Discovery of the Eucalyptus canker pathogen Chrysoporthe cubensis on native Miconia (Melastomataceae) in Colombia

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Chrysoporthe cubensis is one of the most serious canker pathogens on commercially grown Eucalyptus species in the tropics and subtropics. During recent surveys for native hosts of C. cubensis in Colombia, fungi with fruiting structures similar to those of C. cubensis were found on native Miconia theaezans and Miconia rubiginosa, both members of the Melastomataceae. These fungi were identified based on morphology and DNA sequences of the ITS1/ITS2 region of the rDNA operon and the β-tubulin genes. The majority of isolates from M. theaezans and M. rubiginosa grouped together with South American C. cubensis isolates from Eucalyptus species and Syzygium aromaticum (clove). However, some of the isolates from M. theaezans grouped with isolates of Chrysoporhella hodgesiana, another anamorph species linked to Chrysoporthe, from Tibouchina spp. in Colombia. Pathogenicity of these fungi was assessed on various Melastomataceae. Miconia rubiginosa was more susceptible to infection by C. cubensis than two Eucalyptus clones. Isolates of C. cubensis and Chrysop. hodgesiana were mildly pathogenic on the various hosts included in the pathogenicity trials, and most pathogenic on Tibouchina urvilleana and Tibouchina lepidota.

Keywords: Chrysoporthe cubensis, Chrysoporhella hodgesiana, Colombia, Diaporthales, Miconia rubiginosa, Miconia theaezans

Introduction

Chrysoporthe cubensis, previously known as Cryphonectria cubensis (Gryzenhout et al., 2004), is one of the most serious pathogens of Eucalyptus spp. (Myrtaceae) in various South American countries (Boerboom & Maas, 1970; Hodges et al., 1976, 1979; Hodges, 1980), including Colombia (Van der Merwe et al., 2001). The associated canker disease has also been reported from other parts of the world with tropical or subtropical climates, mostly Africa (Gibson, 1981; Myburg et al., 2003; Roux et al., 2003), Southeast Asia (Sharma et al., 1985; Florence et al., 1986; Hodges et al., 1986; Myburg et al., 2003), Hawaii (Hodges et al., 1979; Myburg et al., 2003) and Australia (Davison & Coates, 1991; Myburg et al., 1999, 2003). In these regions, canker caused by C. cubensis is most severe in areas with high rainfall and temperature (Boerboom & Maas, 1970; Hodges et al., 1976, 1979; Sharma et al., 1985).

Cankers caused by C. cubensis are usually found at the base or on the lower stems of trees, but they may also occur higher up on the trunks (Hodges et al., 1976, 1979; Sharma et al., 1985). The pathogen kills the cambium, and in severe cases can result in tree death (Hodges et al., 1976, 1979; Sharma et al., 1985). The only practical management option for the disease is to plant resistant Eucalyptus species and clones (Hodges et al., 1976; Alfenas et al., 1983; Sharma et al., 1985).

Until recently, C. cubensis was known as Cryphonectria cubensis, and the disease that it causes was commonly referred to as cryphonectria canker of Eucalyptus (Wingfield, 2003; Gryzenhout et al., 2004). The fungus was transferred to the new genus Chrysoporthe, distinct from Cryphonectria, based on phylogenetic groupings arising from comparisons of ribosomal operon and β-tubulin gene sequences (Gryzenhout et al., 2004; Myburg et al., 2004). Species of Chrysoporthe are also morphologically distinct from Cryphonectria and are characterized by their dark-coloured, pyriform conidiomata and extending perithecial necks, covered in dark tissue (Gryzenhout et al., 2004; Myburg et al., 2004). Cryphonectria species have orange, pulvinate conidiomata, and perithecial necks extending...
from the stromatal surface are covered in orange tissue (Myburg et al., 2004).

In addition to C. cubensis, two other species have been described in *Chrysoporthe* (Gryzenhout et al., 2004). Isolates of the fungus previously known as Cry. cubensis from South Africa, have been named *Chrysoporthe austroafricana*, and isolates from *Tibouchina* spp. in Colombia are known as *Chrysoporthebella hodgesiana*. The latter species could not be described in *Chrysoporthe* because no teleomorph is known for it. However, DNA sequence data clearly show that *Chrysop. hodgesiana* resides in *Chrysoporthe*, and it was thus necessary to describe the new anamorph genus, *Chrysoporthebella*, to accommodate this species (Gryzenhout et al., 2004).

Until recently, *C. cubensis* has been known to occur only on trees belonging to the Myrtaceae. These hosts are predominantly species of *Eucalyptus* and also include clove (*Syzygium aromaticum*) (Hodges et al., 1986). *C. cubensis* also occurs naturally on strawberry guava (*Psidium cattleianum*) (Hodges, 1988), but it is not known whether isolates from this host reside in the same phylogenetic group as other isolates of *C. cubensis* from South America.

Previously, *C. cubensis* was believed to be present on *Tibouchina urvilleana* and *Tibouchina lepidota* in Colombia, which are members of the Melastomataceae native to South America (Wingfield et al., 2001). More recent studies have shown that the fungus considered by Wingfield et al. (2001) represents *Chrysop. hodgesiana* and not *C. cubensis* (Gryzenhout et al., 2004). There has been a subsequent report of a fungus resembling *C. cubensis* on *Tibouchina granulosa* in Brazil (Seixas et al., 2004). However, it is not yet known whether this fungus from Brazil represents *C. cubensis* or *Chrysop. hodgesiana*. Furthermore, *C. austroafricana* is also known to occur on ornamental *T. granulosa* in South Africa (Myburg et al., 2002a).

Recent surveys of cankers on Melastomataceae in Colombia have led to the discovery of fungi resembling *Chrysoporthe* spp. on a number of tree species that have not previously been implicated as natural hosts. The aim of this study was to identify these fungi based on morphology and DNA sequences. Pathogenicity of representative isolates was also tested on the hosts of origin and on *Eucalyptus grandis*.

**Materials and methods**

**Symptoms and collection of samples**

Disease surveys were conducted in two areas of Colombia having a wide range of different altitudes and precipitation (Fig. 1). Specimens were collected from *Miconia theaezans* (niguito) in a natural forest, with no *Eucalyptus* plantations nearby, alongside the farm La Selva [5°35′34″W and 4°47′26″N, 3143 mm year⁻¹, 2048 masl (m above sea level)]. This farm, belonging to Smurfit Carton de Colombia, is situated near the city of Pereira (Risaralda province). Cankers covered in conidiomata and ascomata were also found on *M. rubiginosa* trees (mortitio) of different ages on the farm Vanessa (76°35′15″W and 3°5′42″N, 2365 mm year⁻¹, 1000 masl), near the city of Timba (Cauca province). These trees were coppiced and occurred within a *Eucalyptus* plantation where *C. cubensis* has previously been collected (Van der Merwe et al., 2001).

Disease symptoms on the *Miconia* spp. included branch die-back, and cankers on branches, trunks or the tree bases that often resulted in the death of trees or tree parts. In the case of *M. rubiginosa*, trees were not killed by the disease but many *E. grandis* trees in plantations adjacent to naturally occurring *M. rubiginosa* were seriously affected by cankers. Cankers on the *Miconia* spp. were generally associated with parts of plants where branches and stems were physically wounded. Fruiting structures were abundant around the edges of the actively growing canker margins.

Specimens collected from cankers were transported to the laboratory for further analysis. Isolations from single conidia were made from the fruiting structures using malt extract agar MEA (20 g L⁻¹ malt extract agar, Biolab). Isolates used in this study have been preserved at 5°C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, and representative isolates have also been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). The original bark specimens from which isolations were made have been deposited (Table 2) in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).
<table>
<thead>
<tr>
<th>Isolate number(^a)</th>
<th>Alternative isolate number(^b)</th>
<th>Species identity</th>
<th>Host</th>
<th>Origin</th>
<th>Collector</th>
<th>GenBank accession numbers(^b)</th>
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Chrysoporthe cubensis on Miconia in Colombia

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CMW 9995c CBS 115730 Chrysop. hodgesiana T. semidecandra Colombia R. Arbelaez AY 956969, AY 956977, AY 956978
CMW 10625c CBS 115744 Chrysop. hodgesiana M. theaezans Colombia C. A. Rodas AY 956970, AY 956979, AY 956980
CMW 10626c CBS 115745 Chrysop. hodgesiana M. theaezans Colombia C. A. Rodas AY 262392, AY 262396, AY 262400
CMW 1652 CBS 112914 Cryphonectria parasitica Castanea dentata U.S.A. – AF 046902, AF 273075, AF 273468
CMW 10518 CBS 112919 Cryphonectria nitschkei Quercus sp. Japan T. Kobayashi AF 452118, AF 525706, AF 525713
CMW 10463 CBS 112920 Cryphonectria macrospora Castanopsis cuspidata Japan T. Kobayashi AF 368331, AF 368351, AF 368350

aCMW, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
bAccession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions, respectively.
cIsolates sequenced in this study.

DNA sequence comparisons

Isolates from Miconia spp. and E. grandis in Colombia were included in the DNA sequence comparisons (Table 1). Previously characterized C. cubensis isolates from Eucalyptus spp. (Myburg et al., 2002b; Gryzenhout et al., 2004) and S. aromaticum (Myburg et al., 1999, 2003) from different parts of the world, were included for comparative purposes. Isolates of Chrysop. hodgesiana from T. urvilleana (Wingfield et al., 2001; Gryzenhout et al., 2004) and C. austroafricana from Eucalyptus spp. and T. granulosa (Myburg et al., 2002a, 2002b) were also included. Species of the closely related Cryphonectria, namely Cry. parasitica, Cry. nitschkei and Cry. macrospora, were included as outgroup taxa to root the phylogenetic trees.

Isolates for DNA sequence comparisons were grown in malt extract broth (20 g L⁻¹ malt extract). DNA was extracted from mycelium as described in Myburg et al. (1999). The internal transcribed spacer (ITS) regions ITS1 and ITS2 as well as the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, and two regions within the β-tubulin genes were amplified using the primer pairs and reaction conditions as given by Myburg et al. (1999) and Myburg et al. (2002b), respectively. PCR products were visualized on ethidium bromide-stained 1% agarose gels, using a UV light. Purification of PCR products was done using a QIAquick PCR Purification Kit (Qiagen GmbH, Germany).

The purified PCR products were sequenced with the same primers that were used to amplify the respective DNA regions. An ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (Perkin-Elmer, UK) was used to sequence the amplification products on an ABI PRISM 3100 automated...
DNA sequencer. The resulting raw nucleotide sequences were edited using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., California, USA) software. Sequences were added to the existing dataset (S 1211, M 2095) of Gryzenhout et al. (2004) and manually aligned. Phylogenetic trees were inferred using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford, 1998). A 500 replicate partition homogeneity test (PHT) was done on the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites) to determine whether they could be analysed collectively (Farris et al., 1994).

A phylogenetic tree was inferred from distance analyses. The correct model for the datasets was found with MODEST version 3.5 (Posada & Crandall, 1998), namely the HKY85 model (Hasegawa et al., 1985) with the gamma distribution shape parameter set to 0.2202 (HKY + G). A 1000 replicate bootstrap analysis was executed to assess the confidence levels of the branch nodes of the phylogenetic tree. The sequence data generated in this study have been deposited in GenBank and accession numbers are listed in Table 1.

Morphology
For morphological identifications, fruiting structures from bark specimens were sectioned and studied under the microscope according to the method of Gryzenhout et al. (2004). Colour notations of Rayner (1970) were used. Growth studies were also conducted because Chrysop. hodgesiana can only be distinguished from C. cubensis based on its optimal growth temperature (25°C): C. cubensis and C. austroafricana grow optimally at 30°C (Gryzenhout et al., 2004). Growth rate of representative isolates (CMW 10625, CMW 10626, CMW 10022) from the Miconia spp. was therefore compared in culture to isolates from E. grandis (CMW 10638, CMW 10640) and T. semidecandra (CMW 9994, CMW 9995) to confirm the identifications based on DNA sequence comparisons. For the comparisons of growth in culture, the procedure described by Gryzenhout et al. (2004) was used, except that the temperature range tested in the current study was from 20°C to 35°C.

Pathogenicity tests
Glasshouse inoculation trials
Three isolates (CMW 10638, CMW 10639, CMW 10640) of C. cubensis from E. grandis in Colombia and two isolates (CMW 10625, CMW 10626) of Chrysop. hodgesiana from M. theaezans were screened for pathogenicity on 7-month-old T. urvilleiana plants in a contained glasshouse with natural light at −25°C. Trees were planted in plastic containers, watered frequently and fertilized when necessary (N-P-K and Boron; 70 and 10 g, respectively, per tree). Five trees were inoculated with each of the test fungi and an equal number of trees were inoculated with sterile water agar (WA; 20 g L−1) plugs. These WA inoculated trees served as negative controls. Inoculations were done by removing a plug of bark at a constant height (~30 cm above the ground) with a cork borer (9 mm diameter) to expose the cambium. Agar discs of the same size were taken from the edges of actively growing cultures and placed inside the wounds with the mycelium facing downwards. The agar discs were covered with tissue paper moistened with sterile water, and secured with masking tape, to reduce desiccation of the inoculum. The masking tape was removed after 10 days.

Trees were inoculated in October 2001 and lesion development was evaluated after 4 weeks. Lesions were exposed by scraping away the bark and the lengths of the lesions were measured. Re-isolations were done from the lesions. A pathogenic isolate of C. cubensis from E. grandis and an isolate of Chrysop. hodgesiana from M. theaezans (CMW 10639 and CMW 10625, respectively) were selected for subsequent field inoculation trials.

In a second glasshouse trial, two C. cubensis isolates (CMW 10022, CMW 10024) from M. rubiginosa were inoculated on T. urvilleiana and E. grandis (clone ZG14), which were 17–24 months old and up to 1.8 m high. A highly pathogenic isolate of C. austroafricana from South Africa (CMW 2113), used in previous pathogenicity studies (Van Heerden & Wingfield, 2001, 2002; Myburg et al., 2002a), was included for comparative purposes. Inoculation procedures were the same as those in the first glasshouse trial and 10 trees were inoculated for each of the three test isolates and for the negative control using WA discs. Inoculations were done as described above, except that a cork borer with a diameter of 6 mm was used. The trees were inoculated in May 2002, and evaluated in June 2002.

Field inoculation trials (Colombia)
The first inoculation trial was conducted at Rancho Grande farm (Fig. 1), Restrepo, Valle (76°30′49″W and 3°51′43″N, 1067 mm year−1, 1469 masl). This trial included reciprocal inoculations with an isolate of C. cubensis from E. grandis (CMW 10639) and an isolate of Chrysop. hodgesiana from M. theaezans (CMW 10625), selected in the first glasshouse trial. Five tree species were used, namely T. semidecandra, T. lepidota, T. urvilleiana, M. theaezans and a clone of E. grandis (clone 274). Trees were 1 year old and 20 of each tree species were inoculated per isolate. An equal number of trees were inoculated with WA discs to serve as negative controls. Inoculations were conducted in a similar way to those for the glasshouse inoculations, but the diameter of the inoculation wound was 4 mm. Trees were inoculated in May 2002 and lesion development was evaluated after 12 weeks. Internal lesion length in the cambium was measured for all field trials and the test fungi were reisolated from the lesions.

The second field trial was at Vanessa farm (Fig. 1), Timba, Cauca province. The C. cubensis isolate CMW 10022 from M. rubiginosa, shown to be pathogenic in the preliminary glasshouse trial, was used. Twenty three-year-old E. grandis trees (clone 275), 20 trees from seeds of a cross between E. grandis and E. urophylla (E. ‘urograndis’
clone 212), and 20 M. rubiginosa trees were inoculated. The M. rubiginosa trees were approximately 6 years old and formed part of the native vegetation surrounding the commercial plantations. Ten trees of each host were inoculated with uninoculated MEA to serve as negative controls. The trial was initiated in June 2002 and lesion lengths were measured after 12 weeks. The same inoculation techniques used in glasshouse and other field trials were applied, except that the inoculation wounds were 6 mm in diameter.

Data for all pathogenicity trials were analysed using a one-way analysis of variance (ANOVA) with SAS (2000) and did not deviate from normality. For the analyses, trees were randomly assigned, reflecting the experimental design. Scatter plots of the lesion measurements on the 20 trees of a species–clone combination exhibited no dependence. A Levene test for homogeneity of variances was applied throughout and showed that heterogeneity was not a factor in the data.

Results

DNA sequence comparisons

Amplification of the ITS1, 5·8S and ITS2 rRNA regions as well as the two regions in the β-tubulin gene resulted in PCR products of approximately 600 and 550 bp, respectively. The aligned DNA sequence of the partial ITS1/ITS2 region (538 bp) consisted of 472 constant characters, 28 parsimony-uninformative and 38 parsimony-informative characters, while the aligned sequence of the β-tubulin gene regions (894 bp) consisted of 716 constant characters, 71 parsimony-uninformative and 107 parsimony-informative characters. The rRNA and the β-tubulin sequence data sets were not fully congruent in the phylogenetic analyses (P = 0·032) because the ITS region could not differentiate C. austroafricana. However, the datasets were combined following Gryzenhout et al. (2004) to strengthen the support of the different clades. The combined data set (1432 bp) consisted of 45 taxa with the Cry. parasitica, Cry. macrospora and Cry. mitchellii isolates as the outgroup (Fig. 2).

The phylogenetic tree (Fig. 2) showed the same four clades as previously characterized (Myburg et al., 2002b, 2003; Gryzenhout et al., 2004). These clades represented Chrysop. hodgesiana, C. austroafricana, and the two morphologically identical clades from Southeast Asia/Zanzibar/Hawaii and South America/Congo, respectively, that define C. cubensis (Gryzenhout et al., 2004). The majority of isolates from M. theaezans (CMW 9980, CMW 9993) and M. rubiginosa (CMW 9996, CMW 10022, CMW 10024, CMW 10025, CMW 10026, CMW 10028) grouped in the South American/Congolese clade of C. cubensis together with isolates (CMW 10638, CMW 10639, CMW 10640) from E. grandis in Colombia (bootstrap support = 94%). However, two isolates from M. theaezans (CMW 10625, CMW 10626) grouped together with isolates of Chrysop. hodgesiana (bootstrap support = 81%).

Morphology

Specimens (PREM 57517, PREM 58307-58309, PREM 58311-58314) for isolates from M. rubiginosa (Table 2), which were identified as C. cubensis based on DNA sequence data (Fig. 2), were available for study. These specimens contained both anamorph and teleomorph structures similar to specimens from Eucalyptus spp. (PREM 57294). These fructifying structures also resembled those previously described for C. cubensis (Bruner, 1917; Hodges et al., 1979; Hodges, 1980; Myburg et al., 2003; Gryzenhout et al., 2004).

A comparison of isolates based on growth in culture confirmed results of the phylogenetic analyses. Isolates CMW 10022 (M. rubiginosa), CMW 10638 and CMW 10640 (E. grandis), which had been identified as representing C. cubensis, grew optimally at 30°C and were able to grow at 35°C. Chrysop. hodgesiana isolates (CMW 9994, CMW 9995) from T. semidecandra displayed optimum growth at 25°C and were not able to grow at 35°C. Isolates CMW 10625 and CMW 10626 (M. theaezans) showed the same growth pattern as isolates CMW 9994 and CMW 9995, confirming that they represented Chrysop. hodgesiana. These results are consistent with those reported by Gryzenhout et al. (2004).

Pathogenicity tests

Glasshouse inoculations

In the first glasshouse trial, inoculation with C. cubensis isolates (CMW 10638, CMW 10639, CMW 10640) from E. grandis and Chrysop. hodgesiana isolates (CMW 10625, CMW 10626) from M. theaezans gave rise to distinct lesions (Fig. 3) from which the test isolates could be reisolated. Lesions associated with the most pathogenic of these isolates (CMW 10625, CMW 10638, CMW 10639) were not significantly different from each other, but differed significantly (P < 0·0014) from the control inoculation. Isolates CMW 10639 (C. cubensis) from E. grandis and CMW 10625 (Chrysop. hodgesiana) from M. theaezans were chosen for field inoculations because they were most pathogenic for each species group.

In the second glasshouse trial, C. cubensis isolates (CMW 10022, CMW 10024) from M. rubiginosa and the South African isolate (CMW 2113) of C. austroafricana resulted in different size lesions (Fig. 4). The isolate of C. austroafricana (CMW 2113) was more pathogenic on the E. grandis clone than the other isolates tested (Fig. 4). This isolate was also less pathogenic on T. urvilleana (Fig. 4) than on the E. grandis clone. A C. cubensis isolate from M. rubiginosa (CMW 10024) was more pathogenic on E. grandis than on T. urvilleana (Fig. 4) and it was also more pathogenic on E. grandis than the other isolate from M. rubiginosa (CMW 10022). Isolate CMW 10022 from M. rubiginosa was equally pathogenic on E. grandis and T. urvilleana (Fig. 4). All isolates produced lesions significantly larger (P = 0·001) than those associated with the control inoculations. Only E. grandis trees infected by the C. austroafricana isolate (CMW
Figure 2 The phylogram generated from a combined data set comprising ribosomal and β-tubulin gene sequences of Chrysoporthe cubensis, C. austroafricana and Chrysoporthe hodgesiana. The phylogram was obtained with distance analyses using the HKY85 parameter model (G = 0.2202). Confidence levels of the tree branch nodes > 70% are indicated and were determined by a 1000 replicate bootstrap analysis. Isolates sequenced in this study are written in bold together with source host and location. Sequences for Cryphonectria parasitica, Cry. nitschkei and Cry. macrospora were used as outgroups.

Figure 3 Comparison of lesion extension in 7-month-old Tibouchina urvilleana trees under glasshouse conditions. The trees were inoculated with Chrysoporthe hodgesiana isolates from Miconia theaezans (CMW 10625, CMW 10626) and Chrysoporthe cubensis isolates from Eucalyptus grandis (CMW 10640, CMW 10638, CMW 10639) in Colombia, and a negative control. Mean length of lesions is shown with 95% confidence limits.
Chrysoporthe cubensis on Miconia in Colombia

2113) produced epicormic shoots below the inoculation points, indicating that the inoculated stems had been girdled.

Field inoculation trials (Colombia)

In the first field trial, lesions were produced on all tree species (T. urvilleana, T. lepidota, T. semidecandra, M. theaezans, E. grandis) in response to inoculation with the C. cubensis isolate CMW 10693 from E. grandis and the Chrysop. hodgesiana isolate CMW 10625 from M. theaezans. The longest lesions were produced on T. urvilleana and T. lepidota, while lesions on T. semidecandra, although smaller, also differed significantly (P = 0.0001) from those associated with the control inoculations (Fig. 5). Lesions on M. theaezans and the E. grandis clone were only slightly longer than the control inoculations (Fig. 5). Lesions produced by the C. cubensis isolate (CMW 10639) and Chrysop. hodgesiana isolate (CMW 10625) were similar in size on each tree species (Fig. 5).

In the second field trial, M. rubiginosa trees (Fig. 6) were more susceptible (P = 0.0001) to the C. cubensis
isolate from M. rubiginosa (CMW 10022) than the E. grandis trees tested (Fig. 6). Inoculations with isolate CMW 10022 on the E. grandis clone (275) and the hybrid clone (212) gave rise to lesions that did not differ from those of the control inoculations (Fig. 6).

Discussion

This study reports on the first discovery of the Eucalyptus canker pathogen C. cubensis on native Miconia species (Melastomataceae) in Colombia. Isolates of the fungus from M. theaezans and M. rubiginosa grouped in the subclade that characterizes C. cubensis occurring in South America, as defined in previous studies (Myburg et al., 1999, 2002b, 2003; Roux et al., 2003; Gryzenhout et al., 2004). Fungal structures on herbarium specimens linked to these isolates had conidiomata and ascomata typical of C. cubensis, and spores were similar in size to those previously reported for this fungus (Hodges, 1980; Myburg et al., 2002b, 2003; Gryzenhout et al., 2004).

In a previous study, Wingfield et al. (2001) reported on the discovery of the Eucalyptus canker pathogen, Cry. cubensis, on native Tibouchina spp. in Colombia. That fungus was shown to be pathogenic to both Eucalyptus and T. urvilleana trees and it was speculated that Tibouchina could represent the host of origin of the fungus. A later study (Gryzenhout et al., 2004), including a large set of isolates collected in recent years, has shown that the fungus studied by Wingfield et al. (2001) represents Chrysop. hodgesiana and not C. cubensis. The present study therefore represents the first discovery in South America of C. cubensis on native hosts, M. rubiginosa and M. theaezans, and where the identity of the fungus has been confirmed with DNA sequence comparisons.

Not all of the isolates collected from Miconia spp. in this study represent C. cubensis. Some isolates from M. theaezans were identified as Chrysop. hodgesiana based on DNA sequences and cultural characteristics. Two species of Chrysoporthe thus appear to occur on this native host in Colombia. Thus far, only one of them, C. cubensis, has been found causing cankers on Eucalyptus, although Chrysop. hodgesiana also appears to have the capability to do so.

Pathogenicity trials in this study included isolates representing both C. cubensis and Chrysop. hodgesiana. It was interesting that there were no significant differences in pathogenicity between isolates of C. cubensis (CMW 10638, CMW 10639, CMW 10640) and Chrysop. hodgesiana (CMW 10625, CMW 10626) in either the glasshouse or field trials. Results of this study confirm those of Wingfield et al. (2001) where the fungus now known as Chrysop. hodgesiana was shown to be able to infect Eucalyptus spp., although it has never been found to occur naturally on this host. Both C. cubensis and Chrysop. hodgesiana appear to represent an equal threat to commercial Eucalyptus plantations in Colombia.

Field inoculation trials showed that species of native Melastomataceae in Colombia differ in their susceptibility to infection by C. cubensis and Chrysop. hodgesiana. In a field trial where five different host species were tested, T. urvilleana and T. lepidota were the most susceptible to the isolates of both C. cubensis and Chrysop. hodgesiana. In contrast, M. theaezans trees were relatively tolerant to infection. Tibouchina semidecandra was less susceptible to infection by C. cubensis and Chrysop. hodgesiana than the other two species of Tibouchina, but it was more susceptible than M. theaezans. Generally, these results reflect a high level of susceptibility amongst various species of Miconia and Tibouchina to infection by C. cubensis and Chrysop. hodgesiana. Miconia and Tibouchina are native plants in Colombia and their relative susceptibility to the two pathogens might differ in different regions of the
country. Results of artificial inoculation tests give an indication of susceptibility, but they can also be misleading, and should be viewed within the context of the objectives specified for the inoculation tests.

Results of the pathogenicity trials in this study suggest that C. cubensis is more pathogenic on native Melastomataceae, especially Tibouchina spp., than on E. grandis. It is generally believed that pathogens are less virulent on their native hosts than susceptible exotic species (Leppik, 1970; Newhouse, 1990). Therefore, the E. grandis clones used in the trials were expected to be more susceptible to C. cubensis than the Tibouchina and Miconia spp. However, these commercially grown clones have been subjected to intensive selection for resistance to disease in recent years and it is possible that the clones or seed lots chosen for these trials have a high degree of tolerance to the pathogen.

Isolates of C. cubensis from native Miconia spp. in Colombia could have originated on these trees. However, it is possible that these fungi were introduced into the country and later adapted the capacity to infect native Melastomataceae. In this study, C. cubensis was found on M. theaezans in native vegetation that was far removed from Eucalyptus plantations. It therefore seems more likely that this fungus originated on Miconia spp. in Colombia than elsewhere. In the case of the site where C. cubensis was found on M. rubiginosa, these trees were coppiced when Eucalyptus stands were established and have hardly been affected by C. cubensis. In contrast, the Eucalyptus trees in the area have been seriously damaged by C. cubensis and it seems that the origin of C. cubensis, in this case, is more likely to be M. rubiginosa than Eucalyptus. However, resolving the question of original host was not an objective of this study and will need to be answered through a comprehensive genetic analysis of a population of isolates.

The occurrence of C. cubensis on M. theaezans and M. rubiginosa, which are native to South America, suggests that this pathogen could possibly be indigenous to that part of the world. Members of the Melastomataceae are common in South America, Central America, the Caribbean islands and Hawaii (Everett, 1981). The occurrence of C. cubensis on species belonging to this family could support the hypothesis (Wingfield et al., 2001) that the fungus occurred widely throughout South and Central America and the Caribbean, prior to the widescale planting of Eucalyptus species. This would explain prior observations (Hodges et al., 1986; Seixas et al., 2004) that Eucalyptus trees were rapidly infected by the pathogen after planting in South American countries. Besides having a possible origin in South America, several alternative hypotheses on the origin of C. cubensis exist (Hodges et al., 1986; Seixas et al., 2004). Although this study reports on the discovery of a potential original host for C. cubensis in South America, more extensive surveys in representative areas of Colombia, and including large numbers of isolates, would be necessary to determine the extent of its occurrence on these native hosts in South America. A constraint to this work is that collecting this material will be difficult due to the sociopolitical climate in this area.

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