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# Cryphonectria canker on *Tibouchina* in South Africa

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*Cryphonectria cubensis* is an important canker pathogen of plantation *Eucalyptus* spp. in tropical and sub-tropical areas of the world, including South Africa. It is best known on *Eucalyptus* spp., but it also occurs on *Syzygium aromaticum* (clove). In 1998, *C. cubensis* was found to cause cankers on the non-myrtaceous hosts *Tibouchina urvilleana* and *T. lepidota* in Colombia. In this study, we report on a similar canker disease that has recently been found in South Africa on *T. granulosa*, commonly grown as an ornamental tree. The identity of the pathogen was determined through morphological comparisons and phylogenetic analyses of ITS and β-tubulin gene sequences. The pathogenicity of the fungus was also tested on *T. granulosa* and *E. grandis*. Morphological as well as DNA sequence comparisons showed that the fungus on *T. granulosa* is the same as *C. cubensis* occurring on *Eucalyptus* spp. in South Africa. Pathogenicity tests on *T. granulosa* and *E. grandis* clones showed that the fungus from *T. granulosa* is able to cause cankers on both hosts.

## INTRODUCTION

*Cryphonectria cubensis* is one of the most important pathogens in *Eucalyptus* plantations in tropical and sub-tropical areas of the world (Boerboom & Maas 1970, Hodges 1980, Wingfield, Swart & Abear 1989). High temperatures and rainfall favor the occurrence of this disease (Hodges *et al.* 1976, Hodges, Geary & Cordell 1979, Sharma, Mohanan & Florence 1985a, b). Reduction of loss due to Cryphonectria canker is usually achieved by the propagation of selected disease tolerant *Eucalyptus* clones and hybrids (Alfenas, Jeng & Hubbes 1983, Conradie, Swart & Wingfield 1990).

*C. cubensis* is known to occur naturally on two host genera other than *Eucalyptus*. In 1986, the fungus was reported from clove (*Syzygium aromaticum*) in Brazil. (Hodges, Alfenas & Ferreira 1986). Subsequently the opportunistic pathogen of clove, *Endothia eugeniae*, was reduced to synonymy with *C. cubensis*. Micales, Stipes & Bonde (1987) confirmed the synonymy of *C. cubensis* and *E. eugeniae* using isozyme analysis, total protein banding patterns and fungal pigments. More recently, *C. cubensis* was reported on native *Tibouchina urvilleana* and *T. lepidota* in Colombia (Wingfield *et al.* 2001). This was an intriguing discovery because it was the first time that the fungus had been found on trees outside the *Myrtaceae*.

Species of *Tibouchina* are widely grown in the warmer parts of South Africa as ornamentals in gardens, parks and along roadsides. In January 1999, a fungus had been found on *T. granulosa* and *T. granulosa* var. *rosea* in KwaMbonambi, South Africa. This disease resulted in the death of branches and die-back of mature trees. Fruiting bodies on the surface of cankers resembled those of the anamorph of *C. cubensis* found on *Eucalyptus* spp. in South Africa (Wingfield *et al.* 1989). The aim of this study was to identify the causal agent of the disease found on *T. granulosa* in South Africa using morphological and molecular tools, and to test its pathogenicity.

## MATERIALS AND METHODS

### *Disease symptoms and collection of samples*

In 1999, *T. granulosa* trees showing branch die-back with girdling cankers were identified in the town of KwaMbonambi, South Africa (28° 22′ S 32° 19′ E).

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**Table 1.** Specimens of *Cryphonectria cubensis* used in morphological comparisons.

| Reference no.[a] | Host | Origin | Date | Collector |
|---|---|---|---|---|
| PREM 49379 | *Eucalyptus grandis* | South Africa | 1988 | M. J. Wingfield |
| PREM 49377 | *E. grandis* | South Africa | 1986 | M. J. Wingfield |
| PREM 49378 | *E. grandis* | South Africa | 1987 | M. J. Wingfield |
| PREM 57357 | *Tibouchina granulosa* | South Africa | 1999 | J. Roux |
| PREM 57358 | *T. granulosa* | South Africa | 1999 | J. Roux |
| PREM 57359 | *T. granulosa* | South Africa | 1999 | J. Roux |
| PREM 57360 | *T. granulosa* | South Africa | 2000 | J. Roux, R. Heath & L. Lombaard |
| PREM 57361 | *T. granulosa* | South Africa | 2000 | J. Roux, R. Heath & L. Lombaard |
| PREM 57362 | CMW 9343 inoculation of GC 747[b] | South Africa | 2000 | J. Roux & R. Heath |
| PREM 57363 | CMW 9343 inoculation of GC 747[b] | South Africa | 2001 | M. Gryzenhout & R. Heath |
| PREM 57364 | CMW 9346 inoculation of GC 747[b] | South Africa | 2001 | M. Gryzenhout & R. Heath |
| PREM 57365 | CMW 9358 inoculation of GC 747[b] | South Africa | 2001 | M. Gryzenhout & R. Heath |
| PREM 57366 | CMW 2113 inoculation of GC 747[b] | South Africa | 2001 | M. Gryzenhout & R. Heath |
| PREM 57367 | CMW 9343 stick inoculation[c] | South Africa | 2002 | R. Heath |

[a] PREM, National Collection of Fungi, Pretoria, South Africa.
[b] An *E. grandis* × *camaldulensis* clone (GC747) inoculated with *C. cubensis* isolate in the field.
[c] Cut stems of an unknown *E. grandis* clone inoculated in the lab.

Bark samples from the surface of cankers bearing fruiting bodies were incubated in moist chambers for 2–3 d to induce production of spores. Single conidial isolations were made onto 2% Malt Extract Agar (MEA) (20 g Biolab Malt Extract, 15 g Biolab Agar, 1 l water) and incubated at 25 °C. These cultures are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

### *Morphological comparisons*

Bark specimens from *Tibouchina granulosa*, with fruiting structures of the fungus, were collected from natural cankers and deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) (Table 1). Bark material from field pathogenicity trials (see Pathogenicity section), was also examined (Table 1). These specimens were compared with specimens of *C. cubensis* from *Eucalyptus* spp. used in a previous study (Wingfield *et al.* 1989) (Table 1). Comparisons were made using standard light microscopic techniques.

Stems (~10–15 mm diam) of an unknown *E. grandis* clone were inoculated with isolate CMW 9343 from *T. granulosa* and isolate CMW 2113 from *E. grandis*. This was done to induce the formation of fresh fruiting structures. These stems were cut into segments (5–8 cm long) and the ends sealed with melted paraffin wax. Bark plugs 6 mm diam were removed from the segments with a cork borer and replaced with mycelial plugs of the same size. Wounds were covered with parafilm and incubated in a moisture chamber until fruiting structures were produced.

Fruiting structures were sectioned using a Leitz 1310K freezing microtome with a KRYOMAT 1700 generator (Setpoint Technologies, Johannesburg) as described in Venter *et al.* (2002). Sections were executed at −30 ° and were 14–20 μm thick. Ten measurements each was taken of relevant structures and are presented as (min −)(mean − SD) − (mean + SD)(− max). For stromata, a range was obtained from three structures. Colour notations described by Rayner (1970) were used to standardise colour annotations.

### *DNA isolation and amplification*

Isolates used to confirm the identity of the fungus from *T. granulosa* are listed in Table 2. DNA was isolated as described by Myburg, Wingfield & Wingfield (1999). The internal transcribed spacer (ITS) regions of the ribosomal RNA operon were amplified using the primer sets ITS1 and ITS4 (White *et al.* 1990). Amplification of two β-tubulin gene regions was done using primer pairs Bt1a/Bt1b and Bt2a/Bt2b (Glass & Donaldson 1995). Amplification of the ITS1 and ITS2 and the two β-tubulin gene regions were as described by Myburg *et al.* (1999) and Myburg *et al.* (2002) respectively. Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Foster City, California). PCR products were visualised on a 1% agarose-ethidium bromide gel using a UV light source.

### *Sequencing and analysis of sequence data*

PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The respective DNA fragments were sequenced in both directions 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) was used for sequencing. DNA sequences were determined with an ABI PRISM 377™ automated DNA sequencer. Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Foster City, CA) was used to translate the DNA sequences to amino-acid sequences. The DNA

**Table 2.** Isolates used for molecular comparison and pathogenicity trials[a].

| Isolate no. | Isolate identity | Host | Origin | GenBank accession numbers |
|---|---|---|---|---|
| CMW 8757 | *Cryphonectria cubensis* | *Eucalyptus grandis* | Venezuela | AF046897[b], AF273069[d], AF273464[d] |
| CMW 1853 | *C. cubensis* | *Syzygium aromaticum* | Brazil | AF046891[b], AF273070[d], AF273465[d] |
| CMW 9927 | *C. cubensis* | *Tibouchina urvilleana* | Colombia | AF265653[c], AF292034[f], AF292037[g] |
| CMW 9928 | *C. cubensis* | *T. urvilleana* | Colombia | AF265654[c], AF292036[f], AF292039[g] |
| CMW 9929 | *C. cubensis* | *T. urvilleana* | Colombia | AF265655[c], AF292035[f], AF292038[g] |
| CMW 2113 | *C. cubensis* | *Eucalyptus grandis* | South Africa | AF046892[b], AF273067[d], AF273462[d] |
| CMW 62 | *C. cubensis* | *E. grandis* | South Africa | AF292041[d], AF273063[d], AF273458[d] |
| CMW 8755 | *C. cubensis* | *E. grandis* | South Africa | AF292040[d], AF273064[d], AF273459[d] |
| CMW 9932 | *C. cubensis* | *T. granulosa* | South Africa | AF273472[e], AF273062[f], AF273457[g] |
| CMW 9327 | *C. cubensis* | *T. granulosa* | South Africa | AF273473[e], AF273060[f], AF273455[g] |
| CMW 9328 | *C. cubensis* | *T. granulosa* | South Africa | AF273474[e], AF273061[f], AF273456[g] |
| CMW 8756 | *C. cubensis* | *E. grandis* | Indonesia | AF046896[b], AF273077[d], AF285165[d] |
| CMW 9903 | *C. cubensis* | *Eucalyptus* sp. | Kalimantan | AF292044[e], AF273066[f], AF273461[g] |
| CMW 9906 | *C. cubensis* | *Eucalyptus* sp. | Kalimantan | AF292045[e], AF273065[f], AF273460[g] |
| CMW 1651 | *C. parasitica* | *Castanea dentata* | USA | AF046901[b], AF273074[d], AF273467[d] |
| CMW 1652 | *C. parasitica* | *C. dentata* | USA | AF046902[b], AF273075[d], AF273468[d] |
| CMW 2498 | *Diaporthe ambigua* | *Malus sylvestris* | Netherlands | AF046906[b], AF273072[d], AF273471[d] |
| CMW 9343 | *C. cubensis* | *T. granulosa* | South Africa | |
| CMW 9346 | *C. cubensis* | *T. granulosa* | South Africa | |
| CMW 9358 | *C. cubensis* | *T. granulosa* | South Africa | |

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. CMW refer to the general and *Cryphonectria* culture collections.
[b] rDNA sequences obtained from Myburg *et al*. (1999).
[c] rDNA sequences obtained from Wingfield *et al*. (2001).
[d] Sequence data obtained from Myburg *et al*. (2002).
[e] rDNA sequences generated in this study.
[f] β-tubulin 1a/1b sequences generated in this study.
[g] β-tubulin 2a/2b sequences generated in this study.

sequences were aligned using CLUSTAL W (Thompson *et al*. 1997) and verified manually.

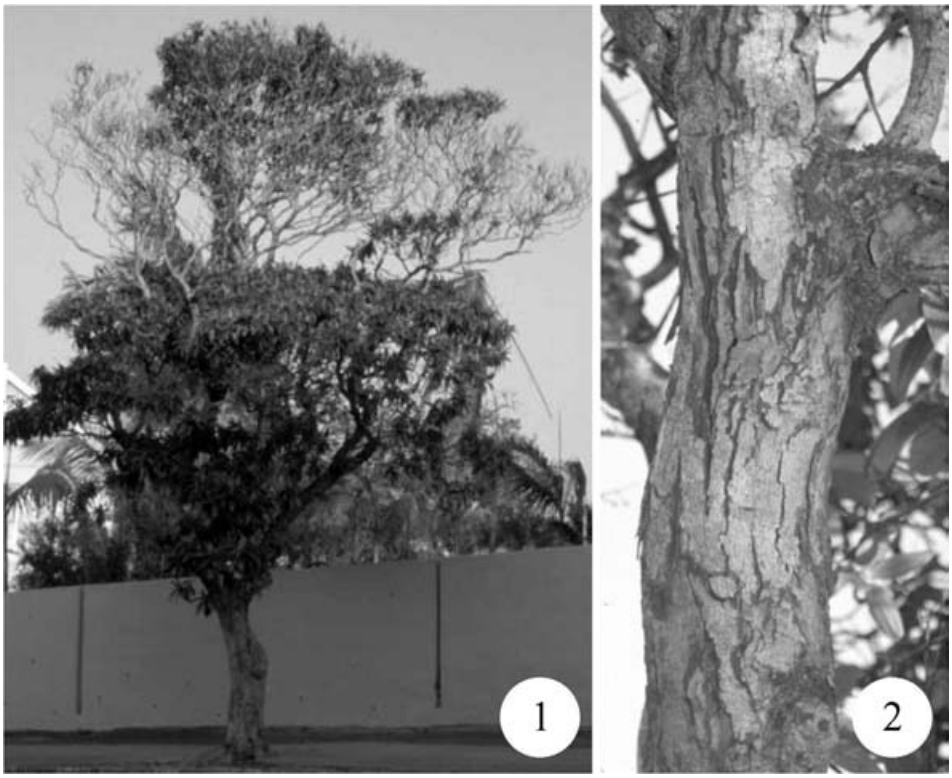
Phylogenetic analyses were performed using PAUP* version 4.0b (Swofford 1998). Heuristic searches with branch swapping (no swapping) and MULTREES (saving all optimal trees) effective were used in parsimony analyses. Gaps inserted during sequence alignment were treated as fifth characters (NEWSTATE). A partition-homogeneity test (PHT) was conducted (500 replicates, heuristic search type) to assess the combinability of the ITS and β-tubulin data sets. Bootstrap analyses (1000 replications) were done to determine the confidence levels of the tree branching points. Previously published sequences for *Cryphonectria parasitica*, the causal agent of chestnut blight (Elliston 1981), were included in the study for comparative purposes. *Diaporthe ambigua* was used as the outgroup taxon to root the phylogenetic tree. All sequences obtained in this study have been deposited in GenBank (accession nos. in Table 2).

### *Pathogenicity*

*Tibouchina granulosa* trees (~10–15 mm diam) were kept under greenhouse conditions for two weeks to acclimatise prior to inoculation. The greenhouse was subjected to natural day/night conditions and a temperature setting of ~25 °. Mycelial plugs (9 mm diam) were taken from the edges of actively growing cultures and placed, mycelium facing the cambium, into wounds made with a cork borer (9 mm diam). Wounds were sealed with parafilm to protect the inoculated fungus and cambium from desiccation. Ten trees were inoculated for each of the ten selected isolates. Sterile plugs were used for control inoculations on ten *Tibouchina* trees. To determine the relative pathogenicity, lesion lengths were measured on each tree after four weeks and the averages computed. The most virulent isolate was selected and used in a subsequent inoculation study on *T. granulosa* and *E. grandis*.

To assess reciprocal pathogenicity of *Tibouchina* and *Eucalyptus* isolates, the most virulent isolate (CMW 9343) from *Tibouchina* and a previously identified virulent isolate (CMW 2113) of *C. cubensis* from *E. grandis* (van Zyl & Wingfield 1999), were inoculated on 20 *T. granulosa* and 20 *E. grandis* (clone ZG 14) trees (~10 mm diam) respectively. The same inoculation technique as described above was used and results based on lesion length were assessed after 4 weeks. Ten *E. grandis* and ten *T. granulosa* trees were inoculated with sterile MEA plugs to serve as controls. This trial was repeated once. To determine the variance between isolates and among trees, the inoculation data were subjected to analysis of variance using the General Linear Model procedure of SAS (SAS/STAT Users guide, Version 6).

To investigate the pathogenicity of the *Tibouchina* and *Eucalyptus* isolates under field conditions, inoculations were performed on trees (~1 yr old) of an established *E. grandis* × *E. camaldulensis* clone (GC 747). These inoculations were performed at KwaMbonambi (KwaZulu-Natal) (28° 22′ S 32° 19′ E) where




**Figs 1–2.** Disease symptoms. **Fig. 1.** *Tibouchina* tree showing branch die-back caused by *C. cubensis*. **Fig. 2.** Girdling canker on the main stem of a *Tibouchina* tree infected by *C. cubensis*.

*C. cubensis* is known to occur in *Eucalyptus* plantations. Twenty trees (100–150 mm diam) each were inoculated with the three most virulent isolates from *Tibouchina* (CMW 9343, CMW 9346, CMW 9358) and one *C. cubensis* isolate from *E. grandis* (CMW 2113). Twenty trees were also inoculated with sterile MEA plugs to serve as controls. Results based on lesion length were measured after 6 months and the entire trial was repeated once. Statistical significance of results was determined as described above.

## RESULTS

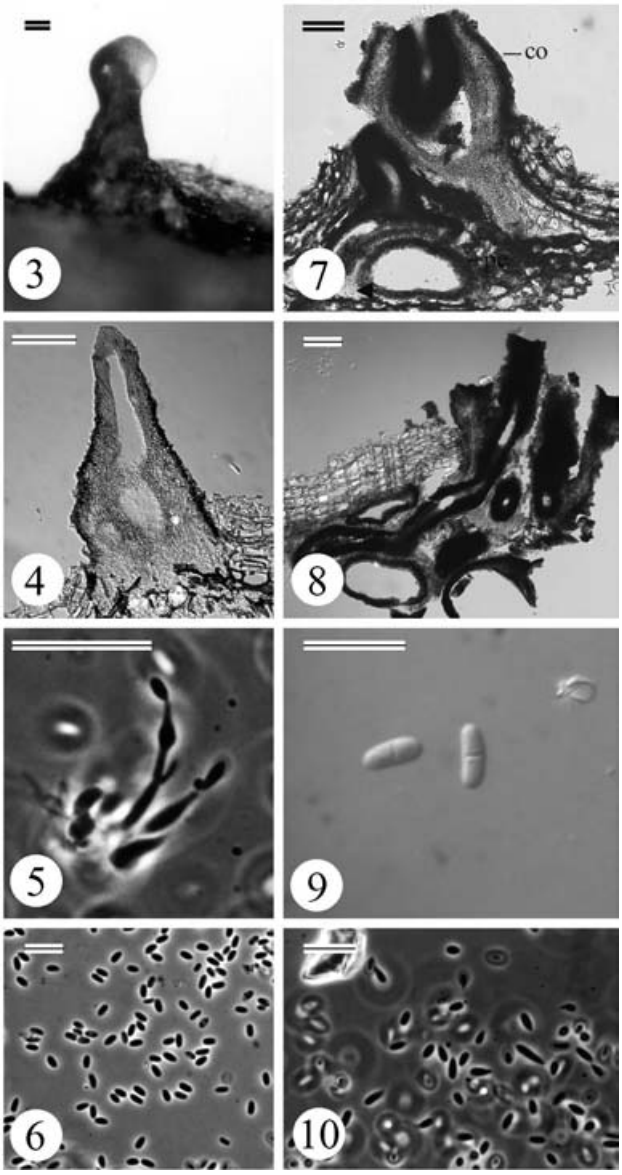
### *Disease symptoms and collection of samples*

During the first disease survey of *Tibouchina granulosa* trees, ten trees showing branch die-back were found in KwaMbonambi. One infected tree was also found in Richards Bay during a preliminary survey. Disease symptoms included branch die-back, cracking of the bark (Figs 1–2), and the development of girdling stem cankers. Fruiting bodies of a fungus resembling the anamorph of *Cryphonectria cubensis* were found between the cracks and on dead areas of the stem.

### *Morphological comparisons*

Anamorph structures of the fungus occurring on *Tibouchina granulosa* were superficial to slightly immersed on the bark, pyriform to clavate (Fig. 3). Longitudinal sections revealed a unilocular to occasionally multilocular, convoluted eustroma (Fig. 4) similar to those observed in Myburg *et al*. (2002). Structures were blackened with a luteous (17) interior, 192–310 μm wide; base 248–477 μm long, base above the bark surface 167–477 μm long (Fig. 3). One to four necks, either originating from a single locule or from more than one locule, occurred on a single superficial eustroma, and were up to 240 μm long, 105–124 μm wide. Conidiophores were hyaline, septate, with or without branching underneath the septum, 9.5–14(–15.5) × 1.5 μm (Fig. 5). Conidia were exuded as bright luteous (17) spore tendrils or drops (Fig. 3). Conidia were hyaline, nonseptate, cylindrical to oblong to oval, 3–4.5(–5.5) × 1–1.5 μm (Fig. 6).

Perithecia were rarely seen, and formed either below the conidioma (Fig. 7) or on their own (Fig. 8). When perithecia developed on their own, they were semi-immersed and surrounded with weakly developed, orange (15), predominantly prosenchymatous stromatic tissue, which was often slightly erumpent (Fig. 8). When the perithecia were produced below the blackened eustroma of the conidioma, the same type of stromatic tissue as that observed for the single ascomata was produced around the bases of the perithecial necks (Fig. 7). This tissue was different to that of the conidiomata, which was umber (15 m), *textura globulosa*. Perithecia were black, globose, 267–310 μm diam. Black, periphysate perithecial necks protruded through the stromatal surface for up to 500 μm. Necks were 62–105 μm wide and




**Figs 3–10.** Light micrographs of the anamorph and teleomorph of *Cryphonectria cubensis* from *Tibouchina granulosa* in South Africa. **Fig. 3.** Conidioma on bark. **Fig. 4.** Vertical section through condioma. **Fig. 5.** Conidiophores. **Fig. 6.** Conidia. **Fig. 7.** Perithecia (pe) with stromatic tissue (arrow) below a conidioma (co). **Fig. 8.** Ascoma. **Fig. 9.** Ascospores. **Fig. 10.** Conidia produced by isolate CMW 9343 after inoculation into a cut stem of an unknown *Eucalyptus* clone. Bars Figs 3–4, 7–8 = 100 μm; Figs 5–6, 9–10 = 10 μm.

covered in brown, *textura porrecta* tissue as it extends beyond the stroma, with the width of the extended parts being 112–200 μm. Ascospores were hyaline, septate, fusoid to oblong, sometimes slightly curved, (5–)5.5–7.5(–8) μm long, 1.5–2 μm wide (Fig. 9). No asci were seen in the available material.

The anamorph of the fungus from *T. granulosa* is similar to *Cryphonectria cubensis* from *Eucalyptus* spp. in South Africa (Myburg *et al.* 2002, Wingfield *et al.* 1989). The presence of the teleomorph of this fungus in South Africa has been reported only once (Wingfield *et al.* 1989). The teleomorph material observed in this study conforms to the description provided by these authors. The anamorph and teleomorph of the fungus on *T. granulosa* were also similar to those described for *C. cubensis* from other parts of the world (Hodges *et al.* 1976, Hodges *et al.* 1979, Hodges 1980, Wingfield *et al.* 2001).

Identification of the fungus on *T. granulosa* as *C. cubensis* is supported by the morphology of the structures produced on plants used in the inoculation studies. Fruiting structures formed by isolates from *T. granulosa* (PREM 57362, PREM 57363, PREM 57364, PREM 57365, PREM 57367) on the *Eucalyptus* clone (GC 747) were similar to those formed by isolate CMW 2113 (isolated from *E. grandis*) on the same host (PREM 57366). Only anamorph structures were produced in these inoculations.

Morphology of conidia from conidiomata formed on the artificially inoculated stems and field inoculations (PREM 57362, PREM 57363, PREM 57364, PREM 57365, PREM 57366, PREM 57367) (Table 1) was variable. Conidia produced by isolates CMW 9343 and CMW 2113 on the *Eucalyptus* stems varied from clavate to cylindrical to allantoid (Fig. 10). The conidia were also variable in size and longer 3.5–5.5(–8) × 1–1.5(–2) μm, than those found on material collected from natural infections. Such variation in spore morphology was not observed for pycnidia from natural infections. Many of the conidia in the inoculated material also had papillate apices (Fig. 10), although these were rarely seen in the natural material on *T. granulosa*. The papillate apices on conidia of South African material have been noted before, but were thought inordinately rare and not taxonomically significant (Myburg *et al.* 2002).

### *Sequencing and analysis of sequence data*

A PHT value of $P = 0.2$ showed that the data partitions (ITS1, 5.8S, ITS2, Bt1a/1b and Bt2a/2b) could be combined as one data set in the phylogenetic analyses. Alignment of the combined sequences resulted in a data set of 1529 characters, consisting of 955 constant characters, 338 parsimony informative characters and 196 variable characters that were parsimony uninformative. A strict consensus tree (50 % majority rule) (length of tree = 671 steps, consistency index/CI = 0.96 and retention index/RI = 0.95) was generated from the 196 variable characters (Fig. 11).

The phylogenetic tree based on the combined DNA sequences (Fig. 11) showed that the fungi from *T. granulosa* and *Eucalyptus* in South Africa, South America and South-east Asia/Australia resided in separate groups to *C. parasitica* and the outgroup *D. ambigua* (bootstrap support 100 %). The *C. cubensis*/*Tibouchina* clade is subdivided into sub-groups, consistent with geographical origins of the isolates. The South African isolates thus formed a clade separate (bootstrap support 98 %) from the South-east Asian and South American isolates. The South American *C. cubensis* strains

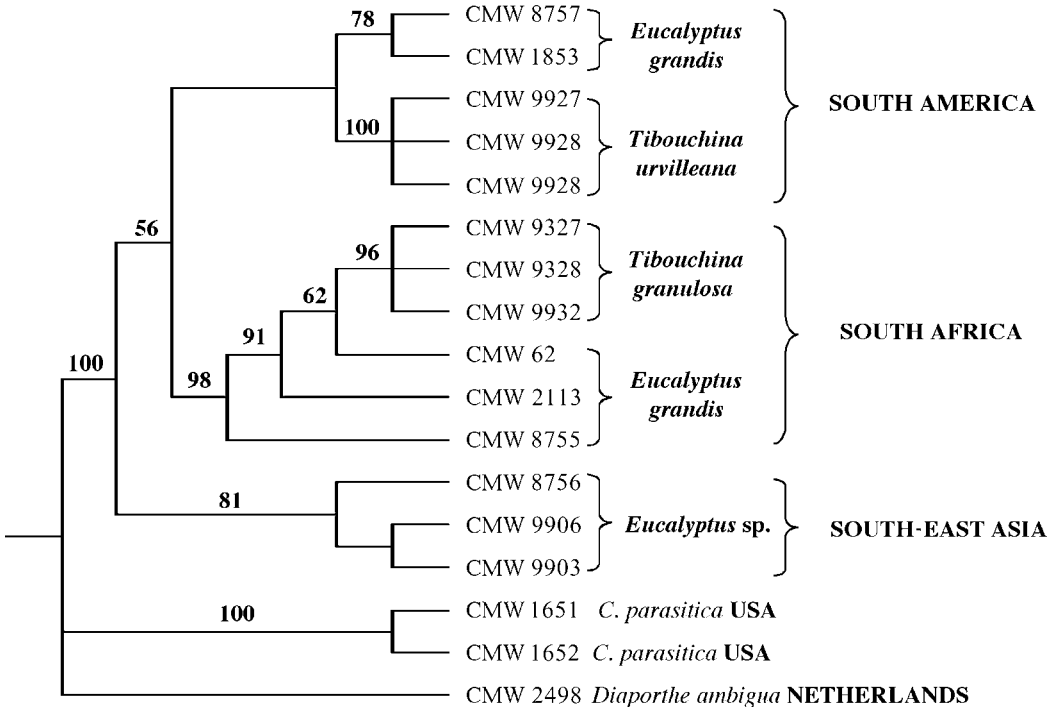



**Fig. 11.** Phylogenetic tree generated from a combined data set including ribosomal DNA (ITS1/ITS2) and β-tubulin gene sequence data. One strict consensus tree (length of tree = 671 steps, CI = 0.96 and RI = 0.95) was generated from heuristic searches performed on the combined data set. Bootstrap values (1000 replicates) are indicated above the branches and those lower than 50 % are not shown. *Diaporthe ambigua* was used to root the tree.

**Table 3.** Lesion lengths on 1 yr-old *Eucalyptus grandis* (clone ZG 14) and *Tibouchina granulosa* four wk after inoculation in the greenhouse.

| | [a]Lesion length (mm) (1st trial) | | [a]Lesion length (mm) (2nd trial) | | [a]Lesion length (mm) (3rd trial) | |
|---|---|---|---|---|---|---|
| Isolate | *Tibouchina* | ZG 14 | *Tibouchina* | ZG 14 | *Tibouchina* | ZG 14 |
| CMW 2113 | 49.9 | 146.5 | 65.6 | 975 | 867 | 155.5 |
| CMW 9343 | 108.9 | 133.0 | 34.2 | 104.1 | 90.9 | 129.3 |
| Control | 10 | 10 | 9.7 | 9.6 | 10 | 10 |

[a] Each value is the average of 20 measurements for each isolate.
$P > 0.0001$.
CV = 43.23666.
R-Square = 0.806038.
F = 7.45.

isolated from *Tibouchina* spp. grouped together with the South American *C. cubensis* isolates from *Eucalyptus* spp. The *C. cubensis* isolates from South-east Asia/Australia grouped together (bootstrap support 81 %). The bootstrap support for the branch node separating the South American and South-east Asian/Australian groups is 56 %, suggesting that these two groups are closely related.

### *Pathogenicity*

Greenhouse inoculation of *Tibouchina granulosa* seedlings and the *Eucalyptus grandis* clone (ZG 14) resulted in the formation of extensive lesions on both the hosts within 4 wk (Table 3). Some of the seedlings were already dropping their leaves, dying and producing epicormic shoots on the stems within this time period. The control inoculations showed no lesions. Both isolates from *Tibouchina* and *Eucalyptus* were more pathogenic to *Eucalyptus* than to *Tibouchina* ($P > 0.0001$) (Table 3).

Field inoculations resulted in the formation of girdling cankers, cracking bark and fungal sporulation around the lesions. The same symptoms were seen on the naturally infected *Tibouchina* trees (Figs 1–2). The analysis of data from the field inoculations, however, showed no significant differences in pathogenicity between isolates ($P > 0.1464$).

## DISCUSSION

This study represents the first report of a serious canker and die-back disease of ornamental *Tibouchina* trees in

**Table 4.** Lesion length on 18 month-old GC 747 trees grown in the plantation after inoculation with *Cryphonectria cubensis* isolates from *Tibouchina* and *Eucalyptus*.

| Isolate | [a]Lesion length (mm) (1st trial) | [a]Lesion length (mm) (2nd trial) |
|---|---|---|
| CMW 2113 | 77.9 | 56.1 |
| CMW 9343 | 83.4 | 45.9 |
| CMW 9346 | 50.8 | 92.5 |
| CMW 9358 | 68 | 54 |
| Control | 10 | 10 |

[a] Each value is the average of 20 measurements for each isolate.
$P > 0.1464$.
CV = 77.84968.
R-Square = 0.555225.
F = 1.24.

South Africa. To the best of our knowledge, this is the first disease to be recorded on this important ornamental tree in South Africa. Our results clearly show that the disease is caused by the well-known *Eucalyptus* canker pathogen *Cryphonectria cubensis*. The fungus has recently been reported as a serious pathogen of native *Tibouchina* spp. in Colombia (Wingfield *et al*. 2001) and the current study represents the first report of the disease on a species of *Tibouchina* outside South America.

In South Africa, *C. cubensis* on *Eucalyptus* is mostly characterised by the occurrence of the asexual fruiting structures. The sexual state of *Eucalyptus* has been found only once (Wingfield *et al*. 1989, van Heerden *et al*. 1997). It was, therefore interesting that perithecia of *C. cubensis* were occasionally found on *T. granulosa* in South Africa. This is in contrast to the situation in Columbia where only asexual fruiting structures were found on *T. urvilleana* and *T. lepidota* (Wingfield *et al*. 2001), while the sexual state is the dominant form on *Eucalyptus* spp. in that country (van der Merwe *et al*. 2001). Wingfield *et al*. (2001) suggested that the differences in occurrence of the sexual state could be the result of differences in biology of *C. cubensis* on different hosts. This hypothesis is supported by our data, where the South African *C. cubensis* rarely produces sexual structures on *Eucalyptus*.

Sequence data generated in this study confirm the fact that the fungus from *T. granulosa* in South Africa is *C. cubensis*. Our results further support a recent report that *C. cubensis* isolates from South Africa reside in a well resolved and strongly supported group different from that accommodating South American and Southeast Asian *C. cubensis* isolates (Myburg *et al*. 2002). Isolates from *T. granulosa* in South Africa cluster together with the South African *C. cubensis* isolates, separate from the *Tibouchina* isolates in Colombia. These data suggest that South African *C. cubensis* on *Tibouchina* and *Eucalyptus* has a similar, but probably different origin to *C. cubensis* found in other parts of the world.

Greenhouse inoculation trials conducted in this study suggest that the *Eucalyptus* clone used is more susceptible to infection by the South African *C. cubensis* than is *T. granulosa*. After four weeks, both tree species were producing epicormic shoots and had begun to die. This is in contrast to Colombian trials (Wingfield *et al*. 2001), where none of the *Eucalyptus* trees died and the *Tibouchina* trees were dead or dying after four months. It also appears that the South African *C. cubensis* isolates are more virulent than *C. cubensis* from *Tibouchina* in Colombia, since trees had begun to die within four weeks of inoculation.

At the present time, the origin of the South African form of *C. cubensis*, now known to occur on *Eucalyptus* and *Tibouchina*, is uncertain. Although morphologically almost identical to the fungus from Asia and South America (Myburg *et al*. 2002), biological and molecular evidence provide robust support for the notion that the South African fungus represents a discrete taxon. This leads us to speculate that the fungus on *Eucalyptus* and *Tibouchina* in South Africa will have an origin different to that of the fungus in Asia and South America. A likely origin of the fungus would be on native *Myrtaceae* in South Africa and surveys have been initiated to consider this hypothesis.

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