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Botryosphaeria eucalyptorum sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa

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Abstract: Two morphologically similar fungi are associated with canker and die-back of eucalypts in South Africa, one of which was identified as part of the *Botryosphaeria dothidea*-complex. In this study, the identity of the other fungus was determined by comparing morphology, pathogenicity and DNA sequence analysis of isolates of both taxa. Based on these results, a new species, *Botryosphaeria eucalyptorum*, and its anamorph *Fusicoccum eucalyptorum*, are described. Although the teleomorph is morphologically similar to other taxa in the *B. dothidea*-complex, conidial characteristics of the anamorph are distinct, as well as the sequences of the nrDNA internal transcribed spacers ITS1 and ITS2. Like *B. dothidea*, the fungus is pathogenic to *Eucalyptus*, although there do not appear to be clear differences in pathogenicity between these two species.

Key Words: canker pathogen, endophyte, systematics

INTRODUCTION

Botryosphaeria dothidea (Moug.: Fr.) Ces. & De Not. was first described by Cesati and de Notaris in 1863 when they established the genus *Botryosphaeria*. The fungus has a cosmopolitan distribution and is associated with diseases of at least 70 plant genera (Smith 1934, Punithalingam and Holliday 1973), including *Eucalyptus* and *Pinus* species (Davison and Tay 1983, Hodges 1983, Webb 1983, Barnard et al 1987, Shearer et al 1987, Smith et al 1994). *Botryosphaeria dothidea* causes die-back and canker symptoms on various *Eucalyptus* species in South Africa (Smith et al 1994) and is thus of concern to commercial forestry in this country.

Considerable controversy exists surrounding the taxonomic status of *B. dothidea* and *Botryosphaeria ribis* (Tode & Fr.) Grossenb. & Duggar. Some authors regard the species as synonyms (Arx and Müller 1954, Witcher and Clayton 1963, English et al 1975, Spiers 1977, Brown and Hendrix 1981, Maas and Uecker 1984, Pusey 1989), while others have treated them as separate taxa (Smith 1934, Punithalingam and Holliday 1973, Rumbos 1987, Rayachhetry et al 1996). Pennycook and Samuels (1985) recognized the diversity within *B. dothidea*, and consequently referred to it as *B. dothidea* sensu lato. If the proposed synonymy of *B. dothidea* and *B. ribis* is upheld (Arx and Müller 1954), the name *B. dothidea* (1863) would have priority, as it predates *B. ribis* (1911) (Witcher and Clayton 1963). However, recent molecular data (Jacobs and Rehner 1998) suggest that separate species exist in this complex. Whether these species can be attributed to *B. dothidea* or *B. ribis*, however, remains to be determined.

The teleomorphs of *Botryosphaeria* are rarely encountered in nature, and much confusion has surrounded the taxonomic classification of the anamorphs. The general similarity based on the morphological descriptions of the anamorphs, and difficulties encountered in inducing strains to sporulate in culture, has resulted in confusion relating to the delimitation of species. This is aptly illustrated by the fact that different species of *Fusicoccum* Corda or *Dothiorella* Sacc. have in the past been linked to either *B. dothidea* or *B. ribis* (Arx and Müller 1954, Grossenbacher and Duggar 1911, Webb 1983, Gardner and Hodges 1990).

Fusicoccum aesculi Corda, the accepted name of the anamorph of *B. dothidea* (Arx and Müller 1954, Sutton 1980, Pennycook and Samuels 1985), has been

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reported as a common endophyte from asymptomatic leaves of various *Eucalyptus* spp. in South Africa (Smith et al 1996a, b). Recently, a species of *Botryosphaeria* resembling species in the *B. dothidea*-complex, but with an anamorph morphologically distinct from *F. aesculi,* consistently has been isolated from stem cankers of *Eucalyptus* spp. in South Africa. Thus, two species of *Botryosphaeria* appear to occur within the same niche on *Eucalyptus* in this country.

The internal transcribed spacers (ITS1 and ITS2) of the nrDNA operon have been successfully employed to analyze intra- and interspecific relationships in various fungi (Berbee and Taylor 1993). In some cases these studies have allowed species delimitation where morphological characters have not been useful (Anderson and Stasovski 1992, Rehner and Uecker 1994, Witthuhn et al 1998).

The aim of the present study was to determine whether there were one or two species of *Botryosphaeria* on *Eucalyptus* in South Africa, and if two, whether they were equally pathogenic to *Eucalyptus.*

MATERIALS AND METHODS

Isolates.—Most isolates included in this study were collected in 1995 during a survey of eucalypt diseases in the Mpumalanga Province of South Africa. Three isolates were collected from indigenous Myrtaceae growing in the KwaZulu-Natal Province (TABLE I). Isolations were made from twigs with die-back symptoms as well as from branch and main stem cankers.

Morphology and cultural characteristics.—Isolates were placed on 2% malt extract agar (MEA) (20 g/L malt extract and 12 g/L agar; Biolab, Midrand, Johannesburg) under continuous fluorescent light for up to 3 wk at 25 C to promote sporulation. Growth rate was determined by placing three single conidial isolates on MEA plates in the dark, at temperatures ranging from 5–35 C at 5 C intervals. Three replicate plates were used per isolate for each temperature. Two perpendicular measurements were obtained after 4 d for each colony, and averages determined. Colony colors (upper surface and reverse) were determined using the color charts of Rayner (1970). Wherever possible, thirty measurements were made of mature structures mounted in lactophenol, the 95% confidence intervals determined, and the extremes given in parentheses. Type specimens are lodged at the National Collection of Fungi, Pretoria (PREM), and ex-type cultures maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA isolation.—Isolates (TABLE I) were grown on MEA in Petri dishes for 4 d at 25 C in the dark. Template DNA was obtained by an extraction method modified from Raeder and Broda (1985). Mycelium was scraped from the colony surface with a scalpel and transferred to sterile Eppendorf tubes (1.5 mL) and 100 µL of an extraction buffer (200 mM Tris-HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5%

TABLE I. Identity and origin of isolates studied

Isolate No.	GenBank No.	Teleomorph	Anamorph	Host	Location	Collector
BOT 275	AF283689	Unknown	*Sphaeropsis sapinea*	*Pinus patula* Schl. & Cham.	Indonesia	M. J. Wingfield
CMW 3141	AF283675	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Cercis canadensis* L.	Columbia, U.S.A.	K. A. Jacobs
BOT 682	AF283680	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Syzygium guinesee* (Willd.) DC.	KwaZulu-Natal, R.S.A.	H. Smith
BOT 681	AF283676	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Heteropyxis natalensis* Harvey	KwaZulu-Natal, R.S.A.	H. Smith
BOT 683	AF283677	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Syzygium cordatum* Hochst.	KwaZulu-Natal, R.S.A.	H. Smith
BOT 7	AF283678	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Eucalyptus grandis* Hill: Maid.	Mpumalanga, R.S.A.	H. Smith
BOT 21	AF283681	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Eucalyptus grandis* Hill: Maid.	Mpumalanga, R.S.A.	H. Smith
BOT 30	AF283682	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Eucalyptus grandis* Hill: Maid.	Mpumalanga, R.S.A.	H. Smith
BOT 19	AF283683	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*Eucalyptus smithii* R. T. Bak.	Mpumalanga, R.S.A.	H. Smith
BOT 25	AF283679	*Botryosphaeria dothidea*	*Fusicoccum aesculi*	*E. grandis*	Swaziland	H. Smith
BOT 11	AF283684	*Botryosphaeria* sp.	*Fusicoccum* sp.	*E. grandis*	Mpumalanga, R.S.A.	H. Smith
BOT 16	AF283687	*Botryosphaeria* sp.	*Fusicoccum* sp.	*E. grandis*	Mpumalanga, R.S.A.	H. Smith
BOT 2	AF283688	*Botryosphaeria* sp.	*Fusicoccum* sp.	*Eucalyptus nitens* (Deane et Maid.) Maid.	Mpumalanga, R.S.A.	H. Smith
BOT 24	AF283686	*Botryosphaeria* sp.	*Fusicoccum* sp.	*E. grandis*	Mpumalanga, R.S.A.	H. Smith
BOT 32	AF283685	*Botryosphaeria* sp.	*Fusicoccum* sp.	*E. grandis*	Mpumalanga, R.S.A.	H. Smith
CMW 3025	AF283690	*Mycosphaerella africana*	—	*Eucalyptus viminales* Labill.	Stellenbosch, R.S.A.	P. W. Crous

SDS) was added to each tube. Tubes were immersed in liquid nitrogen and mycelium was ground with a pestle until a homogenous solution was obtained. At this stage, a further 400 µL of extraction buffer were added to each tube. Extraction of template DNA was achieved by repeated addition of phenol (350 µL) and chloroform (150 µL) with centrifugation (13 000 rpm, 1 h). Template DNA was precipitated overnight at −20 C with isopropanol (0.54 volume) and 3 M NaAc (0.1 volume). The DNA was pelleted at 13 000 rpm for 10 min at 4 C. The resulting pellets were washed with cold 70% ethanol (100 µL) and dried. The dried pellets of template DNA were resuspended in sterile water (100 µL).

PCR amplifications were performed with the primers ITS1 and ITS4 (White et al 1990). The amplified fragments included the 3′ end of the small subunit (SSU) rRNA gene, the 5.8S gene, part of the large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS) regions 1 and 2. The PCR reaction mixture contained 2.5 units of *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany), the buffer supplied with the enzyme, 250 µM dNTPs, 6.25 mM $MgCl_2$ and 0.5 µM of each primer. Initial denaturation was performed at 93 C for 3 min, followed by 35 cycles of primer annealing at 58 C for 45 s, chain elongation at 72 C for 90 s and denaturation at 92 C for 30 s. Final chain elongation took place at 72 C for 15 min. The PCR products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light.

The PCR fragments amplified were approx 560 bp in size. These were purified using the QIAquick PCR purification kit. Both strands of the PCR products were sequenced using the Thermo Sequenase Dye Terminator Cycle Sequencing Pre-mix Kit (Amersham Life Science). Samples were run on an ABI Prism 377 DNA sequencer and the sequence analyzed using Sequence Navigator (Perkin-Elmer). The primers ITS1, ITS4, CS2 and CS3 (Wingfield et al 1996) were used in sequencing reactions. The nucleotide sequences were aligned using CLUSTAL W (Thompson et al 1994), improved manually where necessary, and analyzed using PAUP* version 4.0b2a (Swofford 1999). The alignment was deposited in TreeBase (S539). Maximum parsimony trees were generated using the heuristic search option with 1000 random addition replicates with the tree bisection reconnection (TBR) algorithm. Support for the clades was assessed by 1000 bootstrap replicates (Felsenstein 1985). *Mycosphaerella africana* Crous & MJ Wingf. was used as outgroup because *Botryosphaeria* and *Mycosphaerella* are both placed in the Dothideales (Crous 1998). Sequence data of isolates were deposited in GenBank (AF283675—283690).

Pathogenicity.—Isolates BOT 7, 19, 21, 25, 30 (*B. dothidea*) and BOT 2, 11, 16, 24 and 32 (*Botryosphaeria* sp.) were evaluated for their pathogenicity in inoculation trials. Inoculations were conducted on a susceptible *E. grandis* clone (ZG 14) in the Kwambonambi area, KwaZulu-Natal province in early spring (Sep 1997). The 6-mo-old trees had stem diameters of between 4 and 6 cm. Trees were actively growing during the time that the trials were conducted and no environmental stresses were apparent. Bark disks (5-mm diam) were removed with a cork borer (10 trees per isolate, 1 isolate per tree) and replaced with an agar disk colonized with mycelium from 4-d-old isolates growing on MEA. Controls were inoculated with sterile MEA disks. Wounds were sealed with masking tape to reduce desiccation. Lesion lengths (mm) were recorded 1 mo after inoculation. The entire trial was repeated once on trees in the same plantation.

RESULTS

Morphology and cultural characteristics.—All isolates of the unnamed *Botryosphaeria* sp. produced *Fusicoccum* anamorphs as defined by Crous and Palm (1999). The unnamed *Botryosphaeria* sp. had fusoid ascospores (20–)23–26(–28) × (7–)8–9(–11) µm, overlapping somewhat with the more ovoid to ellipsoid ascospores of *B. ribis* (17–23 × 7–10 µm) (Punithalingam and Holliday 1973) and *B. dothidea* (13–)19–27(–35) × (6–)8–11(–14) µm) (Pennycook and Samuels 1985). The *Fusicoccum* conidia of the unnamed *Botryosphaeria* sp. [(20–)22–25(–28) × (6–)7–8 µm in vivo, (18–)20–23(–25) × 7–8(–12) µm in vitro] were similar in length, but slightly wider than those of *F. aesculi* (anamorph of *B. dothidea*) [18–25(–30) × 4–4.5(–5) µm (immature type in vivo, Crous and Palm 1999); (15–)20–26(–32) × (4–)5–6(–9) µm in vitro (Pennycook and Samuels, 1985)], and the *Fusicoccum* anamorph of *B. ribis* (16–31 × 4.5–8 µm in vivo, Grossenbacher and Duggar 1911; 14–23 × 3–4.5 µm in vitro, Morgan-Jones and White 1987). Conidia of *B. dothidea* and *B. ribis* vary from being fusiform to narrowly ellipsoidal (widest just above the middle), while conidia of the unnamed *Botryosphaeria* sp. are ovoid to clavate, generally having their widest point closer to the apex.

ITS sequence data and phylogeny.—The PCR of all isolates consistently produced amplification products of approx. 560 bp. The DNA sequences of all the *Botryosphaeria* isolates were found to be similar. It was possible to align the data manually by inserting gaps in the sequence data. With *M. africana* as the outgroup, 152 most parsimonious trees of 302 steps were produced from the aligned 529 bp sequence data, using the heuristic search option of PAUP* (FIG. 1) (CI = 0.904, HI = 0.096, RI = 0.854). Gaps were treated as a fifth character (gapmode = newstate). The trees differed only in the position of isolates within terminal groupings and not among clades. The overall topology of all 152 most parsimonious trees was, therefore, identical. Four principal clades were formed, designated as clades I to IV. All the branch points of the clades had bootstrap values (Hillis and Bull 1993) greater than 70%.

Clade I had a bootstrap support value of 97% and included asexual *Fusicoccum* isolates that matched

TABLE II. Lesion lengths produced by *Botryosphaeria dothidea* and *B. eucalyptorum* after inoculation of the *Eucalyptus grandis* clone (ZG 14)

Isolate No.	Identification	Lesion length (mm)[a]
BOT 7	*B. dothidea* (ITS clade I)	109 a
BOT 19	*B. dothidea* (ITS clade I)	98 a
BOT 21	*B. dothidea* (ITS clade I)	90 a
BOT 2	*B. eucalyptorum* (ITS clade III)	66 b
BOT 32	*B. eucalyptorum* (ITS clade III)	57 b
BOT 16	*B. eucalyptorum* (ITS clade III)	55 b
BOT 24	*B. eucalyptorum* (ITS clade III)	46 b
BOT 11	*B. eucalyptorum* (ITS clade III)	45 b
BOT 30	*B. dothidea* (ITS clade I)	23 c
BOT 25	*B. dothidea* (ITS clade I)	22 c

[a] Lesion lengths represent the mean of two replications of ten trees each. Numbers followed by a different letter are significantly different ($P = 0.05$).

the description of *F. aesculi*. Clade II has 100% bootstrap support and consisted of two *Fusicoccum* isolates also are part of the *B. dothidea*-complex (Jacobs and Rehner 1998, AF027750 and AF027746). Clade III had 99% bootstrap support and included 5 isolates of the unnamed *Botryosphaeria* sp. from *Eucalyptus*. Clade IV consisted of a single isolate of *F. luteum* (Jacobs and Rehner 1998, AF027745).

Pathogenicity.—All isolates screened in this study produced lesions in the secondary phloem 1 mo after inoculation (TABLE II). The most virulent isolate was BOT 7, while BOT 25 was least virulent. Both of these isolates belonged to the *F. aesculi* (= *B. dothidea*) group (clade I) (FIG. 9). Isolates representing the unnamed species of *Botryosphaeria* (clade III) did not differ significantly from one another based on lesion length ($P = 0.05$). The five *F. aesculi* (= *B. dothidea*) isolates tested (clade I) caused significantly longer lesions (species mean = 34.3 mm) than the five isolates of the unnamed *Botryosphaeria* sp. (clade III) (species mean = 26.9 mm; contrast testing $P = 0.05$).

Based on the morphological differences observed (primarily in conidium taper), as well as differences in pathogenicity and DNA phylogeny, we describe the unnamed species from *Eucalyptus* as new.

Botryosphaeria eucalyptorum Crous, H. Smith et M. J. Wingf. sp. nov. FIGS. 1–8

Anamorph. **Fusicoccum eucalyptorum** Crous, H. Smith et M. J. Wingf. sp. nov.

Ascostromata in contextu hospitis inclusa, usque ad 300 µm diametro, erumpescentia, solitaria, botryosa, stromatiformia, atrobrunnea vel nigra, cum ostiolis centralibus nigris. Asci clavati, inter paraphyses filiformes interspersi, 70–140 × 15–21 µm, octosporati, bitunicati cum loculo apicali bene evoluto. Ascosporae irregulariter biseriatae, hyalinae, unicellulares, granulares, cum aetate pallide brunnescens, (20–)23–26(–28) × (7–)8–9(–11) µm, juventute valde inaequilaterae, maturitate minus ita, fusoides, medio latissimae, fundis obtusis, apicibus obtusis vel subobtusis. Pycnidia in contextu hospitis inclusa, solitaria vel botryosa, stromatiformia, globosa, usque ad 450 µm diametro; paries pycnidii e stratis 6–8 formata, e textura angulari brunnea composita, ad intima hyalinescens. Cellulae conidiogenae holoblasticae, hyalinae, subcylindricae, 10–25 × 3.5–6 µm, percurrenter cum 1–2 proliferationibus prolificentes, vel in plano eodem periclinaliter minuter incrassatae. Conidia hyalina, granularia, ovoidea vel subclavata, apicibus obtusis, in fundo subtruncato vel obtuse rotundato angustata, interdum cum fimbria marginali minuta, in conidiis junioribus manifesta, (18–)23–25(–28) × (6–)7–8 µm.

Ascostromata embedded in host tissue, up to 300 µm diam, becoming erumpent, solitary or botryose, stromatic, dark brown to black, with central, black ostioles. *Asci* clavate, interspersed amongst filiform paraphyses, 70–140 × 15–21 µm, 8-spored, bitunicate with a well-developed apical chamber (FIGS. 3–6). *Ascospores* irregularly biseriate, hyaline, nonseptate, granular, becoming light brown with age, (20–)23–26(–28) × (7–)8–9(–11) µm, prominently inequilateral when young, less so when mature, fusoid, widest in the middle, base obtuse, apex obtuse to subobtuse (FIG. 2). *Pycnidia* embedded in host tissue, solitary or botryose, stromatic, globose, up to 450 µm diam.; pycnidial wall, 6–8 cell layers thick, composed of brown *textura angularis*, becoming hyaline towards the inner region. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, 10–25 × 3.5–6 µm, proliferating percurrently with 1–3 proliferations, or proliferating at the same level with minute periclinal thickening (FIG. 1). *Conidia* hyaline, granular, ovoid to slightly clavate, apex obtuse, tapering towards a subtruncate or bluntly rounded base, sometimes with a minute marginal frill visible on younger conidia, (20–)22–25(–28) × (6–)7–8(–9) µm in vivo, (18–)20–23(–25) × 7–8(–12) µm in vitro (FIGS. 1, 7, 8). *Cultures* producing iron gray colonies (25''''k) (reverse), and olivaceous gray (25''''i) (surface), with extensive gray aerial mycelium, and smooth margins. Colonies obtaining a radius of 21–24 mm diam on MEA after 4 d in the dark at 25 C. Cardinal temperatures for growth were min above 5 C, max below 35 C, opt 25 C.

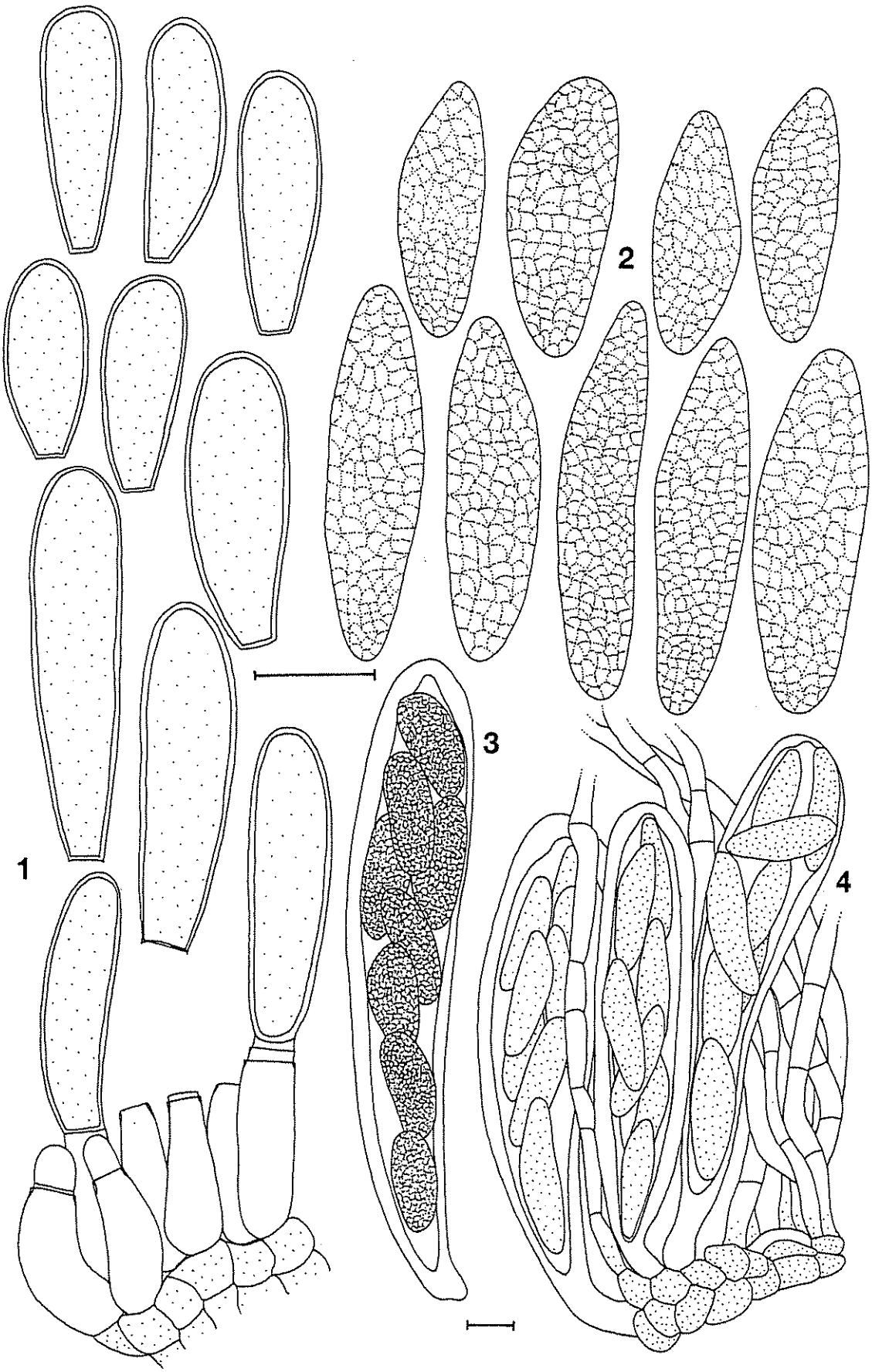
Anamorph. Fusicoccum eucalyptorum Crous, H. Smith et M. J. Wingf. sp. nov.

Etymology. In reference to its host, *Eucalyptus*.

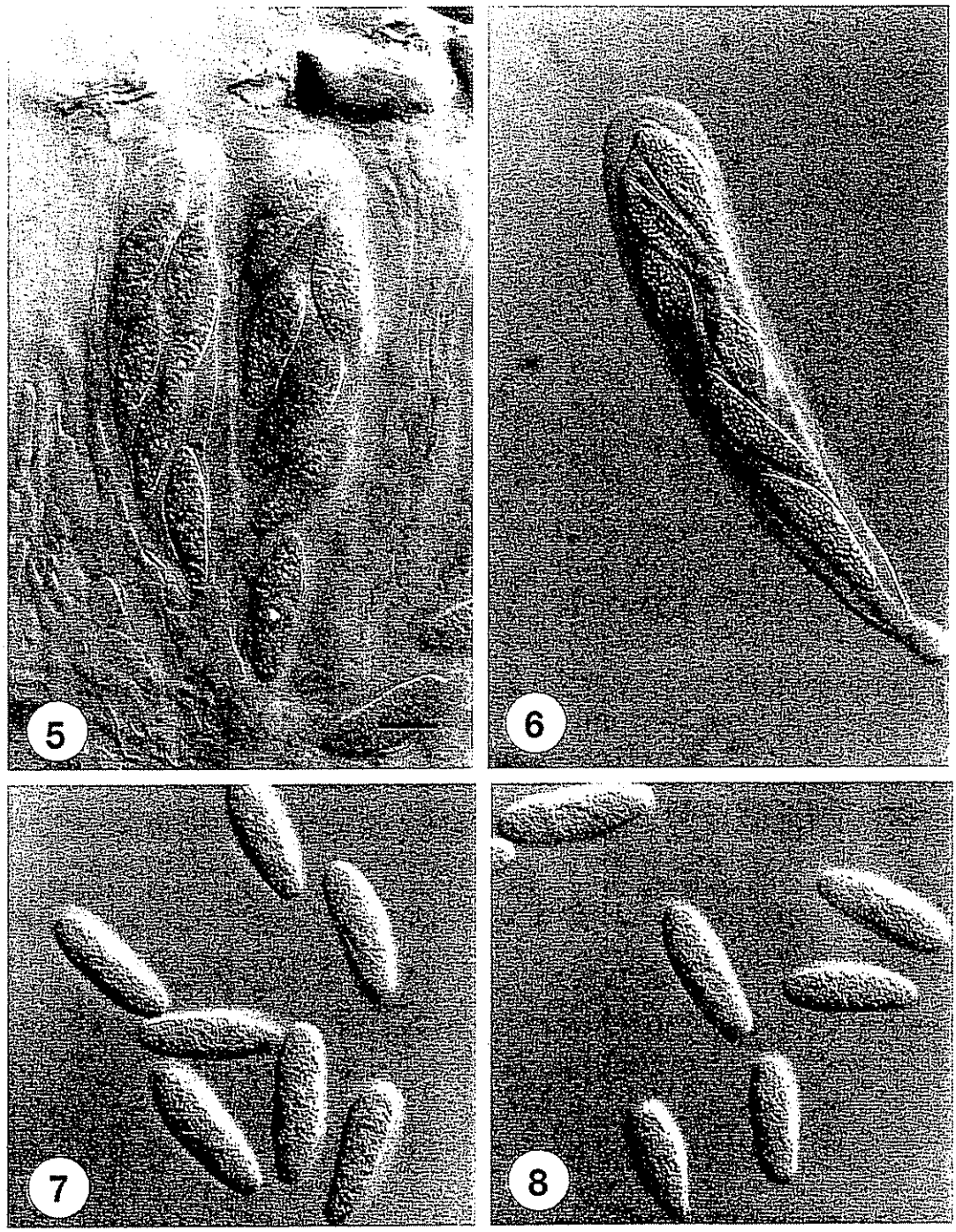
Hosts. Eucalyptus grandis Hill: Maid. and *E. nitens* (Deane et Maid.) Maid.

Distribution. Mpumalanga (White River, Sabie), South Africa.

Specimens examined. SOUTH AFRICA. MPUMA-



FIGS. 1–4. *Botryosphaeria eucalyptorum* and its anamorph *Fusicoccum eucalyptorum*. 1. Conidia and conidiogenous cells. 2. Ascospores. 3. Mature ascus. 4. Developing asci and pseudoparaphyses. Bars = 10 μm.



FIGS. 5–8. Differential interference micrographs of *Botryosphaeria eucalyptorum* and its anamorph *Fusicoccum eucalyptorum*. 5. Developing asci with pseudoparaphyses. 6. Young ascus. 7, 8. Conidia. Bar = 10 μm.

LANGA: Sabie, *Eucalyptus grandis*, 1995, *H. Smith*, (HOLOTYPE of *B. eucalyptorum*, PREM 56603), (HOLOTYPE of *F. eucalyptorum*, PREM 56604).

DISCUSSION

We conclude that two species of *Botryosphaeria*, namely *B. dothidea* and *B. eucalyptorum*, occur on *Eucalyptus* in South Africa, based on morphological and cultural charcateristics, pathogenicity and partial nrDNA ITS sequence data.

Botryosphaeria eucalyptorum closely resembles *B. ribis* (Punithalingam and Holliday 1973, Sivanesan 1984) and *B. dothidea* (Pennycook and Samuels 1985), but the ascospores of *B. eucalyptorum* are more fusoid and slightly longer. Conidia of *F. euca-*

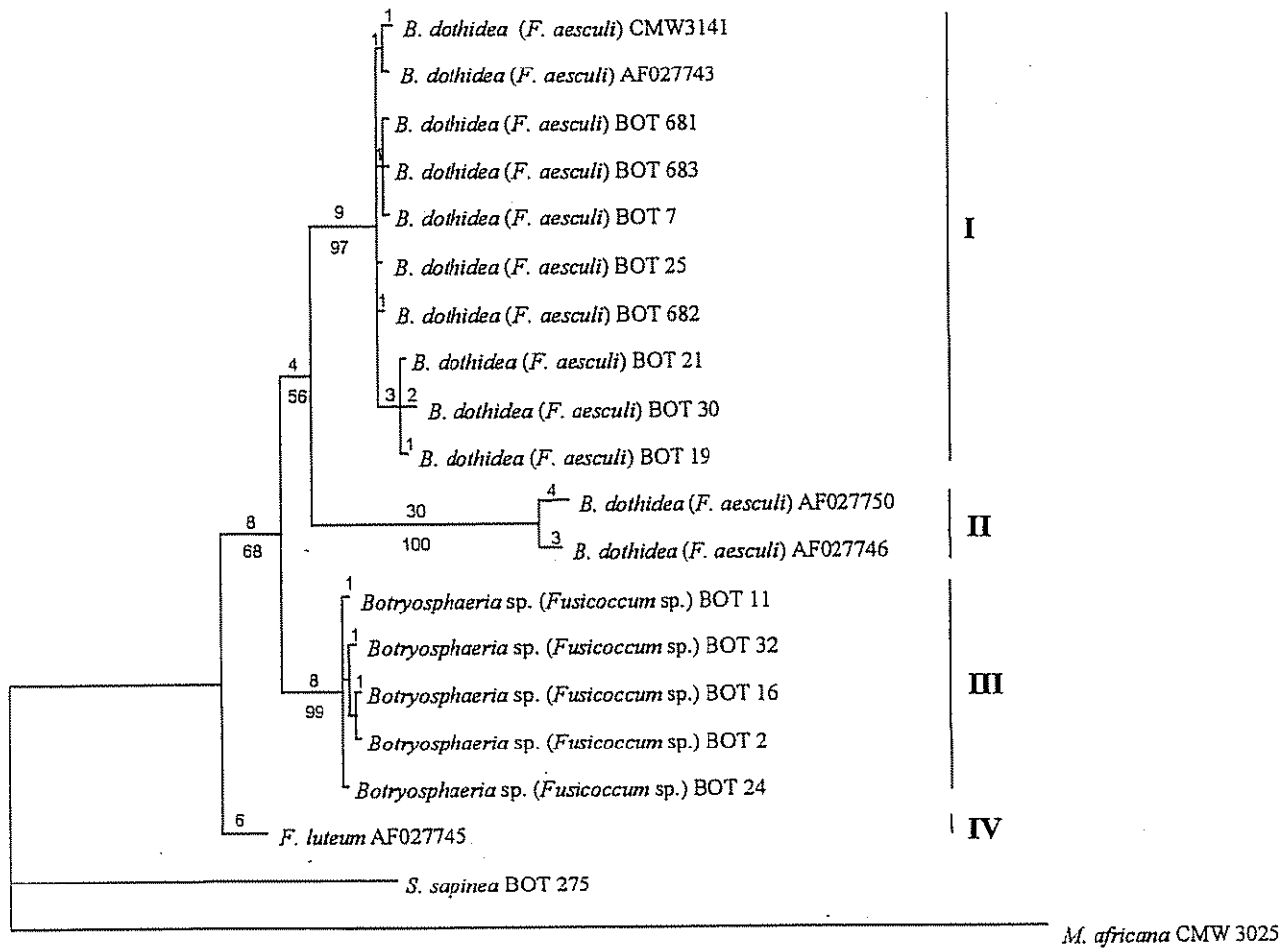


FIG. 9. Phylogenetic relationships amongst *Botryosphaeria dothidea, B. eucalyptorum* and related taxa based on parsimony analyses of the ITS1 and ITS2 rRNA operon DNA sequence data. The phylogram is rooted to *Mycosphaerella africana*. Bootstrap frequencies of higher than 55% are indicated (1000 replications) below internodes, and branch lengths, proportional to the number of steps, are indicated above internodes. Roman numerals represent groupings as used in the text and tables.

lyptorum, the anamorph of *B. eucalyptorum*, are consistently wider, and have a more distinct taper, compared to the smaller, more fusoid conidia of anamorphs (especially *F. aesculi*) in the *B. dothidea*-complex.

The validity of *B. ribis* as a separate species from *B. dothidea* has been a matter of debate for many years (Arx and Müller 1954, Rumbos 1987, Rayachhetry et al 1996). Sequence data of Jacobs and Rehner (1998) supports observations of the above mentioned authors, that isolates described as *B. dothidea* and *B. ribis* respectively, group together in strongly supported clades. This provides further proof that more than one species occurs in the *B. dothidea*-complex. Whether any of these taxa can now be attributed to *B. ribis* remains to be shown. At present no ex-type cultures of either species are available for comparison with isolates thought to represent *B. dothidea* or *B. ribis*. What is necessary is an epitypification of these species and a collection of representative isolates for molecular study. Until such time, we suggest that the synonymy proposed by Arx and Müller (1954) are followed, as no conclusive evidence has yet been presented that *B. ribis* and *B. dothidea* are separate taxa.

Isolates of *B. eucalyptorum* used in this study were found to have limited variability in their pathogenicity. Lesions produced after inoculations did not differ significantly amongst isolates. In contrast, isolates of *B. dothidea* produced lesions that differed significantly between isolates. The fact that these data are based on only five isolates of each species could explain the lack of variability amongst the *B. eucalyptorum* isolates. Based on the available data, isolates of *B. eucalyptorum* were less virulent than that of *B. dothidea*. It is, however, clear that *B. eucalyptorum* is pathogenic to eucalypts.

DNA sequence data was used successfully in this

study to separate isolates of *B. eucalyptorum* from others in the *B. dothidea*-complex. The variability among isolates representing *F. aesculi* (= *B. dothidea*) as reported by Jacobs and Rehner (1998) was reaffirmed in this study. Isolates of *F. aesculi* were found to be present in two distinct, but closely related terminal clades (clades I and II, FIG. 9). This is in spite of the fact that these isolates originated from different hosts and continents. Isolates of *F. aesculi* could be distinguished as distinct from those of *B. eucalyptorum* (= *F. eucalyptorum*). The overall phylogeny indicates, however, that all *Fusicoccum* species represented in this study are closely related.

Botryosphaeria eucalyptorum has thus far been associated only with cankers on the main stems of *E. grandis* and *E. nitens*. Although this fungus appears to be less pathogenic than *B. dothidea*, the fact that it is pathogenic should be considered by the Forestry Industry as being significant. *Botryosphaeria dothidea* is a common endophyte of eucalypt leaves in South Africa (Smith et al 1996a), and continuous monitoring could in future reveal that *B. eucalyptorum* also occurs as an endophyte. This study contributes to the current understanding of *Botryosphaeria* and more specifically to species in the *B. dothidea*-complex. Much work is, however, still needed to fully understand and reclassify this variable group of fungi.

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