* C morphotype (CBS 109725) in only one substitution. Because of little sequence variation of *D. pinea* to all *D. seriata* isolates (two substitutions in ITS and one in EF-1α) and additional substitutions within both species, there was only little bootstrap support (62%) for the grouping of the four isolates with *D. pinea*. But these differences in sequence were reflected in morphological differences. While the conidia size of all *D. seriata* isolates found on *Prunus* wood matched the size given for this species (22–26 × 10–12 μm, Shoemaker 1964) the conidia of these four isolates were larger, (24–)30–37(–43) × (10–)12.5–17(–21) μm, and often were paler than those of *D. seriata* isolates. This corresponded to the description of *D. pinea* conidia that are yellowish brown, eventually turning dark brown, measuring 30–45 × 10–16 μm (Punithalingam and Waterston 1970). In addition the fungi have a fluffy white to gray mycelium and sporulated less abundantly than the *D. seriata* isolates. Palmer et al (1987) described morphotype A as having a fluffy white to gray-green mycelium and conidia 34.3–39.4 × 12.6–12.8 μm and morphotype B with a white to black mycelium closely appressed to the agar surface and conidia 33.5–34.3 × 11.6–12.1 μm. Sequence and cultural characteristics of our isolates were different from *D. pinea* morphotype B, described as *D. scrobiculata* (de Wet et al 2003), but similar to morphotypes A and C (de Wet et al 2000), which differ in distribution, host specificity, virulence (de Wet et al 2003) and conidial size (de Wet et al 2000).

According to isozyme comparisons by Swart et al (1991), RAPD analysis by Stanosz et al (1999) and de Wet (2000) and simple sequence repeat marker analyses by Burgess et al (2001), *D. pinea* isolates from *Pinus* spp. in South Africa and neighboring

countries group with the A morphotype of *D. pinea*. Because sequences of the *Prunus* isolates were identical to those of the *D. pinea* A morphotype we conclude that these isolates also are representative of morphotype A.

Diplodia pinea is an important pathogen on *Pinus* spp. worldwide (Punithalingam and Waterston 1970), including South Africa, where it has been known since 1912 (Fisher 1912), and it also has been reported on *Abies*, *Araucaria*, *Chamaecyparis*, *Cupressus*, *Larix*, *Picea*, *Pseudotusga*, *Thuja* (Coniferae, Gymnospermae) (Punithalingam and Waterston 1970, Sutton 1980). The fungus also has been reported on *Eucalyptus* spp. and native species of Myrtaceae in Uruguay (Bettucci et al 1999, 2004), although these reports are unconfirmed. This study is the first report of *D. pinea* on *Prunus*. While the other species were isolated mainly from necrosis inside the wood of *Prunus* trees, three of the four *D. pinea* isolates obtained in this study were derived from pycnidia on the bark of pruning debris. The fungus caused lesions on nectarine shoots that were significantly longer than the control but considerably shorter than those from most of the other species tested.

Neofusicoccum australe was reported from grapevine (van Niekerk et al 2004) and plum in South Africa (Slippers et al 2007) but considered to be infrequent and of minimal importance in stone fruits. In the present study it was found commonly on three host species (peach, plum and apricot) in different areas in Western Cape, often in V-shaped necrotic tissue. Taylor et al (2005) isolated *N. australe* (and *D. seriata*) from wedge- and half moon-shaped internal lesions in grapevine wood in Australia where the fungus frequently occurred. This is the first report of *N. australe* on peach and apricot. According to the long lesion lengths on nectarine and plum shoots, the fungus appears to have considerable potential as pathogen of stone fruits.

Neofusicoccum vitifusiforme was isolated from symptoms on plum and peach in two South African provinces. The fungus recently was described from grapevine in South Africa (van Niekerk et al 2004) but had not been isolated previously from *Prunus*.

Dothiorella viticola also is newly reported from *Prunus* and thus far was known from grapevines in South Africa (van Niekerk et al 2004) and Spain (Lurque et al 2005). In this study it also was isolated for the first time from necrotic wood of plum and nectarine.

The conidial dimensions of isolate STE-U 5824 closely correspond to those of *D. mutila*. This isolate also is identical phylogenetically to the reference strain of *D. mutila* (CBS 112553). *Diplodia mutila* was found on necrotic plum wood in Paarl. The fungus was reported on peach in New Zealand by Laundon (1973) and on apricot and peach by Sutton (1980). This is its first report from South Africa, where it was found to occur in plum wood.

Diplodia africana initially was identified as *D. mutila*. However by comparing sequence data and conidial measurements of the different isolates found on *Prunus* and those in GenBank and in the literature it became obvious that it is related closely to *D. mutila*, *D. corticola* and the *Diplodia* anamorph of ''*B.*'' *quercuum* but is different from them as well as from other *Diplodia* species and most probably is new to science. DNA sequence data (ITS and EF-1α) of *D. africana* differ from all known species of Botryosphaeriaceae. The fungus was found several times in one peach orchard in Paarl, Western Cape, South Africa, and was shown to be potentially pathogenic to nectarine and plum shoots.

While *L. theobromae* is the most common *Lasiodiplodia* species in tropical regions (Burgess et al 2006) and a common cause of blister canker of peach (Britton and Hendrix 1982), this species was not found on any of the *Prunus* species studied. However another *Lasiodiplodia* species was isolated from plum wood that is distinct morphologically and phylogenetically from *L. theobromae* and all four recently described *Lasiodiplodia* spp. (Pavlic et al 2004, Burgess et al 2006). Morphological differences were found in conidial size, paraphyses size, septation and shape and pycnidial color. Conidia of *L. plurivora* are larger than those of *L. theobromae* and smaller than those of *L. gonubiensis*. Paraphyses are septate as in *L. venezuelensis* and *L. crassispora* but much longer and frequently branched.

The ITS sequence of *L. plurivora* (STE-U 5803) is identical to the ITS of isolate STE-U 4583 from *V. vinifera* in South Africa (van Niekerk et al 2004), isolates WAC11080 (GenBank AY727846) and WAC11081 (AY727847) from *Vitis vinifera* in Western Australia (Taylor et al 2005) and isolate MAMB-5 (AY612337) from a stem canker on an eucalypt tree in Australia (Barbosa et al 1995). Isolate STE-U 4583 (AY343482), formerly identified as *L. theobromae*, was included in this study and could be confirmed to be *L. plurivora* based on conidial dimensions and EF-1α sequence data. Conidial dimensions of WAC11080 and WAC11081 were also the same as in *L. plurivora*, (27–)30–32(–34) × (15–)16–18(–20) and (26–)30–32(–34) × 16–18, respectively (Taylor et al 2005). Concerning isolate MAMB-5, electrophoretic examination of intracellular marker genes (esterases, phosphatases) and laccases (Vasconcelos et al 2001) revealed that isolate MAMB-5 was different genetically from *L. theobromae*. The newly described fungus *L.*

plurivora occurs on plum, grapevine and eucalypt wood in South Africa and Australia. Due to its morphological similarity to *L. theobromae* and similar ITS sequence this fungus had been reported erroneously as *L. theobromae* and further collections are now required to resolve its distribution, host range and relative importance on woody hosts.

According to the results obtained in the pathogenicity test (TABLE II) *Diplodia mutila*, *L. plurivora*, *N. australe*, *D. africana*, *D. seriata* and *N. vitifusiforme* can be considered potentially pathogenic to *Prunus salicina* while *D. pinea* and *Dothiorella viticola* appear to be nonpathogenic to this host under the conditions tested here. All species, excluding *Dothiorella viticola*, also can be considered potentially pathogenic to *P. persica*. While the two *Lasiodiplodia* isolates from grapevine from Australia produced only small lesions on grapevine in the pathogenicity trial by Taylor et al (2005), isolate STE-U 5803 produced long lesions on plum and peach. These observations correspond to results pertaining to *Lasiodiplodia* isolate MAMB-5, which produces high amounts of lignocellulose-degrading enzymes (Dekker et al 2001). The amounts of extracellular laccase production of this isolate were much higher than that of *B. ribis* and *L. theobromae* (Vasconcelos et al 2001). It appears therefore that *L. plurivora* is well equipped for biodegradation of woody plant material, and based on our preliminary pathogenicity tests it appears to be highly virulent on nectarine and plum shoots. However further pathogenicity tests should be conducted on living plants to satisfy Koch's postulates.

In this study several species of Botryosphaeriaceae were found on a variety of *Prunus* species in South Africa. Most were reported for the first time on these host plants and most of them were shown to be potentially pathogenic to *Prunus* species. Compared with other reports on *Prunus* spp. (Pusey et al 1995, Slippers et al 2007) the species composition of Botryosphaeriaceae in stone fruit orchards in South Africa seems to be different and comprises a broader variety of species. The impact of these species on the productivity of stone fruit orchards in South Africa has not been studied yet. It should be noted also that most of the species found on *Prunus* spp. in this study are known grapevine pathogens in South Africa and/or other regions in the world (van Niekerk et al 2004, Taylor et al 2005). Because the orchards chosen for sampling were situated next to vineyards these species could have spread from grapevine plants to stone fruit trees. On the other hand infected stone fruit trees and debris could act as an inoculum source from which grapevines could be infected and/or stone fruit trees could act as alternative hosts in the absence of grapevine plants.

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