Discovery of the Canker Pathogen *Chrysoporthe austroafricana* on Native *Syzygium* spp. in South Africa

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ABSTRACT

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Chrysoporthe canker is one of the most important diseases of plantation-grown *Eucalyptus* spp. in tropical and subtropical regions worldwide. For many years, the disease was reported to be caused by the fungal pathogen *Cryphonectria cubensis*. Recent DNA-based studies have shown that the fungus in South Africa is not conspecific with *Chr. cubensis* and it was recently described in the new genus *Chrysoporthe* as *Chrysoporthe austroafricana. Chr. austroafricana* is known only from South Africa, where it causes severe cankers on *Eucalyptus* spp. and on ornamental *Tibouchina* trees, both of which have been introduced into South Africa. The origin of *Chr. austroafricana* is unknown, but it is possible that it expanded its host range from native trees related to *Eucalyptus* and *Tibouchina* spp. to these exotic hosts. Subsequent surveys of some indigenous South African Myrtales led to the discovery of fruiting structures resembling those of *Chr. austroafricana* on native *Syzygium cordatum* and *S. guineense*. The fungus from these *Syzygium* spp. was identified as *Chr. austroafricana* based on morphological characteristics and β-tubulin gene sequences. Pathogenicity trials showed that *Chr. austroafricana* is more virulent on exotic *Eucalyptus* spp. than on native *S. cordatum*. This study represents the first report of *Chr. austroafricana* from native hosts in South Africa and adds credence to the view that the fungus could be native to this country.

Additional keywords: Diaporthales, forestry, Myrtales

Cryphonectria cubensis (Bruner) Hodges was considered one of the most important stem canker pathogens of plantation-grown *Eucalyptus* spp. in tropical and subtropical parts of the world (27). The pathogen first became a threat in Brazil at a time when *Eucalyptus* plantation forestry was expanding (27). Much later, *C. cubensis* was discovered in South Africa in the late 1980s, causing basal cankers and mortality of juvenile *Eucalyptus grandis* clones (28).

C. cubensis has been reported from three host genera in two families of the order Myrtales. The fungus was first reported on *Eucalyptus* in Cuba (1). This was followed by reports on a wide range of *Eucalyptus* spp. (Myrtaceae) worldwide, including those from Australia (4), Asia (5,21), South and Central America (9), and Africa (6,20,29). *C. cubensis* also has been described as causing dieback of *Syzygium aromaticum* (L.) Merr. & Perry (clove) (Myrtaceae) in Brazil and Indonesia (9).

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In 2001, *C. cubensis* was reported on native *Tibouchina urvilleana* (DC.) Cogn. and *T. lepidota* Baill. from Colombia, where it causes girdling cankers and dieback of stems and branches of these ornamental trees (28). More recently, *C. cubensis* also has been found on *T. granulosa* Cogn. in northern KwaZulu-Natal, South Africa (13).

Based on sequence data for the internal transcribed spacer (ITS) region of the ribosomal DNA operon, Myburg et al. (15) showed that isolates of *C. cubensis* from South East Asia and South America group in discrete clades, with South African isolates grouping with those from South America. Biological differences between *C. cubensis* isolates from South Africa and the rest of the world (14) prompted subsequent studies using multiple gene trees, including the ITS and β-tubulin genes. These studies showed that the South African fungus is distinct from the fungus of the same name occurring in the rest of the world. Gryzenhout et al. (8) confirmed this fact in a later study based on morphological and DNA sequence data, and assigned *C. cubensis* to a new genus as *Chrysoporthe cubensis* Gryzen. & M.J. Wingf. (10). Isolates from South Africa subsequently were assigned to *Chr. austroafricana* Gryzenhout & M. J. Wingf. One other species was identified in the genus, but was described in the new anamorph genus for *Chrysoporthe*, as *Chrysoporthella hodgesiana* Gryzenhout & M. J. Wingf. because its teleomorph was not seen (8).

Of the three species residing in the genus *Chrysoporthe, Chr. austroafricana* has been reported only from exotic *Tibouchina* and *Eucalyptus* spp. in South Africa (10). In contrast *Chr. cubensis* occurs on a number of native and exotic hosts residing in the Myrtales in South East Asia (5), Central Africa (20), and South America (1,9,19). *Chrysoporthella hodgesiana* is a newly described species that occurs on *Tibouchina* spp. in Colombia (8,19).

Very little is known regarding the origin of *Chr. austroafricana* in South Africa. However, the fact that the fungus is now recognized as unique and different from *C. cubensis* and is found only in South Africa does suggest that it could be native to Africa. If this were true, *Chr. austroafricana* could have originated on native trees and crossed to exotic *Eucalyptus* and *Tibouchina* trees. This would be the same as the case with the *Eucalyptus* rust pathogen *Puccinia psidii* that is native to species of Myrtaceae in South and Central America and that has become a serious pathogen of *Eucalyptus* spp. in that part of the world (3). There are various native trees in South Africa belonging to the Myrtaceae and some, such as species of *Syzygium*, are commonly found growing in close association with *Eucalyptus* plantations.

Recognition of the fact that the canker disease in South Africa is caused by a unique fungus different from *Chr. cubensis* has prompted speculation as to its origin. This led to surveys of native *Syzygium* spp. for canker pathogens similar to *Chr. austroafricana*. These surveys led to the discovery of a fungus resembling *Chr. austroafricana* in the KwaMbonambi area of the KwaZulu-Natal Province. The aim of this study was to survey additional *Syzygium* spp. adjacent to *Eucalyptus*-growing areas in South Africa and to determine the occurrence of *Chrysoporthe* spp. on native Myrtaceae in the country. The resulting fungi were studied using morphological characteristics, comparison of β-tubulin gene sequences, and pathogenicity studies.

MATERIALS AND METHODS

Disease symptoms and collection of samples. *S. cordatum* and *S. guineense*

trees showing signs of dieback and the formation of bark cracks and cankers were identified in the Zululand (KwaZulu-Natal Province), Tzaneen (Limpopo Province), Hazyview, and Sabie (Mpumalanga Province) areas of South Africa (Fig. 1). These symptoms were different from those observed on *Eucalyptus* spp. (27). Segments of bark and branches bearing fruiting bodies resembling those of *Chrysoporthe* spp. were collected for further analysis. The material was incubated in moist chambers to induce the production of fruiting bodies. Single conidial and ascospore isolations were made on 2% malt extract agar (MEA) (Biolab, Merck, Midrand, South Africa) and incubated at 25ºC. Isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Bark specimens from the *Syzygium* spp. have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) (Table 1).

Morphology. Bark specimens with fruiting structures collected from cankers on *Syzygium* spp. were compared with specimens of *Chrysoporthe* spp. used in previous studies (8,13,14,29; Table 1). For these comparisons, fruiting structures were embedded in Leica embedding medium and sectioned using a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa). Specimens were sectioned at –20ºC to a thickness of 12 µm. The sectioned structures were mounted in lactophenol and subsequently studied using standard light microscopy. To obtain the sizes of fruiting structures, minimum and maximum sizes were obtained of the smallest and largest structures. Twenty measurements were taken of conidiophores, conidia, asci, and ascospores for each collection and were presented as (min-) mean – st.dev. – mean + st.dev. (-max). Color notations used were those described by Rayner (18).

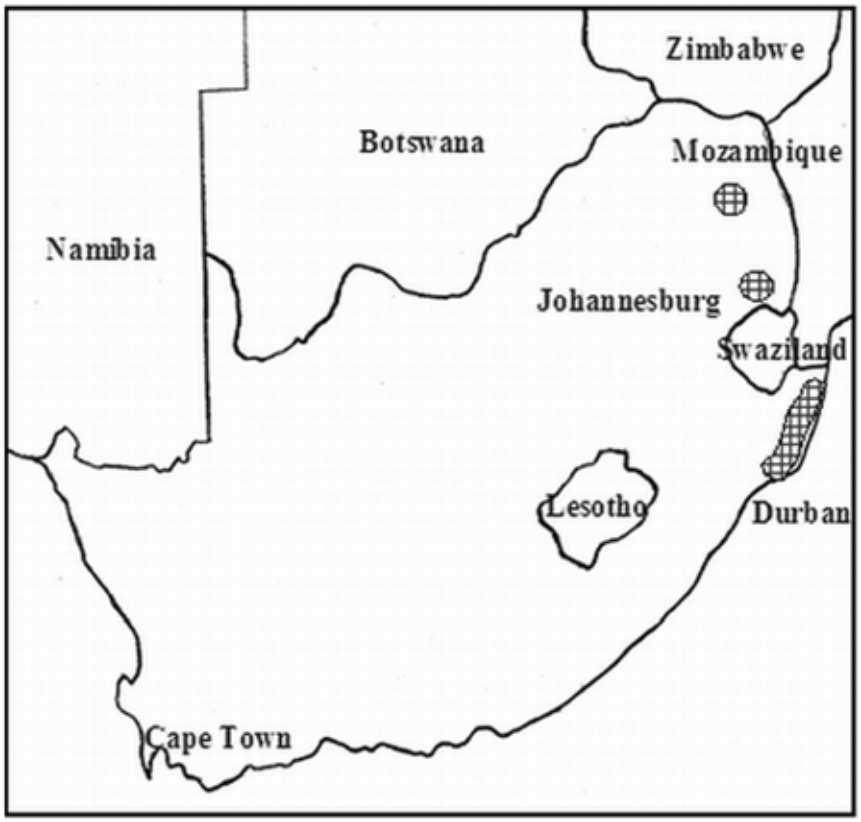

Fig. 1. Map of South Africa indicating areas where *Chrysoporthe austroafricana* isolates were collected.

DNA isolation and amplification. Isolates (Table 2) were inoculated into 1.5-ml microcentrifuge tubes containing 3% malt extract broth (750 µl) and incubated at 25ºC for 4 days. DNA was isolated as described by Van der Merwe et al. (24). Two β-tubulin gene regions were amplified using primer pairs Bt1a/Bt1b and Bt2a/Bt2b (7). Amplification of the β-tubulin gene regions followed the protocols of Myburg et al. (14). Amplification reactions were performed on a Perkin-Elmer GeneAmp polymerase chain reaction (PCR) System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Inc. Foster City, CA). PCR products were visualised on a 2% agarose-ethidium bromide gel using ultraviolet light.

DNA sequencing. PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany). DNA fragments were sequenced with the same primer pairs used in the amplification reactions. An ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin-Elmer, Warrington, UK) was used for the sequencing, using an ABI PRISM 3100 automated sequencer. Sequences were aligned using ClustalX (23) and manually adjusted using Sequence Navigator (version 1.0.1; Perkin-Elmer Applied BioSystems Inc.). All sequences obtained in this

Table 1. Specimens used in the morphological comparisons

Herbarium no.[a]	Identity	Host	Origin	Date	Collector
PREM 58023 (holotype)	*Chrysoporthe austroafricana*	*Eucalyptus grandis*	South Africa	1989	M. J. Wingfield
PREM 49379	*Chr. austroafricana*	*E. grandis*	South Africa	1988	M. J. Wingfield
PREM 49377	*Chr. austroafricana*	*E. grandis*	South Africa	1986	M. J. Wingfield
PREM 49378	*Chr. austroafricana*	*E. grandis*	South Africa	1987	M. J. Wingfield
PREM 57293	*Chr. austroafricana*	*E. grandis*	South Africa	2001	M. Gryzenhout
PREM 57357	*Chr. austroafricana*	*Tibouchina granulosa*	South Africa	1999	J. Roux
PREM 57358	*Chr. austroafricana*	*T. granulosa*	South Africa	1999	J. Roux
PREM 57474	*Chr. austroafricana*	*Syzygium cordatum*	South Africa	2001	R. N. Heath & M. Gryzenhout
PREM 57475	*Chr. austroafricana*	*S. guineense*	South Africa	2001	M. Gryzenhout
PREM 57476	*Chr. austroafricana*	*S. cordatum*	South Africa	2001	R. N. Heath & M. Gryzenhout
PREM 57477	*Chr. austroafricana*	*S. cordatum*	South Africa	2002	R. N. Heath & J. Roux
PREM 57478	*Chr. austroafricana*	*S. cordatum*	South Africa	2002	R. N. Heath & J. Roux
PREM 57479	*Chr. austroafricana*	*S. cordatum*	South Africa	2002	R. N. Heath & J. Roux
PREM 57480	*Chr. austroafricana*	*S. cordatum*	South Africa	2002	R. N. Heath & J. Roux
PREM 57294	*Chr. cubensis*	*E. grandis*	Colombia	2000	M. J. Wingfield
PREM 57297	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	2001	M. J. Wingfield

[a] PREM, National Collection of Fungi, Pretoria, South Africa.

study have been deposited in GenBank (Table 2).

Data analyses were performed using phylogenetic analysis using parsimony (PAUP*) version 4.0b (22). A partition-homogeneity test (PHT) was performed using PAUP* version 4.0b for the two β-tubulin gene region data sets (22). Analyses were done using the heuristic search option with tree-bisection-reconnection (TBR) branch swapping. Gaps inserted during sequence alignment were treated as fifth character (NEWSTATE). A bootstrap analysis (50% majority rule, 1,000 replications) was done to determine the confidence levels of the tree-branching points. Previously published sequences for *Chr. austroafricana*, *C. cubensis,* and *Chrysoporthella hodgesiana* (8,13,14) were used for comparative purposes. *Cryphonectria parasitica* was used as an outgroup taxon to root the phylogenetic tree (8).

The credibility of the branch nodes was tested using Markov Chain Monte Carlo Algorithms (MCMC) (10) in Bayesian analysis (12). Random trees were obtained through 2,000,000 generations, with every 100th tree sampled. The first 9,000 trees were discarded as the burnin period. MODELTEST (17) was used to determine that a general time reversal model, including invariable sites, best fit the data set, and four MCMC chains were run simultaneously in the analysis, with three heated chains and one cold chain. The sampled trees were summarized in a consensus tree showing posterior probabilities of the branches.

Pathogenicity. In order to determine whether the fungus isolated from the *Syzygium* spp. was the causal agent of the cankers and dieback observed on trees, and to consider the pathogenicity of the fungus on *Eucalyptus* spp., infection studies were performed. The *S. cordatum* and *E. grandis* trees used for inoculation had stem diameters ranging from 10 to 23 mm and were arranged in a completely randomized design. All trees were maintained under greenhouse conditions for a 2-week acclimatization period prior to inoculation. The greenhouse was subjected to natural day/night conditions (approximately 13 h of daylight and 11 h of darkness) and a temperature of approximately 25ºC was maintained.

In order to assess the reciprocal pathogenicity of isolates on *S. cordatum* and *Eucalyptus* spp., the fastest growing isolate from *S. cordatum* (CMW 9364) and an isolate (CMW 2113) from *E. grandis*, previously shown to represent a higher level of pathogenicity in a population of *Chr. austroafricana* isolates (25), each were inoculated into the stems of 20 *S. cordatum* and 20 *E. grandis* (clone ZG14) trees. This *Eucalyptus* clone was selected because it is known to be highly susceptible to infection by *Chr. austroafricana* and has been used previously in pathogenicity trials (2,13). Ten trees per species were inoculated with sterile MEA plugs to serve as controls. Wounds were made on the stems of trees approximately 150 mm from the soil level, using a 10-mm-diameter cork borer. Mycelial plugs of a similar size were taken from the actively growing edges of 7-day-old cultures and placed in the wounds with the mycelium toward the cambium. Wounds were sealed with laboratory film (Parafilm "M"; American National Can, Chicago) to protect the inoculated fungus and cambium from desiccation.

Lesion lengths associated with the inoculations were measured after 6 weeks. To determine the variance between isolates and between trees, inoculation data were subjected to analysis of variance using the General Linear Model procedure of SAS (24). All inoculation tests were repeated and the data analyzed together. To determine whether the inoculated fungi were responsible for the lesion development, Koch's postulates were applied by re-isolating from the lesions and confirming the identity of the inoculated fungi based on morphology.

RESULTS

Disease symptoms and collection of samples. A number of trees showing branch dieback and stem and branch cankers were found in all of the areas surveyed. Asexual fruiting bodies of a fungus, resembling those of *Chr. austroafricana,* were found on dead areas of the stems and branches of these trees. Teleomorph structures, morphologically similar to *Chr. austroafricana,* also were common on trees in Limpopo Province and the Zululand area. No teleomorph structures were found on trees in the Mpumalanga Province. A total of three isolates were obtained from Limpopo Province, two isolates from Mpumalanga Province, and 68 isolates from the Zululand area. Only one isolate was collected per tree.

Morphology. The fungus found on native South African *Syzygium* spp. had a morphology similar to that of *Chr. austroafricana* described from *Eucalyptus* spp. and *T. granulosa* (8,13) in South Af-

Table 2. Isolates used for phylogenetic analysis and pathogenicity trials

Culture no.[a]	Isolate identity	Host	Origin	GenBank accession number
CMW 9364	*Chrysoporthe austroafricana*	*Syzygium cordatum*	South Africa	AY149284[b]
CMW 9366	*Chr. austroafricana*	*S. cordatum*	South Africa	AY149285[b]
CMW 10036	*Chr. austroafricana*	*S. cordatum*	South Africa	AY149286[b]
CMW 10046	*Chr. austroafricana*	*S. cordatum*	South Africa	AY149287[b]
CMW 10076	*Chr. austroafricana*	*S. cordatum*	South Africa	AY149288[b]
CMW 10086	*Chr. austroafricana*	*S. cordatum*	South Africa	AY149289[b]
CMW 10092	*Chr. austroafricana*	*Syzygium guineense*	South Africa	AY149290[b]
CMW 62	*Chr. austroafricana*	*Eucalyptus grandis*	South Africa	AF273063; AF273458[c]
CMW 2113	*Chr. austroafricana*	*E. grandis*	South Africa	AF273067; AF273462[c]
CMW 8755	*Chr. austroafricana*	*E. grandis*	South Africa	AF273064; AF273459[c]
CMW 9327	*Chr. austroafricana*	*Tibouchina granulosa*	South Africa	AF273060; AF273455[c]
CMW 9328	*Chr. austroafricana*	*T. granulosa*	South Africa	AF273061; AF273456[c]
CMW 9932	*Chr. austroafricana*	*T. granulosa*	South Africa	AF273062; AF273457[c]
CMW 9927	*Chrysoporthella hodgesiana*	*T. urvilleana*	South America	AF292034; AF292037[c]
CMW 9928	*Chr. hodgesiana*	*T. urvilleana*	South America	AF292036; AF292038[c]
CMW 9929	*Chr. hodgesiana*	*T. urvilleana*	South America	AF292035; AF292038[c]
CMW 8758	*Chrysoporthe cubensis*	*E. grandis*	South America	AF273068; AF273463[c]
CMW 1853	*Chr. cubensis*	*S. aromaticum*	South America	AF273070; AF273465[c]
CMW 8756	*Chr. cubensis*	*E. grandis*	Southeast Asia	AF273077; AF375606[c]
CMW 9906	*Chr. cubensis*	*Eucalyptus* sp.	Southeast Asia	AF273069; AF273464[c]
CMW 9903	*Chr. cubensis*	*S. aromaticum*	Southeast Asia	AF273070; AF273465[c]
CMW 7047	*Cryphonectria parasitica*	*Quercus virginiana*	United States	AF273469; AF273073[d]
CMW 7048	*C. parasitica*	*Q. virginiana*	United States	AF273470; AF273076[d]

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.
[b] β-Tubulin 1 and 2 sequence data generated in this study.
[c] β-Tubulin 1 and 2 sequence data obtained from Myburg et al. (16,17).
[d] β-Tubulin 1 and 2 sequence data obtained from Venter et al. (30).

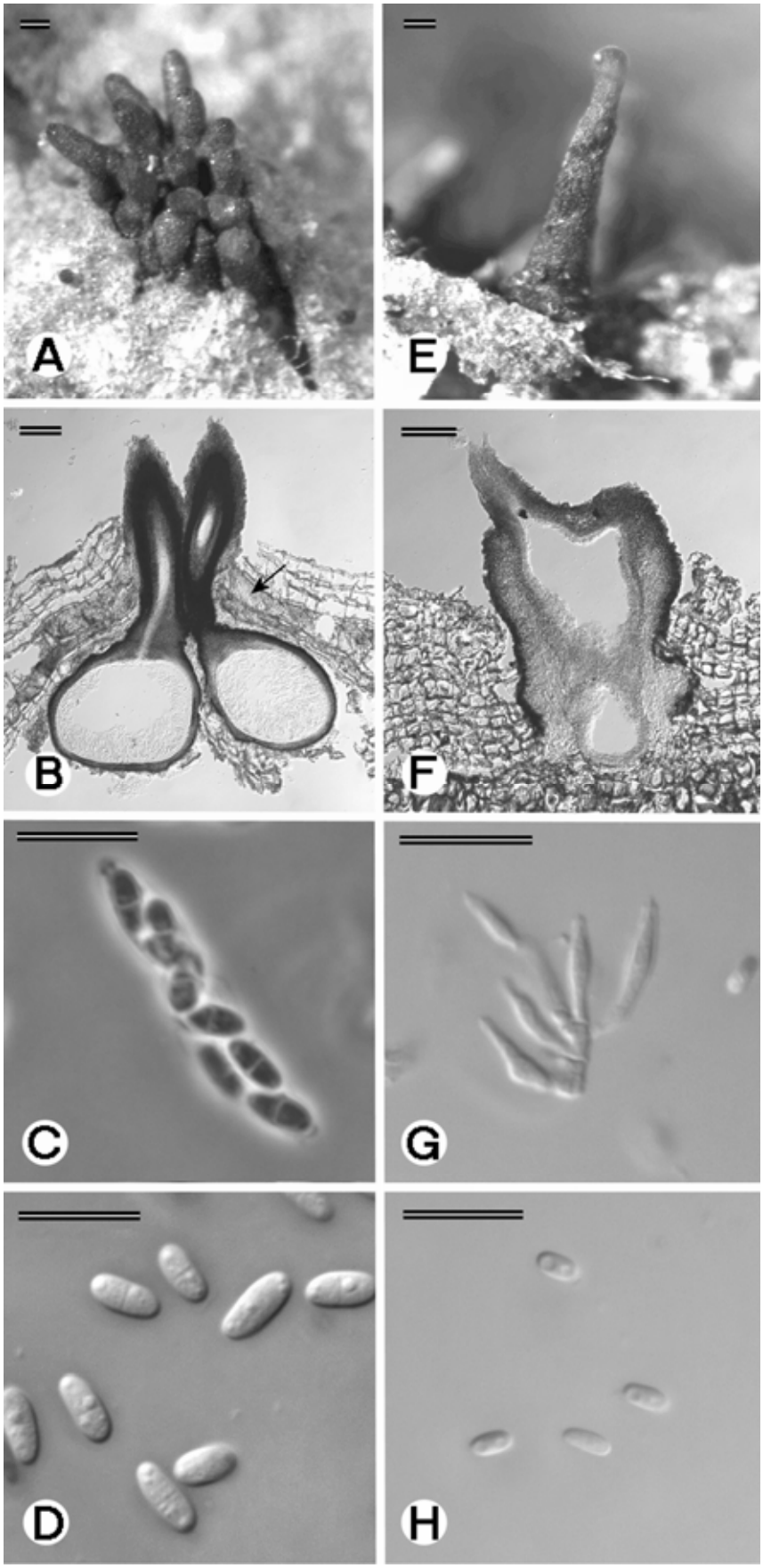

Fig. 2. Light micrographs of the fruiting structures of *Chrysoporthe austroafricana* from *Syzygium* spp. in South Africa. **A,** Ascoma on bark. **B,** Longitudinal section through ascoma showing clypeus (arrow). **C,** Ascus. **D,** Ascospores. **E,** Conidioma on bark. **F,** Longitudinal section through conidioma. **G,** Conidiophore. **H,** Conidia. Bars: **A, B, E,** and **F** = 100 µm; **C, D, G,** and **H** = 10 µm.

rica. Ascomata were characterized by long perithecial necks emerging from the bark with weakly developed, predominantly prosenchymatous, cinnamon- to orange-colored stromatic tissue at the bases of the necks (Fig. 2A and B). Perithecia were semi-immersed in the bark, black, base globose, and about 250 to 320 µm in diameter (Fig. 2B). Perithecial necks were periphysate, up to 1,220 µm long as they emerged through the bark, and covered in umber tissue, giving them a fuscous black appearance as they extended beyond the stromata (Fig. 2B). Extended parts of the perithecial necks were about 100 to 155 µm wide, and the width of the actual perithecial neck was approximately 65 to 113 µm. Asci were fusoid to ellipsoid, nonstipitate, with nonamyloid refractive rings, (26-) 27 – 31.5 (-32) × 6 to 7 (-7.5) µm in size, and contained eight ascospores (Fig. 2C). Ascospores were hyaline, septate, fusoid to oval, (5-) 5.5 – 6.5 (-7) × (1.5-) 2 – 2.5 (-3) µm in size, with rounded ends (Fig. 2D), which is the morphological feature distinguishing *Chr. austroafricana* from *Chr. cubensis* (8).

Conidiomata were superficial to slightly immersed, pyriform to clavate (Fig. 2E and F), uni- to multilocular (Fig. 2F), and fuscous black, with an umber interior base approximately 280 to 500 µm long and a base above the bark surface 60 to 370 µm long and 300 to 450 µm wide. One to three necks (Fig. 2E) connected to single or separate locules occurred in each structure, and these were 110 to 170 µm wide. Conidiophores were hyaline, cylindrical or flask shaped with attenuated apices, frequently septate, branching irregularly, and (7.5-) 9.5 – 15.5 µm long (Fig. 2G). Conidiogenous cells were phialidic, determinate, and 1 to 1.5 µm wide (Fig. 2G). Conidia were exuded as bright luteous spore tendrils or droplets, hyaline, nonseptate, oblong to oval, and 3 – 3.5 × 1.5 µm in size (Fig. 2H).

DNA sequencing. PCR amplification with the two primer pairs resulted in fragments of 537 bp (Bt1a/Bt1b) and 495 bp (Bt2a/Bt2b). The fragments were sequenced and resulted in sequences of 464 bp (Bt1a/Bt1b) and 425 bp (Bt2a/Bt2b) before alignment. After alignment, a partition homogeneity test on the two separate data sets gave a PHT value of $P = 0.2$, showing that the two data sets (Bt1a/1b and Bt2a/2b) were congruent and could be combined in the phylogenetic analysis. Aligned sequences of the combined data sets resulted in a data set of 976 characters, consisting of 809 constant characters, 25 parsimony-informative characters, and 142 variable characters that were parsimony uninformative. The heuristic search produced four most parsimonious trees with most variation in the clade, including the *Chr. austroafricana* isolates (Fig. 3). A strict bootstrap consensus tree (length of tree = 198 steps, CI = 0.889, RI = 0.909, RC = 0.808) was generated from the 100

variable characters and most branches were well supported with high bootstrap values (Fig. 3). Posterior probability values calculated for the branch nodes were as high as the bootstrap values.

Results of the DNA sequence analysis supported the outcome of the morphological comparison, with isolates from *Syzygium* spp. grouping with those from *Tibouchina* and *Eucalyptus* spp. in South Africa. This confirms the identification of the fungus as *Chr. austroafricana* (Fig. 3). The *Syzygium* isolates grouped within the *Chr. austroafricana* clade and separate from isolates in the *Chr. cubensis* and *Chrysoporthella hodgesiana* clades (bootstrap support 98%, posterior probability 100%). The larger *Chrysoporthe* clade grouped separately from *Cryphonectria parasitica* (bootstrap support 100%, posterior probability 100%).

Pathogenicity. Inoculation of *S. cordatum* and *E. grandis* with *Chr. austroafricana* resulted in lesions on both hosts within 6 weeks after inoculation (Table 3). Some of the *Eucalyptus* trees showed signs of decline and produced epicormic shoots on the stems within this time period. Epicormic shoots were present on some *S. cordatum* trees, but no dieback was visible. No lesions were produced on control trees of either host. Isolates from both *Syzygium* and *Eucalyptus* spp. were more pathogenic on *Eucalyptus* than on *S. cordatum* ($P > 0.0001$) (Fig. 4; Table 3). Isolates from *Syzygium* spp. were more pathogenic than those from *Eucalyptus*. The test organism was isolated consistently from the lesions on both hosts after 6 weeks.

DISCUSSION

Canker on *Eucalyptus* spp., caused by *Chr. austroafricana* (previously *Cryphonectria cubensis*), has been known in South Africa since 1989, and it is recognized as a serious threat to the future of plantations of these trees (28,30). Recent recognition that the causal agent of this disease in South Africa, *Chr. austroafricana*, represents a species different from the well-known *Chr. cubensis* (previously known as *Cryphonectria cubensis*), has raised the intriguing question as to whether the fungus in South Africa might not be native to this region. Collection of *Chr. austroafricana* from native *Syzygium* spp. covering a wide natural distribution of the tree in this study provides support for this hypothesis.

Analysis of DNA sequence data as well as morphological characteristics presents robust evidence that the fungus on native *Syzygium* spp. is the same as that found on *Eucalyptus* and *Tibouchina* spp. in South Africa. It has been suggested previously that *Chr. austroafricana*, previously reported under the name *Cryphonectria cubensis*, was accidentally introduced into South Africa (2,26). This view was based on the fact that a South African population of the fungus showed a low genetic diversity based on vegetative compatibility groups (26). Results of the present study suggest that *Chr. austroafricana* is native to Southern Africa or, alternatively, that it could have been introduced into the country from an as yet unknown origin.

Collections of fungi from dieback symptoms on native *Syzygium* spp. were planned specifically to broadly cover the natural distribution range of these trees in South Africa. *Chr. austroafricana* was collected from all three major geographical areas considered. The occurrence of the fungus on *Syzygium* spp. in KwaMbonambi on the Zululand coast, a major *Eucalyptus* forestry region where canker disease caused by *Chr. austroafricana* resulted in serious losses during the 1990s (28), might have suggested that the fungus spread from *Eucalyptus* to *Syzygium* spp. However, the other regions from which collections were made on the Zululand coast (Amanzimgwenya, Kosi bay, and Kosi mouth) are more isolated from *Eucalyptus* plantings. Furthermore, the occurrence of the fungus in the northern and eastern regions of the country, where cankers associated with *Chr. austroafricana* are not common on *Eucalyptus* spp. even though susceptible trees are planted, was surprising.

The teleomorph of *Chr. austroafricana* was found frequently on samples collected from native *Syzygium* spp. This is particularly interesting, because teleomorph structures are rare on *Eucalyptus* and *Tibouchina* spp. in South Africa. For example, Van Heerden et al. (25) conducted an extensive survey of *Chr. austroafricana* (then known as *Cryphonectria cubensis*) in *Eucalyptus* plantations and failed to detect the sexual structures of the causal agent. However, sexual structures of the pathogen occasion-

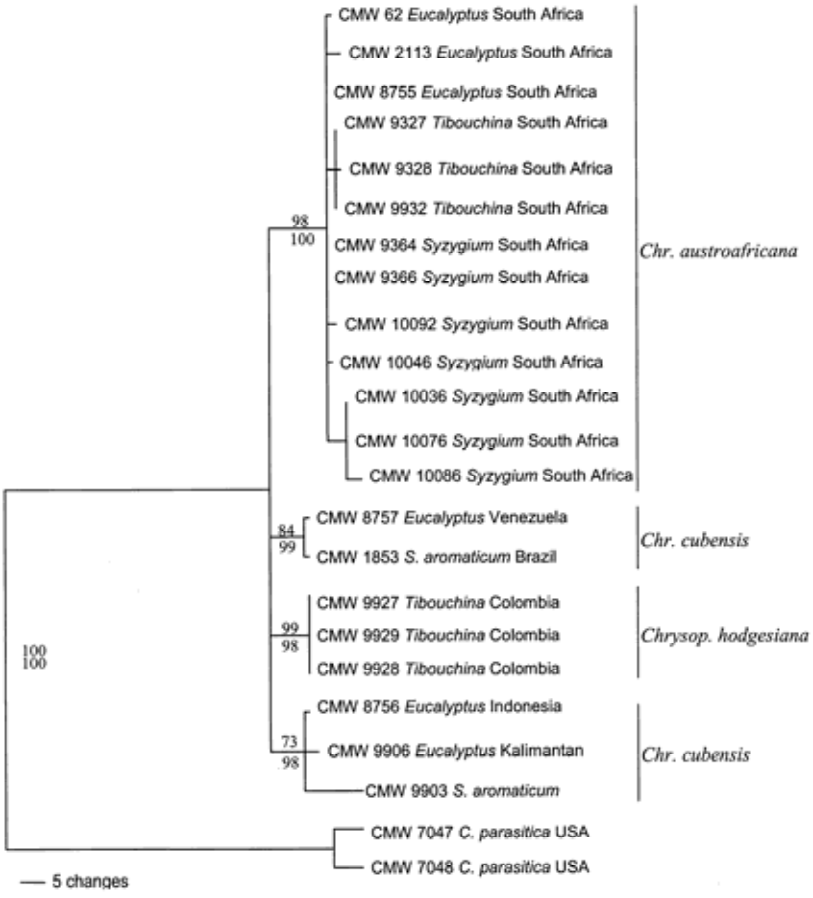

Fig. 3. Phylogenetic tree based on parsimony generated from a data set including β-tubulin gene sequence data. Strict consensus tree (length of tree = 198 steps, CI = 0.889, RI = 0.909, RC = 0.808) generated from heuristic searches using parsimony. Bootstrap values (1,000 replicates) are indicated above the branches with those lower than 50% not shown. Posterior probability values are indicated below the branches. *Cryphonectria parasitica* was used to root the tree.

Table 3. Lesion lengths resulting from inoculation with *Chrysoporthe austroafricana* on 1-year-old *Eucalyptus grandis* (ZG 14 clones) and *Syzygium cordatum* trees 6 weeks after inoculation in the greenhouse

	Lesion length (mm)[a]	
Isolate	***S. cordatum***	***E. grandis***
CMW 2113[b]	49.9	146.5
CMW 9364[c]	108.9	133.0
Control	10	10

[a] Each value is the average of 40 measurements for each isolate.
[b] Isolated from *Eucalyptus* sp.
[c] Isolated from *Syzygium* sp.; the isolate from *Syzygium* was more pathogenic than the one from *Eucalyptus* ($P > 0.0001$).

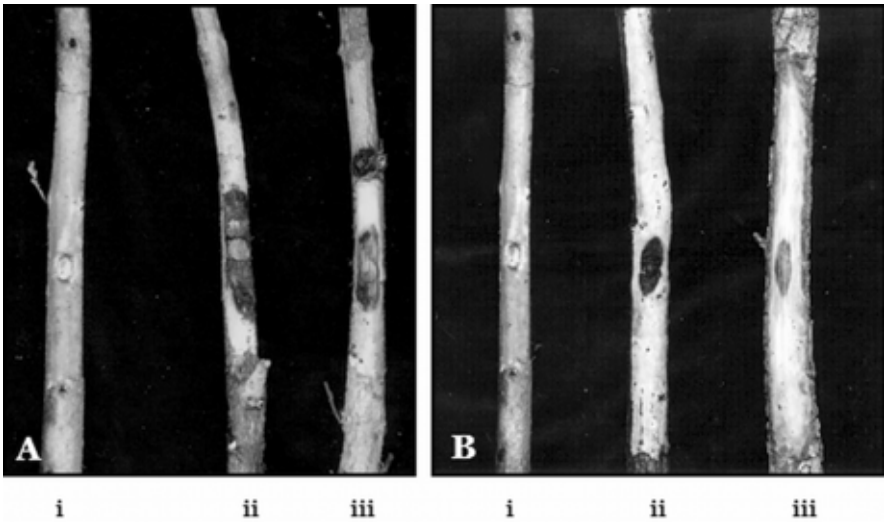

Fig. 4. Lesions resulting from pathogenicity trials. **A,** Lesions formed on *Eucalyptus grandis* clones (**A**ii = CMW 2113; **A**iii = CMW 9364) with control inoculation producing no lesions (**A**i). **B,** Lesions formed on *Syzygium cordatum* trees (**B**ii = CMW 2113 and **B**iii = CMW 9364) with control inoculation producing no lesions (**B**i).

ally have been found on *Eucalyptus* spp. (8,29) and a limited number of times on *Tibouchina* spp. (13). If the fungus is native on *Syzygium* spp. and has adapted to infect *Eucalyptus* spp., recombination may be occurring on native hosts, resulting in new genotypes of the fungus able to infect *Eucalyptus* spp.

Pathogenicity trials in this study showed that *S. cordatum* is more tolerant to infection by *Chr. austroafricana* than the *Eucalyptus* clone tested. This suggests that the fungus may be native, because native hosts would be expected to be more resistant to infection than exotics (11,16). *S. cordatum* trees inoculated in this study all were raised from seed and each plant represented a distinct genotype. In contrast, the *Eucalyptus* trees were all of a single genotype known to be susceptible to infection by *Chr. austroafricana*. Nevertheless, a highly pathogenic isolate from *Eucalyptus* spp. did minimal damage on inoculated *S. cordatum* trees, providing strong evidence for a relatively high level of resistance in the native South African tree. These data further support the view that the fungus could be native on *S. cordatum*.

Discovery of a native host for *Chr. austroafricana* in South Africa and the suggestion that the fungus on *Eucalyptus* originated on *Syzygium* spp. have important implications for forestry internationally. Previous studies have shown that *Chr. austroafricana* is considerably more pathogenic than the closely related *C. cubensis* (19,20). Clearly, the South African fungus is a threat to *Eucalyptus* forestry in the rest of the world. Furthermore, it probably is not present in Australia and, if it were to enter that country, it could have a serious effect on native Australian Myrtaceae. In this regard, it is similar to the *Eucalyptus* rust *P. psidii*, which is considered as a major threat to the Myrtaceae in Australia (3). Thus, every effort should be made to restrict its spread from South Africa. Population diversity studies on the pathogen would also provide insight into the possible origin of *Chr. austroafricana*.

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