CHAPTER 8

Aurapex penicillata gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia

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Aurapex penicillata gen. sp. nov. from native _Miconia theaezans_ and _Tibouchina_ spp. in Colombia

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Abstract: Conidiomata of a fungus resembling *Chrysoporthe cubensis*, a serious canker pathogen of *Eucalyptus* spp. (*Myrtaceae, Myrtales*) in tropical and subtropical parts of the world, was found on *Eucalyptus grandis* in Colombia. Fruiting structures of the fungus could be distinguished from those of *C. cubensis* by their distinctly orange conidiomatal necks. This fungus was also found on several plant species native to Colombia including *Tibouchina urvilleana*, *T. lepidota* and *Miconia theaezans* (*Melastomataceae, Myrtales*). Morphological comparisons as well as those based on sequences of the ITS1/ITS2 region of the ribosomal DNA repeat and the β-tubulin gene, were used to characterize this fungus. Its pathogenicity was also assessed on various plants from which it has been collected, either in field or greenhouse trials. Phylogenetic analyses showed that isolates reside in a clade distinct from the four clades accommodating *Chrysoporthe, Cryphonectria*, *Endothia* and *Rostraureum*. Members of this clade are distinguished by the presence of orange

conidiomatal necks with black bases, and a unique internal stromatal structure. No teleomorph has been found for this fungus, for which we have provided the name *Aurapex penicillata* gen. sp. nov. *A. penicillata* produced only small lesions after inoculation on young *T. urvilleana*, *M. theaezans* and *E. grandis* trees, and appears not to be a serious pathogen.

Taxonomical novelties: *Aurapex* Gryzenh. & M. J. Wingf. gen. nov. nom. prov., *Aurapex penicillata* Gryzenh. & M. J. Wingf. sp. nov. nom. prov.

Key words: *Aurapex*, *Chrysoporthe*, Colombia, *Diaporthales*, *Eucalyptus*, *Melastomataceae*

INTRODUCTION

The *Melastomataceae* represents a family of flowering plants common to Neotropical America and Hawaii (Everett 1981). This family resides in the *Myrtales*, which also accommodates the *Myrtaceae* (Conti *et al.* 1996). The *Myrtaceae* includes the genus *Eucalyptus*, many species of which are grown as a source of pulp and timber in plantations around the world (Turnbul 2000).

Chrysoporthe cubensis (Bruner) Gryzenh. & M. J. Wingf. (formerly *Cryphonectria*) is a serious canker pathogen of *Eucalyptus* spp. (Boerboom & Maas 1970, Hodges 1980, Sharma *et al.* 1985, Wingfield 2003) and *Syzygium aromaticum* (clove, also *Myrtaceae*) (Hodges *et al.* 1986), in the tropics and sub-tropics. Intriguingly, this pathogen has recently been shown to cause disease on members of the *Melastomataceae* such as *Miconia theaezans* (niguito) and *Miconia rubiginosa* (mortiño) in Colombia (Rodas *et al.* 2005). A second fungus, *Chrysoporthella*

hodgesiana Gryzenh. & M. J. Wingf., a species of *Chrysoporthe* based on phylogenetic data but known only by its anamorph, also occurs on Colombian *Melastomataceae* such as *M. theaezans* (Rodas *et al.* 2005), *Tibouchina urvilleana*, *Tibouchina lepidota* and *Tibouchina semidecandra* (Gryzenhout *et al.* 2004/Chapter 1 in this thesis). Recognition of *C. cubensis* on hosts residing in the *Melastomataceae* has substantially altered views regarding the origin and distribution of this important tree pathogen (Wingfield *et al.* 2001, Wingfield 2003, Rodas *et al.* 2005).

During the course of surveys in Colombia to assess the occurrence of *Chrysoporthe* spp. on trees other than *Eucalyptus* spp., a fungus similar to *C. cubensis* and *Chrysop. hodgesiana* was found on *Tibouchina* spp. These trees were planted as ornamentals in parks or on farms. The unknown fungus produced only conidiomata that were black and pyriform, in a shape reminiscent of *Chrysoporthe* spp. The fruiting bodies, however, differed from those of *Chrysoporthe* spp. in that the apices of the conidiomatal necks were orange. Subsequent surveys led to the discovery of the fungus on native *M. theaezans* as well as on *Eucalyptus grandis*. The aims of this study were to define the phylogenetic position of this fungus using DNA sequence comparisons, as well as to produce a taxonomic description and generic key. In addition, the pathogenicity of the new fungus was assessed in greenhouse and field inoculation experiments.

MATERIALS AND METHODS

Symptoms and collection of samples

Structures of the unknown fungus were first found in 1996 on *T. urvilleana* and *T. lepidota* in Colombia. These trees were growing in La Culebra park in El Peñol, on a

private farm near Granada (Antioquia Province), and on the Argentina farm of Smurfit Cartón de Colombia near Riosucio (Caldas Province). In a subsequent disease survey in 1998, similar fruiting structures were found on *Miconia theaezans* occurring in native vegetation on La Selva farm near Pereira (Risaralda province). In 2002, this fungus was discovered for the first time on basal cankers on *E. grandis* on the Libano farm near Pereira, as well as on *M. theaezans* at the same location.

The fruiting structures of the unknown fungus occurred around the periphery of cankers on the stems and branches of trees, which occasionally led to branch die-back. In some cases, fruiting structures of *C. cubensis* and *Chrysop. hodgesiana* occurred on the same plant. On *E. grandis*, fruiting structures of the fungus were also found on branches that were in the process of senescence, and the fungus also appeared to colonize branch stubs.

Bark specimens containing conidiomata were collected from cankers and taken to the laboratory for isolation. Single conidial isolates were obtained from spore suspensions on 2 % Malt Extract Agar (MEA) (20 g Biolab Malt Extract, 15 g Biolab Agar, 1 L water, Merck, Midrand, South Africa) and incubated at 25 ºC. The cultures have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). Original bark specimens from which isolations had been made, were used for morphological characterization and have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Morphology

Conidiomata were cut from bark specimens, rehydrated for one min in boiling water, and sectioned at –20 ºC with a Leica CM1100 Cryostat after embedding in Leica mountant (Setpoint Premier, Johannesburg, South Africa). Sections approximately 12 µm thick were mounted on microscope slides in lactophenol. Fifty measurements of ascospores, asci, conidia and conidiophores were taken for the holotype specimen and these are presented as (min–)(average – std. dev.) – (average + std. dev.)(–max) µm. Ten structures were sectioned to observe the internal morphology of the fruiting bodies and a range was obtained for the eustromatic bases, necks and conidial locules. Micrographs were taken with a HRc Axiocam digital camera and accompaning Axiovision 3.1 software (Carl Zeiss Ltd., Germany). The color charts of Rayner (1970) were used to define colors of cultures and morphological structures.

Culture morphology of the ex holotype strain CMW 10030 and CMW 11296 (Table 1), was characterized on MEA (20 g/L malt extract agar, Biolab, Merck). These studies were conducted using the technique described by Venter *et al.* (2002). Growth tests were at temperatures ranging from 15 to 35 ºC at 5 º intervals, and cultures were grown in the dark.

DNA sequence comparisons

Representative isolates of the fungus from Colombia were used in the DNA sequence comparisons (Table 1). Sequences of *C. cubensis* isolates from *Miconia* and *Eucalyptus* spp. in Colombia (Gryzenhout *et al.* 2004, Rodas *et al.* 2005), and *Chrysop. hodgesiana* isolates from *Miconia* and *Tibouchina* spp. in Colombia (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004, Rodas *et al.* 2005) were also used. Sequences for isolates of *C. cubensis* from other parts of the world, those of

Chrysoporthe austroafricana Gryzenh. & M. J. Wingf. (Myburg *et al.* 2002a, 2003) and those for recognized members of *Cryphonectria* and *Endothia*, which are closely related to *Chrysoporthe* (Venter *et al.* 2002, Myburg *et al.* 2004a, 2004b), were also included (Table 1). Sequences from the recently described *Rostraureum tropicale* Gryzenh. & M. J. Wingf., a pathogen of *Terminalia* in Ecuador (Gryzenhout *et al.* 2005/Chapter 7 in this thesis), were included in the dataset. *Rostraureum* contains the fungus previously known as *Cryphonectria longirostris* (Earle) Micales & Stipes (Gryzenhout *et al.* 2005). Two *Diaporthe ambigua* Nitschke isolates, which also reside in the *Diaporthales* but have been shown to reside in a different family to that of *Cryphonectria* and related taxa (Castlebury *et al.* 2002), were used as a single outgroup to root the phylogenetic tree generated in this study. The sequence matrix (study accession number = S1128, matrix accession number = M1935) from the study of Myburg *et al.* (2004a) was used as template for the alignment.

DNA was isolated from the fungi as described in Myburg *et al.* (1999). PCR amplification of the ITS1, conserved 5.8S and ITS2 regions of the rRNA operon as well as two regions in the β-tubulin gene, was performed as described in Myburg *et al.* (1999) and Myburg *et al.* (2002a) respectively. Primer pairs ITS1/ITS4 (White *et al.* 1990) were used for the ITS1/ITS2 region, and primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995) were used to amplify two β-tubulin gene regions. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were sequenced in both directions using the same primers that were used in the amplification reactions. Sequencing reactions were performed using a PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.). Nucleotide sequence data were generated with an ABI PRISM 3100™ automated DNA

sequencer. The raw sequence data were manipulated using the Sequence Navigator version 1.0.1 software package (Perkin-Elmer Applied BioSystems, Foster City, California).

Nucleotide sequences were manually aligned by inserting gaps. Gaps were treated as Newstate in the parsimony analyses, and as missing in the distance analyses. Phylogenetic analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). A Partition Homogeneity Test (Farris *et al.* 1994) was used to determine whether the ribosomal rRNA (ITS1, 5.8S, ITS2) partition and β-tubulin partition could be combined in the phylogenetic analyses. The aligned sequences were analyzed with parsimony by heuristic searches, with the tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) in effect, and sequences added randomly (100 additions). Uninformative sites were excluded and sites were re-weighted according to their individual Consistency Indices (CI) to reduce the number of trees obtained. A distance analysis was also performed using the Tamura-Nei parameter model (Tamura and Nei 1993) with adjusted settings (proportion of invariable site (I) = 0.4334; Gamma distribution (G) = 0.4592; Base frequency 0.2044, 0.3191, 0.2551; Rate matrix 1.00, 2.2953, 1.00, 1.00, 4.5168). This model was chosen as suggested by MODELTEST version 3.5 (Posada & Crandall 1998). Tree branch supports were assessed with a 1000-replicate bootstrap analysis. GenBank accession numbers of sequences generated in this study as well as those from previous phylogenetic studies are listed in Table 1. The DNA sequence alignment has been deposited in TreeBASE (SN 2261).

Pathogenicity tests

Two isolates of the newly described fungus (CMW 10031, CMW 10034) from *M. theaezans* were compared with isolates of *Chrysoporthe* spp. in a contained pathogenicity trial. One *Chrysoporthe* isolate (CMW 2113) represented *C. austroafricana* and has been used in previous pathogenicity tests (Myburg *et al.* 2002b, Van Heerden & Wingfield 2002). The other *Chrysoporthe* isolate (CMW 10639) represented *C. cubensis* from *E. grandis* in Colombia (Gryzenhout *et al.*. 2004).

Pathogenicity of the isolates was compared on each of ten seedlings of *T. urvilleana* and a susceptible *E. grandis* clone (ZG14) in a custom-built phytotron. These trees were approximately 1.5 m tall and 7-mo-old, and they were exposed to natural light conditions and an average daily temperature of ~ 25 ºC. Ten trees were inoculated at a constant height (~ 30 cm above the ground) with sterile water agar (WA, Biolab, Merck) plugs to serve as negative controls. Wounds were made on stems using a cork borer (6 mm diam) to expose the cambium. Discs of the same size were taken from the actively growing edges of colonies and inserted into the wounds with the mycelium facing inwards. Wounds were then covered with plastic film to prevent desiccation and contamination. Trees were inoculated in June 2002 and lesion development was evaluated after 6 wks by measuring lesion lengths below the outer bark.

A field trial to consider the pathogenicity of the fungus under natural conditions was carried out on *M. theaezans* trees and a susceptible clone (018) of *E. grandis* on the Libano farm, Pereira, Risaralda, Colombia. Twenty *E. grandis* and the same number of *M. theaezans* trees were inoculated with isolate CMW 10031 from *M. theaezans* in Colombia. Trees of the *E. grandis* clone were approximately 18-mo-

old (~ 9 m high), while the *M. theaezans* trees formed part of the natural vegetation growing in close proximity to the *E. grandis* trees and were of unknown age (~ 4–6 m high). Ten trees of each species were inoculated with WA as negative controls. Inoculations were carried out in the same way as those described for the phytotron inoculation, except that the inoculation wounds were 4 mm in diam and covered with masking tape. The trees were inoculated in June 2002 and results were evaluated 12 weeks later in September 2002. The lengths of the lesions produced in the cambium were measured and compared after removal of the bark. Data were compared using a one-way Analysis of Variance (ANOVA) computed using the SAS software package, v. 6 (2002). Mean lesion sizes together with 95 % confidence limits were presented graphically.

RESULTS

Morphology

Conidiomata of the fungus on bark specimens of *M. theaezans*, *T. urvilleana* and *E. grandis* were pyriform and superficial with fuscous black bases, and most similar to fruiting structures of *C. cubensis* (Hodges 1980, Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). These structures were thus different to the anamorph structures of all genera in the *Diaporthales* with orange fruiting structures ie. *Cryphonectria*, *Endothia* and *Rostraureum*, which all have completely orange conidiomata (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005). Conidiomata had fuscous-black, globose bases with long, slender necks (Figs 1A, 2A) that might be confused with those of *C. cubensis* in the absence of the orange neck apices. The conidiomatal base cells were *textura globosa*, umber to sienna when sectioned, with

thick walls, while the inner cells were prosenchymatous (Fig. 1C). This is similar to the basal tissue of conidiomata of *C. cubensis* (Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). The minute [(2.5–)3–4(–4.5) × 1–1.5(–2) µm], aseptate conidia (Figs 1I, 2C) were also similar to those of *C. cubensis* (Hodges 1980, Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004).

The fungus from *M. theaezans*, *T. urvilleana* and *E. grandis* in Colombia could be distinguished from *C. cubensis* by various unique characteristics of the conidiomata as well as on the basis of cultural morphology (Table 2). Despite having basal tissue similar to that of *C. cubensis*, tissue surrounding the conidiomatal locules is dark, consisting of larger cells than those of the adjacent prosenchyma (Fig. 1C). Neck tissue differed from that found in *C. cubensis* and consisted of square cells at the outer edge, with *textura porrecta* cells at the center, and thinner *textura porrecta* cells lining the ostiolar canals (Figs 1E–1F). The tissue at the tips of the conidiomatal necks was orange and contained orange crystals (Figs 1A, 1G, 2A). Long, sterile hyphae similar to perithecial periphyses occurred in the ostiolar canals (Fig. 1F), but these are absent in *C. cubensis*. Unique protrusions consisting of three to several cell layers were also formed within the conidiomatal locule lining (Figs 1B, 1D, 2B). Cultures of the fungus from *Tibouchina* and *Miconia* spp. had an olivaceous to isabelline interior that differed from the creamy white cultures patched with cinnamon produced by *C. cubensis*.

No ascomata were observed that were known to have been produced by the newly discovered fungus. A few ascomatal structures were observed on specimen PREM 58575 from *M. theaezans*. However, these structures stained brown in 3 % KOH, and not purple as was the case for the conidiomata of the fungus being studied and other members of this group in the *Diaporthales* (Castlebury *et al.* 2002). These ascomata

probably represent a species of *Valsa* co-infecting this particular host, since the ascospores were allantoid and aseptate.

DNA sequence comparisons

The PCR products generated for the ribosomal and two β-tubulin gene regions were between 550 bp and 600 bp in size, respectively. The PHT test (P = 0.182) indicated no significant conflict between the two data sets for these gene regions, which were thus combined in the phylogenetic analyses. There were also no strongly supported conflicts between the trees obtained for the two gene regions separately. The sequence data set included 32 taxa of which the two *D. ambigua* isolates represented a single outgroup taxon. The β-tubulin dataset (total 952 bp including both regions) consisted of 543 constant, 32 variable parsimony-uninformative and 377 variable and parsimony-informative characters (g1 = –0.780817). The ITS dataset (total 573 bp) consisted of 338 constant, 29 variable parsimony-uninformative and 206 variable and parsimony-informative characters (g1 = –0.863275). The combined set amounted to a total of 1525 characters. The heuristic search produced ten trees (tree length = 1095.3 steps, consistency index of = 0.805, retention index of = 0.918) that differed only in branch length for isolates. The tree obtained with distance analyses showed the same clades as the trees obtained with parsimony, one of which was chosen for presentation (Fig. 3). The same groupings of isolates with equally high bootstrap support, were also obtained when ambiguously aligned portions, which mostly represented the introns of the β-tubulin dataset and the ITS1 region, were excluded from the analyses.

The isolates of the anamorphic fungus on *M. theaezans* formed a distinct clade (bootstrap support 100 %) among the other clades in the phylogenetic tree, although it was apparent that some degree of variation existed between isolates, some originating

from the same location but different fruiting structures. The clade representing the anamorphic fungus was most closely related to *Cryphonectria* (Fig. 3). The other clades represented different closely related genera, namely *Cryphonectria*, *Endothia* and *Rostraureum.* Therefore, the anamorphic fungus grouped separately from the isolates of *C. cubensis* and *Chrysop. hodgesiana* from *Miconia* and *Tibouchina* spp. in Colombia, which grouped within the *Chrysoporthe* clade (Fig. 3).

Taxonomy

The fungus found on *M. theaezans*, *T. urvilleana* and *E. grandis* from Colombia has clearly defined morphological features that distinguish it from *Chrysoporthella*, the anamorph genus of *Chrysoporthe*, to which it is morphologically most similar (Table 2). These features are unlike those of any other coelomycete genus. The fuscous black conidiomata are also different from the uniformly orange fruiting structures of *Cryphonectria*, *Endothia* and *Rostraureum*, although the necks of the undescribed fungus are orange. These differences are supported by DNA sequence data showing that isolates of the morphologically distinct fungus from Colombia group separately from those representing *Chrysoporthe*, *Cryphonectria*, *Endothia* and *Rostraureum*.

No teleomorph was found for the fungus considered in this study. The distinct grouping of the fungus from Colombia, however, indicates that the fungus represents a distinct genus, and not the anamorph of an already existing genus. Phylogenetic data present clear evidence of its affinity to members of *Cryphonectria* and allied genera residing in the *Diaporthales.* In the absence of a teleomorph, the fungus from Colombia cannot be described in an ascomycete genus (ICBN, Art. 59.2, Greuter *et al.* 2000). It is thus described as a new species in a new mitosporic genus, and the following description is provided.

Aurapex Gryzenh. & M. J. Wingf., **gen. nov., nom. prov.**

Etymology. Latin, *aureus*, golden, and *apex*, top, refers to the golden colored tips of the conidiomata.

Conidiomata globosa vel pyriformia, basibus fusconigris collis aurantiacis, superficialia. Collum e textura porrecta factum, cellulis parietalibus ostioli gracilioribus, in ora colli quadratis, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes, hyalinae. Cellulae conidiogenae phialidicae. Conidia obtusa, hyalina, non septata.

Conidiomata eustromatic, globose to pyriform base with one to several, long, cylindrical to attenuated necks with orange tips, superficial to slightly immersed, fuscous-black. *Tissue* at the edges of conidiomal bases of *textura globulosa*, with elongated cells adjacent to conidial lining and prosenchymatous tissue occurring in the center of the basal tissue. Tissue of necks made up of *textura porrecta* with cells lining the ostiole thinner, cells at edge of necks consisting of square cells. *Conidiophores* cylindrical to flask-shaped, hyaline, occasionally septate with or without lateral branches. *Conidiogenous cells* phialidic. *Conidia* obtuse, hyaline, aseptate.

Typus: *Aurapex penicillata* Gryzenh. & M. J. Wingf., 2006.

Aurapex penicillata Gryzenh. & M. J. Wingf., **sp. nov., nom. prov.** Figs 1–2

Etymology. *penicillus*, a painter's brush, refers to the brush-like protrusions formed by the lining of the conidial locules.

Conidiomata pyriformia cum collis, superficialia, basibus fusconigris collis aurantiacis, textura parietali loculorum prominentia e 3– circiter 15 cellulis formans. Textura colli in ora e cellulis quadratis, intus e textura porecta facta, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes apicibus attenuatis, hyalinae. Cellulae conidiogenae phialidicae. Conidia (2.5–)3–4(–4.5) × 1–1.5(–2) µm, obtusa, non septata, hyalina, in forma guttarum sporarum coccinearum exsudata. Coloniae cum hyphis aeriis sparsis, albocremet, intus atro-olivaceae vel isabellinae, celeriter crescentes. temperatura optima 25°C.

Conidiomata single or aggregated, eustromatic, with globose to pyriform bases and attenuated or cylindrical necks, base 120–400 µm high, 300–700 µm wide above bark surface, necks up to ~1800 µm long depending on environmental conditions, 80–225 µm wide, conidiomata superficial to slightly immersed, bases fuscous-black with tips of necks orange (Figs 1A, 1B, 2A, 2B). Unilocular or multilocular (Figs 1B, 2B), locules up to 360 µm diam at widest point, locule lining producing conidiophores forming protrusions consisting of 3 to ~15 cells (Figs 1B, 1D, 2B), locules opening through 1 to 3 necks, each either connected to a single locule or to more than one locule. *Tissue* of base complex with thick-walled cells, *textura globulosa*, umber to sienna at edge, cells around the locules sienna to hazel, larger and more elongated, and almost white prosenchymatous tissue occurring between the edge and the locule (Fig. 1C). Neck tissue consisting of hazel, double-walled, square cells at the edge, with the cells lining the ostiole thinner and those at the center of *textura porrecta* tissue (Figs 1E–1F), long, aseptate filaments, similar to periphyses, occurring inside the ostiolar canals (Fig. 1F), tip of necks of *textura epidermoidea*, containing orange

crystals (Fig. 1G). *Conidiophores* (6–)7.5–13.5(–18.5) × (0.5–)1–1.5(–2) µm, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline (Figs 1H, 2C). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches, collarette and periclinal thickening inconspicous (Figs 1H, 2C). *Conidia* (2.5–)3–4(–4.5) × 1–1.5(–2) µm, obtuse, aseptate, hyaline (Figs 1I, 2C), exuded as scarlet spore droplets.

Cultural characteristics: fluffy with few aerial hyphae, creamy white with a dark olivaceous to isabelline interior, margins even, conidiomata occasionally produced in mature cultures, optimum growth at 25 C, isolates covering the surface of 90 mm plates on day 6 at the optimum temperature.

Distribution: Colombia

Substrate: Miconia theaezans, Tibouchina urvilleana, Tibouchina lepidota, Eucalyptus grandis

Specimens examined. **Colombia**, Risaralda, Pereira, Libano farm (75º 35′ 49″ W and 4º 43′ 13″ N, 2102 msal), bark of *Miconia theaezans*, Sept. 2002, C. A. Rodas, **holotype** PREM 57520, ex-type culture CMW 10030 = CBS 115740, additional cultures CMW 10031, CMW 10034, CMW 10035 = CBS 115742; Libano farm, bark of *Eucalyptus grandis*, Sept. 2002, C. A. Rodas, PREM 58578; La Selva farm (75º 35′ 34″ W and 4º 47′ 26″ N, 2048 msal), bark of *Miconia theaezans*, Nov. 1998, C. A. Rodas, PREM 58572; Quindio, Salento, Andes farm (75° 33′ 16″ W and 4° 41′ 08″ N, 2102 masl), bark of *Miconia theaezans*, May 2000, M. J. Wingfield, PREM 58576, living cultures CMW 11296 = CBS 115801; Antioquia, Granada, Granada farm (75° 8′ 10″ W and 6° 6′ 52″ N, 2050 msal), bark of *Tibouchina urvilleana*, Nov. 1998, C. A. Rodas, PREM 58573; Caldas, Riosucio, La Argentina farm (75º 44′ 55″ W and 5º

22′ 25″ N, 2247 msal), bark of *Tibouchina urvilleana*, Nov. 1998, C. A. Rodas, PREM 58574, PREM 58575; Valle, Darien, Cedral farm (76º 26′ 06″ W and 3º 57′ 06″ N, 1825 masl), bark of *Eucalyptus grandis*, Dec 2001, C. A. Rodas, PREM 58577.

Key to *Cryphonectria, Endothia, Chrysoporthella, Rostraureum* and *Aurapex*

1a. Base of anamorph fruiting structures fuscous-black…..…… 2

1b. Anamorph fruiting structures completely orange……...……... 3

2a. Conidiomata uniformly fuscous-black, locule lining even…… ***Chrysoporthella***

2b. Conidiomata fuscous-black but with tip of neck orange, locule lining have brush-like protrusions…..... ***Aurapex***

3a. Conidiomata rostrate, superficial ……...……..... ***Rostraureum***

3b. Conidiomata pulvinate……..……..……... 4

4a. Conidiomata semi-immersed, ascospores uniseptate……...……..... ***Cryphonectria***

4b. Conidiomata superficial, ascospores aseptate……..……...……...…. ***Endothia***

Pathogenicity

Isolates of *A. penicillata* (CMW 10031, CMW 10034) produced small lesions on both *T. urvilleana* and the *E. grandis* clone in the phytotron trial. These lesions did not differ significantly from the control inoculations (Fig. 4). Conidiomata were produced abundantly on the surfaces of the lesions. Lesions associated with *A. penicillata* were significantly smaller ($P < 0.0001$) than those associated with *C. cubensis* and *C. austroafricana* on *E. grandis* and *T. urvilleana* with those of *C. austroafricana* (CMW 2113) on *E. grandis* longest (Fig. 4). The latter isolate was also

the only one observed to girdle inoculated stems resulting in the production of epicormic shoots below the sites of inoculation.

In the Colombian field trial, the isolate of *A. penicillata* (CMW 10031) gave rise to small lesions on *M. theaezans* that did not differ significantly from the control inoculations (Fig. 5). Lesions produced on the *E. grandis* clone did not differ significantly from those on *M. theaezans* (P = 0.0217) and were extremely variable (Fig. 5). Control inoculations on the *E. grandis* clone also gave rise to small lesions, possibly due to endophytes naturally present in the stems of trees (Fig. 5).

DISCUSSION

This study treats the discovery of a new mitosporic genus in the *Diaporthales*, which thus far contains a single species, *Aurapex penicillata*. All indications are that this fungus is native to South America where it occurs naturally on *Miconia* and *Tibouchina* spp. *Aurapex penicillata* is closely related to *Cryphonectria* and *Endothia*, and the recently described genera *Chrysoporthe* (anamorph *Chrysoporthella*) and *Rostraureum*, which contain species previously in *Cryphonectria*. This was established through DNA sequence comparisons of the ITS region of the ribosomal repeat and β-tubulin genes.

The distinction of *A. penicillata* based on DNA sequence comparisons as a genus separate from *Cryphonectria*, *Chrysoporthe*, *Endothia* and *Rostraureum*, and not an anamorph genus of one of the existing genera, is well supported by morphological characteristics. Although the teleomorph is unknown, the morphological characteristics of the asexual state differ substantially from those of its closest relatives. Conidiomata of *A. penicillata* are fuscous-black, superficial and

pyriform with attenuated necks. These resemble the conidiomata of *Chrysoporthella*, the anamorph of *Chrysoporthe* that also has fuscous black conidiomata. *Aurapex* can, however, easily be distinguished from *Chrysoporthella* based on the distinctly orange tips of the necks and by its unique stromatal tissue. This new fungus can also be distinguished from the conidiomata of *Cryphonectria*, *Endothia* and *Rostraureum* spp., which are completely orange in color. These anamorph differences are consistent with those found in previous studies (Venter *et al.* 2002, Myburg *et al.* 2004a, Gryzenhout *et al.* 2005). These studies showed that stromatic and anamorph morphology are the most informative morphological characters that support the different phylogenetic assemblages, even in the absence of sexual structures. Recognition of these phylogenetic assemblages as different genera is strongly supported by the fact that they are morphological very distinct and they would not comfortably reside in a single genus.

Aurapex penicillata could be confused with the serious pathogens *C. cubensis* and *Chrysop. hodgesiana*, which occur on the same hosts and in the same region. This is especially so when the characteristic orange colored necks of *A. penicillata* become dislodged from their conidiomatal bases. Moreover, fruiting structures of these different fungi can occur on the same piece of bark. Of these three fungi, only *C. cubensis* is known to cause serious disease on *Eucalyptus* (Wingfield 2003, Gryzenhout *et al.* 2004) although *Chrysop. hodgesiana* can also infect *Eucalyptus* trees in artificial inoculations (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004).

Aurapex penicillata gave rise to lesions on *E. grandis* in pathogenicity tests, but there was no evidence of significant pathogenicity, at least in comparison to *C. cubensis*. Although *A. penicillata* occurs on *E. grandis* under natural conditions, the pathogen appears to be mainly associated with dead branch stubs. Because of

differences in pathogenicity and importance, it is necessary to identify *C. cubensis*, *Chrysop. hodgesiana* and *A. penicillata* correctly in *Eucalyptus* plantation disease surveys.

Pathogenicity tests conducted in this study should be seen as preliminary as they were limited by the lack of a complete series of known hosts of *A. penicillata*. Our primary objective was to assess pathogenicity, especially given the fact that the highly pathogenic *C. cubensis* has now been found to cause severe disease on *Melastomataceae* native to South America (Rodas *et al.* 2005). Although preliminary, our results show that *A. penicillata* probably poses no threat to *Eucalyptus* or *Melastomatacea.* Furthermore, the common occurrence of the fungus on native *Melastomataceae* in Colombia adds substance to our view that it is native to hosts in that family. Its ability to infect *Eucalyptus* spp. is probably opportunistic and related to the high levels of inoculum on surrounding native vegetation in Colombia.

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Table 1. Isolates of *Chrysoporthe*, *Cryphonectria*, *Endothia*, *Rostraureum* and *Aurapex penicillata* used in this study. Isolates sequenced in this study are in bold.

Identification	Culture no. [a]	Alternative isolate number[a]	Host	Origin	Collector	Genbank Accession no. [b]
Chrysoporthe cubensis	CMW 2632	—	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
	CMW 1856	—	*Eucalyptus* sp.	Kauai, Hawaii	—	AY 083999, AY 084010, AY 084022
	CMW 11290	CBS 115738	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 214268
	CMW 10774	—	*Syzygium aromaticum*	Zanzibar, Tanzania	—	AF 492130, AF 492131, AF 492132
	CMW 9432	CBS 115724	*Eucalyptus grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
	CMW 8757	—	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
	CMW 1853	—	*Syzygium aromaticum*	Brazil	—	AF 036891, AF 273070, AF 273465

	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 9993	CBS 115728	*Miconia theaezans*	Colombia	C.A. Rodas	AY 214298, AY 214226, AY 214262
	CMW 10024	CBS 115739	*Miconia rubiginosa*	Colombia	C.A. Rodas	AY 262390, AY 262394, AY 262398
Chrysoporthella hodgesiana	CMW 9927	—	*Tibouchina urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265653, AF 292034, AF 292037
	CMW 9995	CBS 115730	*T. urvilleana*	Colombia	R. Arbelaez	AY 956969, AY 956977, AY 956978
	CMW 10625	CBS 115744	*M. theaezans*	Colombia	C.A. Rodas	AY 956970, AY 956979, AY 956980
	CMW 10641	CBS 115854	*Tibouchina semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 8755	—	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273459
Rostraureum tropicale	CMW 9973	CBS 115726	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167427, AY 167432, AY 167437
	CMW 9975	CBS 115727	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167429, AY 167434, AY 167439

	CMW 10796	CBS 115757	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438
Aurapex penicillata	**CMW 10030** (ex-type designated here)	CBS 115740	*M. theaezans*	Colombia	C.A. Rodas	AY 214311, AY 214239, AY 214275,
	CMW 10031	—	*M. theaezans*	Colombia	C.A. Rodas	AY 994511, AY 994513, AY 994514
	CMW 10034	—	*M. theaezans*	Colombia	C.A. Rodas	AY 994512, AY 994515, AY 994516
	CMW 10035	CBS 115742	*M. theaezans*	Colombia	C.A. Rodas	AY 214313, AY 214241, AY 214277,
	CMW 11296	CBS 115801	*M. theaezans*	Colombia	C.A. Rodas	AY 214315, AY 214243, AY 214279
Cryphonectria radicalis	CMW 10455	CBS 238.54	*Quercus suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
Cryphonectria parasitica	CMW 7047	ATCC 48197	*Quercus virginiana*	USA	R.D. Wolfe	AF 368329, AF 273073, AF 273469
Cryphonectria nitschkei	CMW 13742	MAFF 410570	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 697936, AY 697961, AY 697962
Cryphonectria macrospora	CMW 10463	CBS 112920	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

Endothia gyrosa	CMW 2091	ATCC 48192	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
Endothia gyrosa	CMW 10442	—	*Q. palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF 368338
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ATCC = American Type Culture Collection, Manassas, USA; MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

[b] Given as sequences from the ITS region, and regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b respectively.

Table 2. Comparison of morphological features distinguishing *Aurapex penicillata* from *Chrysoporthe* spp.

Morphological features	***Chrysoporthe* spp.**[a]	***Aurapex penicillata***
Stromatic tissue	Cells surrounding locule similar to those in center.	Cells surrounding locule darker, larger.
Neck	Uniformly fuscous-black.	Orange apex.
Neck tissue	Cells at edge *textura globulosa*.	Cells at edge square.
Ostiolar canal	Contains no periphyses.	Contains periphyses.
Locule lining	Even to convoluted.	Forms protrusions consisting of three to several cell layers.
Culture morphology	Creamy white with cinnamon patches.	Creamy white with olivaceous to isabelline interior.

[a] According to Myburg *et al.* (2002a, 2003), Gryzenhout *et al.* (2004).

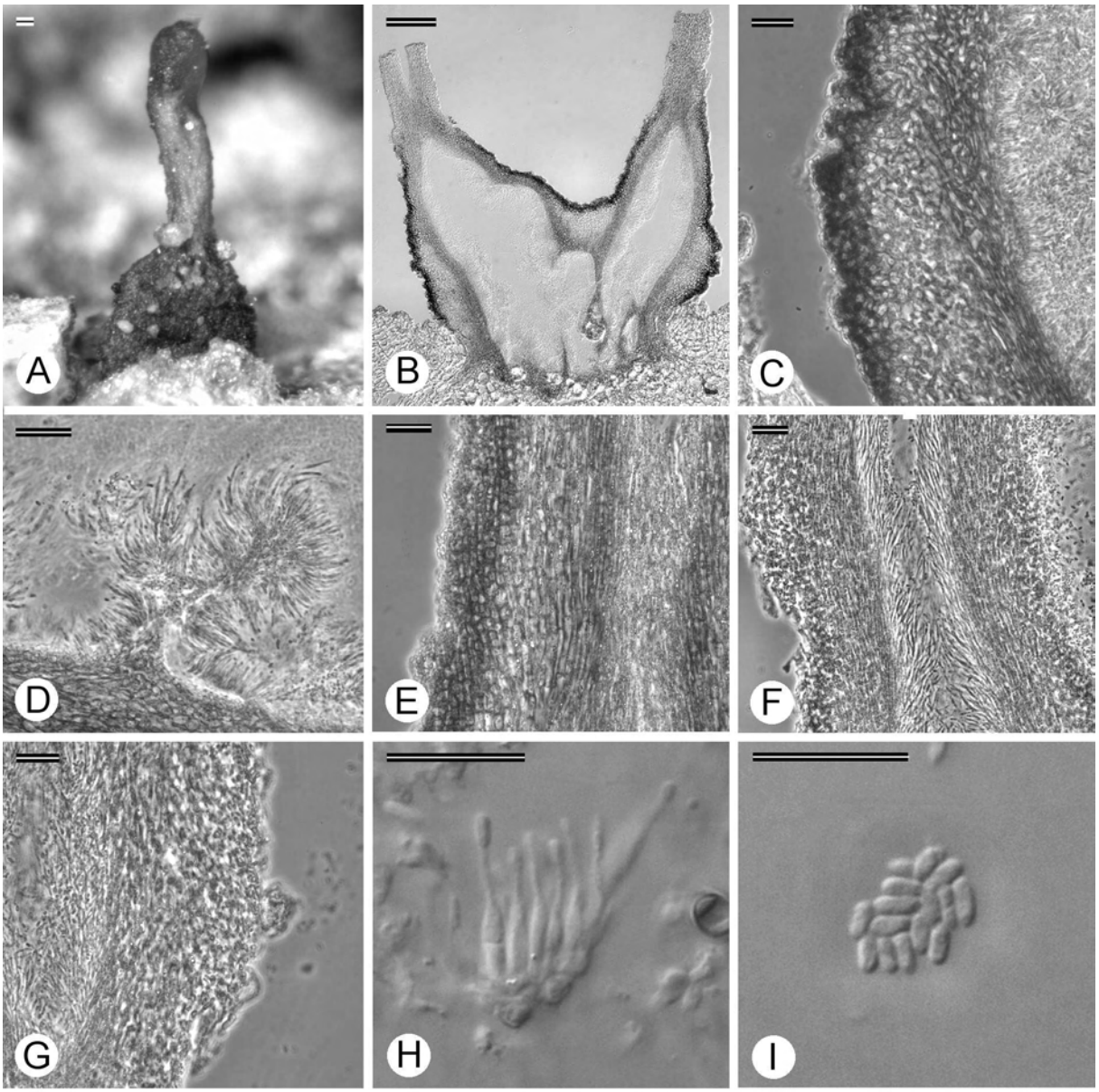

Fig. 1. Fruiting structures of *Aurapex penicillata*. A. Conidiomata on bark and in section (B). C. Tissue at base of conidioma. D. Protrusions in locule lining. E. Tissue of neck and periphyses in ostiolar canal (F). G. Tissue of neck apex. H. Conidiophores. I. Conidia. Scale bars A–B = 100 µm; C–G = 20 µm; H–I = 10 µm.

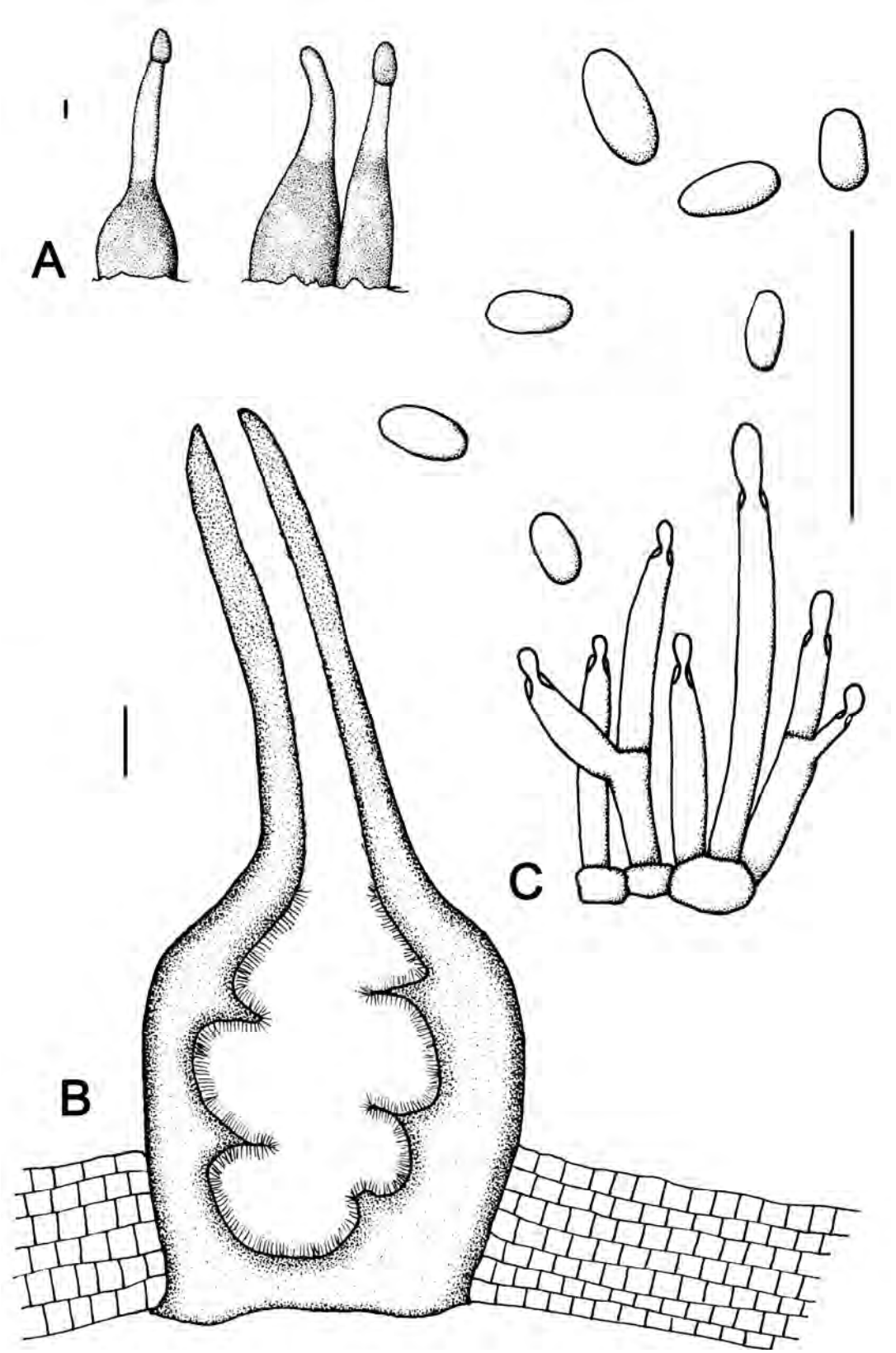

Fig. 2. Schematic drawings of *Aurapex penicillata*. A. Conidiomata on bark. B. Section through conidioma. C. Conidiophores and conidia. Scale bars A–B = 100 µm; C = 10 µm.

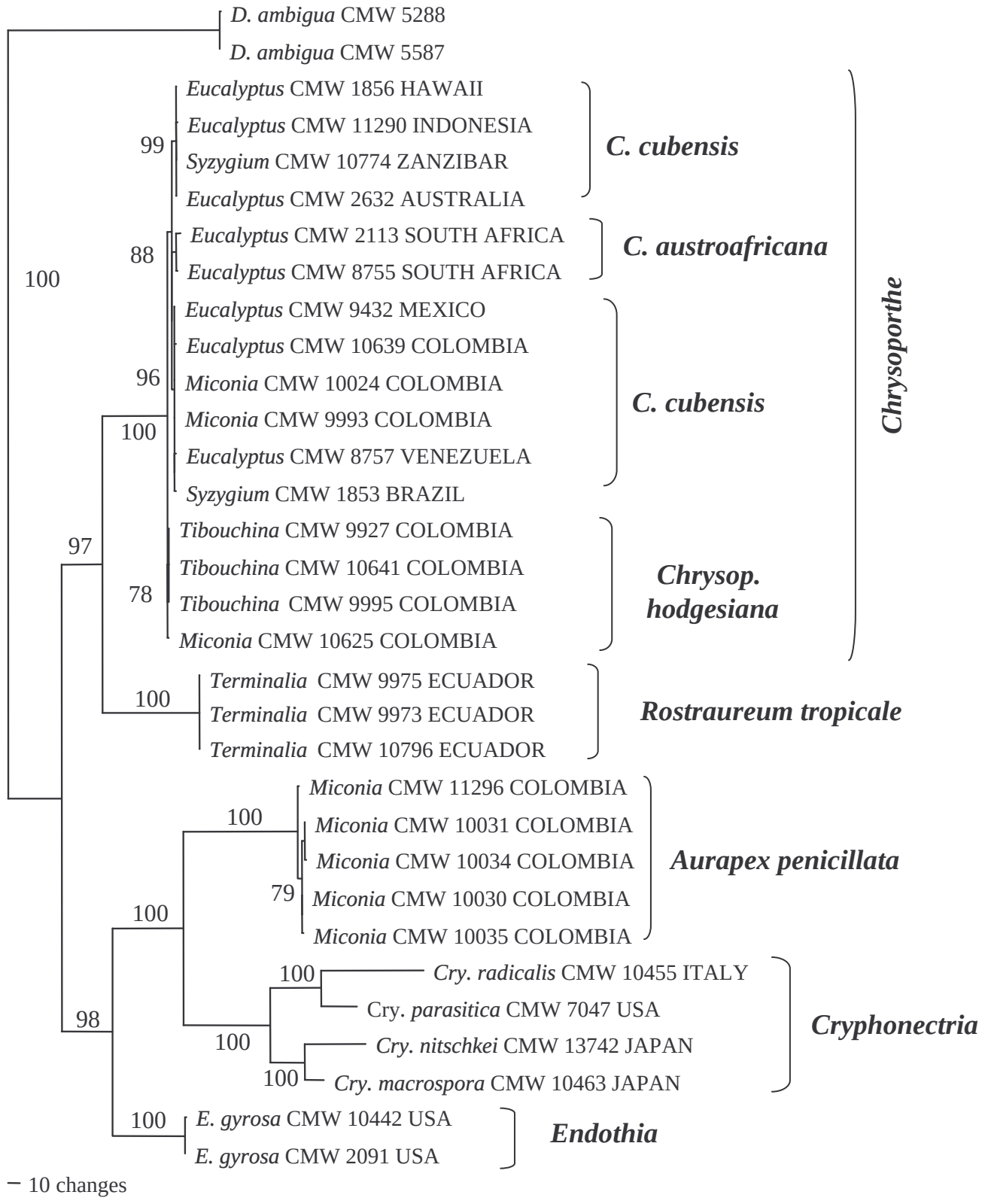

Fig. 3. One of ten most parsimonious trees obtained from a combined data set comprising ribosomal and β-tubulin gene sequences (tree length = 1095.3 steps, consistency index of = 0.805, retention index of = 0.918). Isolates representing the genera *Chrysoporthe*, *Rostraureum*, *Aurapex*, *Cryphonectria* and *Endothia* are represented. Confidence levels >70% determined by a 1000 replicate bootstrap analysis are indicated on the tree branch nodes. Two sequences for *Diaporthe ambigua* were used as an outgroup.

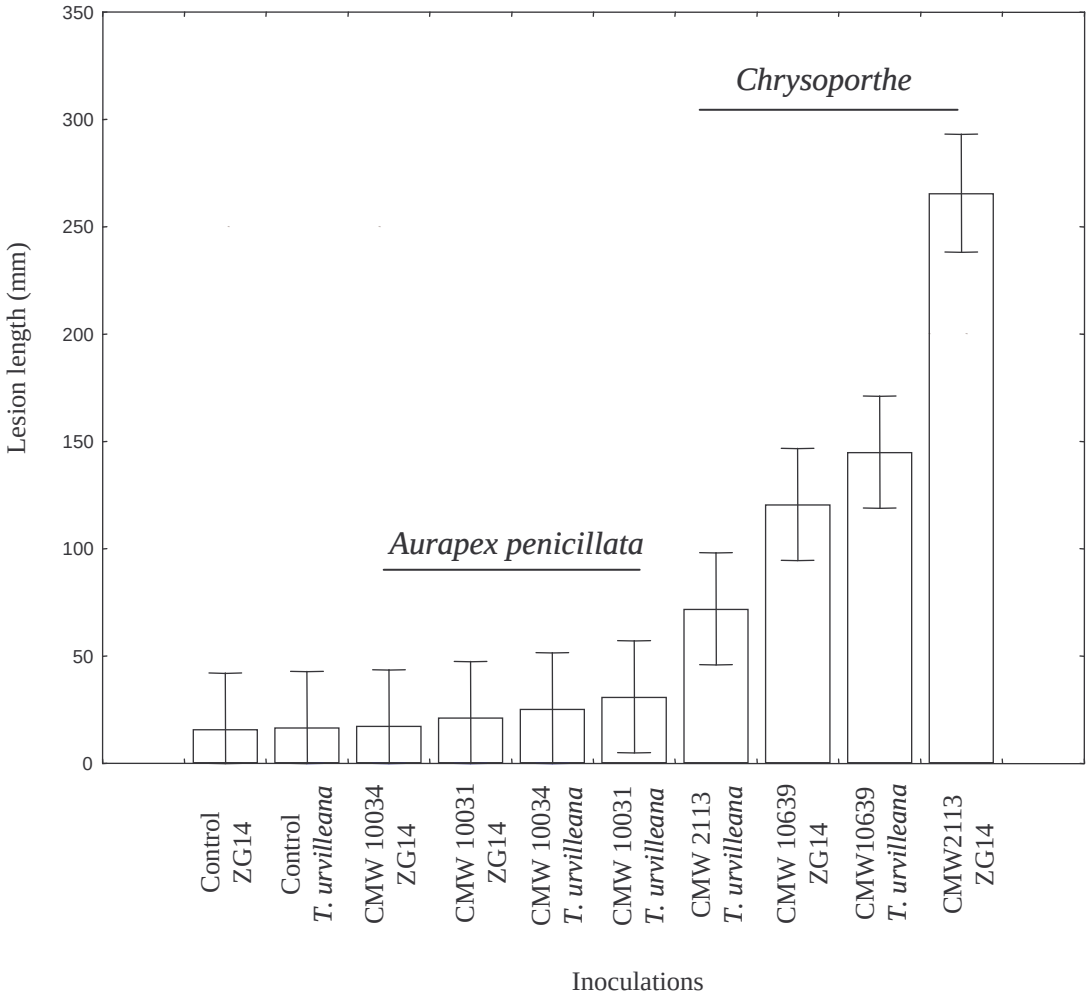

Fig. 4. Mean lesion length in *Tibouchina urvilleana* and a ZG14 clone of *Eucalyptus grandis* resulting from greenhouse inoculations with *Aurapex penicillata* (CMW 10031, CMW 10034), *Chrysoporthe cubensis* (CMW 10639), *Chrysoporthe austroafricana* (CMW 2113) and a negative control. Means are shown with 95% confidence limits.

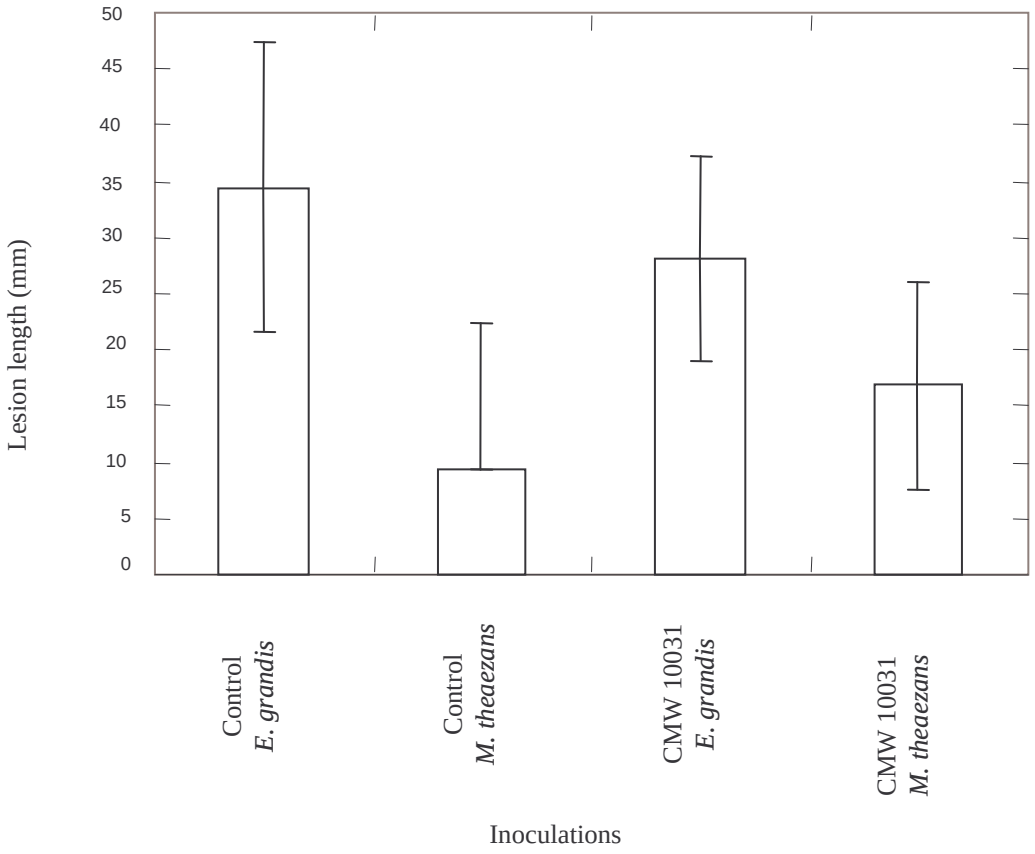

Fig. 5. Comparison of mean lesion length resulting from field inoculations with *Aurapex penicillata* (CMW 10031) and a negative control in *Miconia theaezans* and a susceptible clone (018) of *Eucalyptus grandis*. Means are shown with 95% confidence limits.