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ORIGINAL ARTICLE

Canker disease caused by *Chrysoporthe doradensis* and *C. cubensis* on *Eucalyptus* sp. and *Tibouchina* spp. in Brazil

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Abstract
Chrysoporthe species are known to cause canker on hosts belonging to *Myrtaceae* and *Melastomataceae* families. Cankers occur on tree trunks and branches and may reduce growth and lead to plant death. In this study, the incidence and pathogenicity of *Chrysoporthe cubensis* and other species causing canker on *Eucalyptus* sp., *Tibouchina heteromalla and T. granulosa* were examined. The isolates were collected in Maranhão (MA) and Minas Gerais (MG) in Brazil. Sequence analysis of beta-tubulin and actin genomic regions confirmed the presence of *C. cubensis* and *C. doradensis* on clones of the hybrid *Eucalyptus grandis* x *E. urophylla*, *T. granulosa*, and *T. heteromalla* in Brazil. Morphological characterization enabled the identification of the isolates from both genera primarily based on differences in conidial size and shape. The isolates were pathogenic to ten *Eucalyptus* clones and *Tibouchina* plants. Our results contribute new knowledge of *Chrysoporthe* species causing diseases of woody plants in Brazil of importance to *Eucalyptus* breeding programs when screening for resistance. Furthermore, this is the first report of *C. doradensis* infecting *Eucalyptus* and *T. granulosa*, as well as the first record of *C. cubensis* infecting *T. heteromalla* in Brazil.

Keywords Resistance · *Tibouchina granulosa* · *T. heteromalla* · Diaporthales · Cryphonectriaceae

Introduction

The ascomycete genus *Chrysoporthe* includes several important pathogens of *Eucalyptus* species. *Eucalyptus* canker caused by *Chrysoporthe cubensis* is an important disease of commercial *Eucalyptus* plantations in Brazil, despite several years of improving the crop for resistance to this disease (Ferreira and Milani 2004; Gryzenhout et al. 2009). Canker resistant clones have been selected by means of controlled crosses between species of *Eucalyptus* (Assis and Mafia 2007; Alfenas et al. 2009). *Chrysoporthe cubensis* occurs in the Neotropics, in which conditions of high temperature and relative air humidity prevail (Sharma et al. 1985; Boerboom and Maas 1970; Hodges et al. 1976, 1979). This pathogen also causes canker in several other species in the families Myrtaceae and Melastomataceae. Previously, *C. cubensis* was reported in Brazil only from *T. granulosa* and *Marlierela edulis* (Boerboom and Maas 1970; Hodges et al. 1976, 1979; Sharma et al. 1985; Myburg et al. 2003; Rodas et al. 2005; Seixas et al. 2004; Barreto et al. 2006; Gryzenhout et al. 2006).

The taxonomic positioning of *Chrysoporthe cubensis* has been problematic since its discovery in 1917, and only in 2004 the species was accommodated within the genus *Chrysoporthe* (Gryzenhout et al. 2004). The fungus was originally described as *Diaporthe cubensis* Bruner (1917),

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but changed to *Cryphonectria* because of the cultural and morphological characteristics that resembled the latter (Hodges 1980). However, molecular studies based on the LSU and SSU gene sequences clearly showed that *C. cubensis* was phylogenetically distant from other species within *Cryphonectria* (Zhang and Blackwell 2001; Castlebury et al. 2002; Gryzenhout et al. 2004).

Based on morphological differences and DNA sequences, new species of *Chrysoporthe* were described, and the genus currently comprises eight species. In 2010, a new species (*C. deuterocubensis*) was described to accommodate Asian isolates, which were previously placed in *Cryphonectria cubensis* (van der Merwe et al. 2010). Two other species, both previously placed in *Cryphonectria cubensis*, were described as *Chrysoporthe doradensis* and *Chrysoporthe inopina* (Gryzenhout et al. 2005; Gryzenhout et al. 2006). The description of both *C. doradensis* and *C. inopina* was supported using morphological features which are clearly distinct from *C. cubensis* (Gryzenhout et al. 2005, 2006). Gryzenhout et al. (2006) found that isolates obtained from *Tibouchina* spp. in Colombia had only the asexual state, for which they placed in a new genus *Chrysoporthella,* as *Chrysoporthella hodgesiana.* Two other species from Zambia pathogenic to *Eucalyptus,* also known only from their asexual states, were described as *Chrysoporthella zambiensis* and *Chrysoporthella syzygiicola* (Chungu et al. 2010).

Reports of new *Chrysoporthe* species, causing canker in other locations around the world, have increased over recent years (Gryzehout et al. 2009; van der Merwe 2012). These new reports have been made based on a more accurate species delineation partly resulting from the increasing use of molecular tools to characterize isolates. Thus, phylogenetic studies are important to evaluate differences among taxonomic groups and describe novel species from different regions. Only *Chrysoporthe cubensis* has been reported from Brazil, and little is known about the phylogenetic characterization of Brazilian isolates, despite the importance of canker in *Eucalyptus* plantations. In addition, there are reports of the fungus on *T. granulosa*, probably because of its extensive use in urban forestry in Brazil. Although *T. heteromalla* is used as an ornamental tree in Minas Gerais State (Brazil) and is also naturally found in savanna areas, there are no records of the disease in this species. Species of the genus *Tibouchina* (Melastomataceae) occur in all Brazilian territory (Souza 1986), mainly in the states of the southeast region (Peralta 2002) and the genus stands out from the economic point of view for its ornamental value. Accordingly, the present study aimed to identify *Chrysoporthe* species using morphological and DNA sequencing data of β-tubulin and actin regions from *Eucalyptus*, *T. granulosa,* and *T. heteromalla* in Brazil and to determine their pathogenicity to the three hosts.

Materials and methods

Collection of fungal isolates

Stem and bark fragments with typical cankers and fruiting bodies resembling those of *C. cubensis* were collected from hybrid clones (*Eucalyptus grandis* x *E. urophylla*) in *Eucalyptus* plantations in the Maranhão (MA) and Minas Gerais (MG) states and from *T. granulosa* and *T. heteromalla* ornamental trees in Minas Gerais. Isolations were performed by transferring single ascospore and/or conidial masses from pycnidia to Petri dishes containing 39 g/L Potato Dextrose Agar (PDA; HiMedia Laboratories Pvt. Ltd) medium.

Morphological and molecular characterization

Pure cultures prepared on PDA medium were grown for 10 days to obtain sufficient fungal biomass for DNA extraction. Mycelium was scraped from the surfaces of actively growing cultures and macerated in liquid nitrogen, and DNA extractions performed using the Wizard®Genomic DNA Purification Kit (Promega), according to the manufacturer's instructions.

From the DNA extracted, β-tubulin region was amplified with the primers Bt1a, Bt1b, Bt2a, Bt2b. (Glass and Donaldson 1995) and actin (ACT) region with the primers ACT-512F, ACT-783R (Carbone and Kohn 1999), according to van der Merwe et al. (2010).

All amplification reactions were prepared in a final volume of 25 μl. PCR reactions were performed in a thermocycler (My Cycler™ -BIO-RAD) and conditions were adjusted for each gene as described in Glass and Donaldson (1995) and Carbone and Kohn (1999). The amplified fragments were purified using the Gen Elute PCR Clean-up Kit (Sigma-Aldrich).

Sequencing was performed at the Laboratório de Patologia Florestal, Universidade Federal de Viçosa, and at the Macrogen. The generated electropherograms were edited using SeqAssem Software (Hepperle 2004). Phylogenetic analysis was conducted using a dataset containing sequences of *Chrysoporthe* from previous studies and obtained from GenBank (Table 1).

Multiple alignments of nucleotide sequences were constructed using the CLUSTALW Program (Thompson et al. 1994) and implemented using MEGA v 6.0 (Koichiro et al. 2013). Sequences were manually edited where needed using SeqAssem (Hepperle 2004). Maximum parsimony (MP) analysis was performed using PAUP* 3.2.1 software (Swofford 2002) (Ronquist et al. 2012) with a heuristic search with 1000 random additions and TBR. A total of 1,978,224 heuristic search replicates, with 100 tres retained. A bootstrap analysis (1000 replicates) was also done on the dataset to determine the confidence levels of the branches using 10 random additions and TBR for each replicate. The gaps in the alignment were

Table 1 Information for the isolates of *Chrsyoporthe* used for phylogenetic analysis in this study

				GenBank acession numbers		
Taxon	Isolate number	Origin	Host	ACT	BT1	BT2
Chrysoporthe cubensis	**CE6**	Brazil/MA[c]	*Eucalyptus sp.*	KX603774	KX639097	KX639114
	CE12	Brazil/MA	*Eucalyptus sp.*	KX603765	KX639088	KX639105
	CE22	Brazil/MA	*Eucalyptus sp.*	KX603766	KX639089	KX639106
	CE26	Brazil/MA	*Eucalyptus sp.*	KX603767	KX639090	KX639107
	CC3	Brazil/MA	*Eucalyptus sp.*	KX603768	KX639091	KX639108
	CC4	Brazil/MA	*Eucalyptus sp.*	KX603769	KX639092	KX639109
	CT2	Brazil/MG[d]	*T. granulosa*	KX603770	KX639093	KX639110
	CT24	Brazil/MG	*T. granulosa*	KX603771	KX639094	KX639111
	CT25	Brazil/MG	*T. heteromalla*	KX603772	KX639095	KX639112
	CT30	Brazil/MG	*T. heteromalla*	KX603773	KX639096	KX639113
	CMW[a] 10,028	Colombia	*Miconia rubiginosa*	QG290161	GQ290175	GQ290186
	CMW 10669	Republicof Congo	*Eucalyptus* sp.	GQ290171	GQ290177	GQ290188
	CMW 12734	Mexico	*Rhyncanthera mexicana*	GQ290159	DQ368791	GQ290191
C. austroafricana	CMW 10192	South Africa	*Syzygium cordatum*	GQ290163	GQ290176	GQ290187
	CMW 9327	South Africa	*T. granulosa*	GQ290173	GQ290185	GQ290194
C. inopina	CMW 12727	Colombia	*T. lepidota*	GQ290169	GQ290180	DQ368806
	CMW 12731	Colombia	*T. lepidota*	GQ290168	GQ290182	DQ368811
	CMW 12729	Colombia	*T. lepidota*	GQ290166	DQ368808	DQ368809
C. doradensis	CMW 11287	Ecuador	*E. grandis*	GQ290167	GQ290179	GQ290190
	CMW9126	Ecuador	*E. deglupta*	–	DQ224044	DQ224045
	CMW9125	Ecuador	*E. deglupta*	–	DQ224042	DQ224043
	CMW9123	Ecuador	*E. deglupta*	–	DQ224038	DQ224039
	CE3	Brazil/MA	*Eucalyptus sp.*	KX603764	KX639087	KX639104
	CE34	Brazil/MA	*Eucalyptus sp.*	KX603775	KX639098	KX639115
	CE37	Brazil/MA	*Eucalyptus sp.*	KX603776	KX639099	KX639116
	CE38	Brazil/MA	*Eucalyptus sp.*	KX603777	KX639100	KX639117
	CE45	Brazil/MG	*Eucalyptus sp.*	KX603778	KX639101	KX639118
	CT5	Brazil/MG	*T. granulosa*	KX603779	KX639102	KX639119
	CT6	Brazil/MG	*T. granulosa*	KX603780	KX639103	KX639120
C. deuterocubensis	CMW 8650	Indonesia	*S. aromaticum*	GQ290172	AY084024	GQ290193
	CMW 2631	Australia	*E. marginata*	GQ290174	GQ290184	AF543825
	CMW 12745	Singapura	*T. urvilleana*	GQ290160	GQ290183	DQ368781
	CMW 17178	Tailandia	*T. urvilleana*	GQ290164	DQ368785	GQ290192
C. syzygiicola	CMW29942	Zambia	*S. guineense*	–	FJ805232	FJ805238
	CMW29940	Zambia	*S. guineense*	–	FJ805230	FJ805236
C. zambiensis	CMW29930	Zambia	*E. grandis*	–	FJ858711	FJ805235
	CMW29928	Zambia	*E. grandis*	–	FJ858709	FJ805233
Chrysoporthella hodgesiana	CMW 9995	Colombia	*T. semidecandra*	GQ290162	AY956978	AY956977
	CMW 10625	Colombia	*T. theaezans*	GQ290170	AY262391	AY956979
Amphilogia gyrosa[b]	YMJ91123101	Taiwan		EF025600	EF025615	EF025615

[a] CMW = Forest e Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

[b] Outgroup

[c] MA = Maranhão

[d] MG = Minas Gerais. Isolates in bold were sequenced in this study

treated as missing data. Bayesian inference was used to generate posterior probabilities (PP) for consensus nodes using MRBAYES 3.1 (Huelsenbeck 2001). The analysis was performed using algorithm MCMC (Markov Chain Monte Carlo) (Larget and Simon 1999). Two concurrent analyses of four chains were both run for 10^7 generations, using random starting trees.

Trees were randomly sampled every 1000 generations, and 25% of them were discarded as burn-in. Trees were viewed and edited in FigTree 1.3.1. (http://tree.bio.ac.uk/software Accession code TreeBase 22716). A combined dataset of ACT, BT1, and BT2 sequences was submitted to a partition homogeneity test (PHT; Farris et al. 1994) to determine whether the datasets could be combined. *Amphilogia gyrosa* sequences were defined as outgroup taxon according to van der Merwe et al. (2010).

Fungal structures and fruiting bodies from herbarium material were selected based on the results obtained from phylogenetic analysis and used in morphological characterization. For this purpose, histological sections of fungal structures and fruiting bodies were examined according to the method described by Gryzenhout et al. (2004), wherein 50 asci, ascospores, conidia and conidiophores were measured.

Pathogenicity tests

Inoculation of *Tibouchina granulosa* and *T. heteromalla*

The pathogenicity of the *Chrysoporthe* isolates obtained was determined on *T. granulosa* and *T. heteromalla* using two isolates, CE6 and CE38 selected according to results of sequence analyses of the β-tubulin and actin regions. Seedlings of *T. granulosa* (18-months-old) and *T. heteromalla* (12-months-old) were inoculated and kept under greenhouse conditions. Wounds of 2 cm of length were made with a scalpel by removing the bark to expose the cambium. Subsequently, fungal culture discs (5 mm diameter) were placed in the wound with the mycelium facing the xylem, and then covered with plastic film (Parafilm®) to maintain humidity. This protection was maintained for 30 days after inoculation, as described by Ferreira and Milani (2004). Control treatments consisted of wounded seedlings receiving only a disc of PDA without the fungus. After 75 days, the plants were evaluated by measuring the lesion lenght in the xylem. Koch's postulate was fulfilled by re-isolating the fungus. Pathogenicity tests were conducted in a completely randomized design, with 10 replicates for *T. granulosa* plants, three replicates for *T. heteromalla*, and five replicates for each *Eucalyptus* clone.

Inoculation of *Eucalyptus* clones

Pathogenicity tests were carried out in 6-month-old *Eucalyptus* clonal plants, and inoculation was performed as described above, using 10 different *Eucalyptus* hybrid clones (*Eucalyptus grandis* x *E. urophylla)* (T3, T4, T5, T6, T7, T8, T9, T10, T11 and T12) with the isolates CE6 and CE38. *Eucalyptus* plants were assessed by measuring the lesion length formed in the xylem at seventy-five days after inoculation. Koch's postulate was carried out by pathogen re-isolation.

Statistical analysis

The normality of both tests was analyzed using Shapiro-Wilk test (Shapiro and Wilk's 1965). The means of each treatment were compared and grouped according to the Scott-Knott's test ($p \leq 0.05$). Statistical analyses were performed using SISVAR software (Ferreira 2010).

Results

Collection of fungal isolates

Typical fungal structures were observed on diseased *T. granulosa*, *T. heteromalla*, and *Eucalyptus* plants. Only the asexual state was found on *Tibouchina* species. In species of *Tibouchina*, the main symptoms observed were cankers and the death of branches and trunks (Fig. 1). On *Eucalyptus* sp., we observed thickening of the trunk base, bark cracking, and perithecia and pycnidia of the pathogen on the bark of diseased trees.

Molecular and morphological characterization

Phylogenetic trees were generated by using the maximum parsimony (MP) and Bayesian inference (BI) methods for the amplified BT1, BT2, and ACT regions, using sequences from 17 isolates from *Eucalyptus* sp. and *Tibouchina* spp. Twenty two additional sequences of *Chrysoporthe* spp. obtained from GenBank were included in the analysis for comparison. The trees generated for two gene regions showed similar topologies in both parsimony and Bayesian methods.

The aligned DNA sequence of the BT1 region had an alignment of 410 characters, 198 constant characters, with 196 parsimony non-informative and 16 parsimony informative, produced 100 trees of 230 steps (Consistency index = 0.991, Retention index = 0.986, and Homoplasy index = 0.008). For BT2, the dataset had an alignment of 389 characters produced 4 trees of 45 steps with 343 constant characters, 36 parsimony non-informative and 11 parsimony informative (Consistency index = 0.977, Retention index = 0.989, and Homoplasy index = 0.022). The alignment of the actin gene region had a total 271 characters, with 235 constant, 29 parsimony-uninformative and 7 parsimony

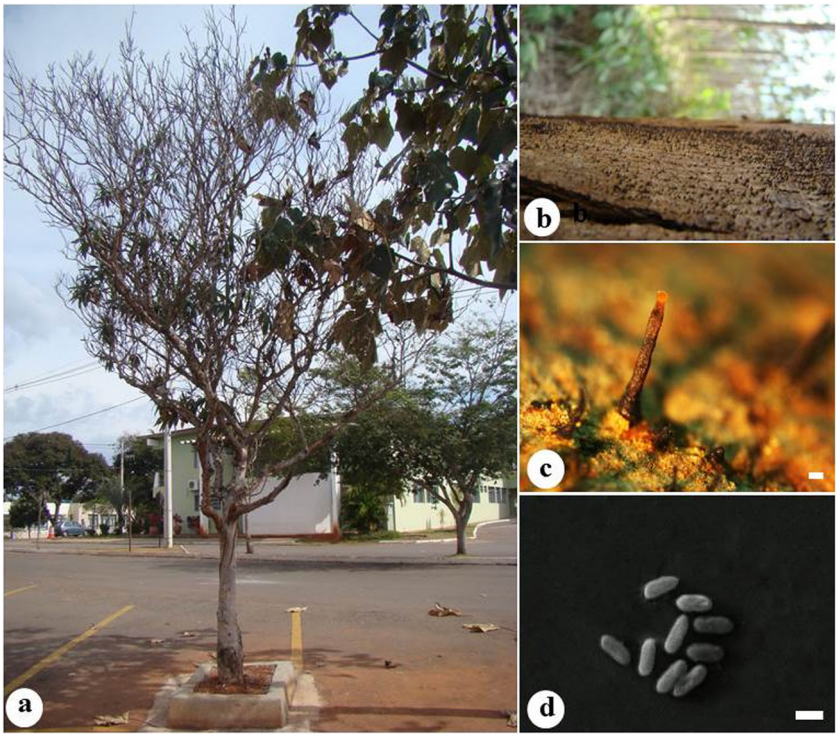

Fig. 1 *Chrysoporthe* spp. hosts: dead *Tibouchina granulosa* tree (**a**), fruiting bodies under the *Eucalyptus* bark (**b**), pycnidium with golden spore mass at the apex (**c**) and *C. doradensis* conidia (**d**). Scale bar: c = 100 μm, d = 2 μm

informative characters produced 10 trees of 37 steps (Consistency index = 1.000, Retention index = 1.000, and Homoplasy index = 0.000). Evolution models GTR + G for alignment to BT1, BT2 and ACT were selected and incorporated into the Bayesian analysis. The combined dataset of β-tubulin region (BT1 + BT2) and actin had an alignment of 1067 characters, with 763 constant, 266 parsimony non-informative and 38 parsimony informative characters.

A consensus tree showed that the isolates sequenced in this study were grouped with *C. doradensis* and *C. cubensis*. Branch was supported by high bootstrap and high posterior probability values for *C. doradensis* being of 86% and 0.99, respectively, and *C. cubensis* with 90% and 0.97, bootstrap and posterior probability values, respectively (Fig. 2).

The isolates CE6 and CE38 were selected for morphological characterization based on the results of the phylogenetic analyses. No variation in color and morphology was observed among isolates when grown on PDA medium. Diseased tissue showed abundant black perithecial or pycnidial necks in *Eucalyptus* and black pycnidial necks on *Tibouchina* species. Isolate CE6 had the typical morphology of *C. cubensis*: ascomata perithecial, subglobose, ostioles rostrate, asci unitunicate, clavate (18.5–24.0 × 4.5–6.5 μm); ascospores ellipsoid (4.5–6.0 × 2.0–2.5 μm), one septa and hyaline. Conidiomata were superficial to slightly immersed, fuscous-black bases, pyriform to clavate, with one to four attenuated necks per structure. Conidiomata were occasionally multilocular, with a single locule connected to one or several necks. Conidiophores were hyaline and with globose rectangular basal cells that are 10.0–12.5 × 17.0–21.0 μm), branched irregularly at the base or above the cylindrical cells with total length between, conidiogenous cells cylindrical to flask-shaped with attenuated apices (1.5–2.0 × 2.5–3.0 μm). Conidia were hyaline (2.5–3.0 × 1.5–2.0 μm), oblong, non-septate, exuded as bright luteous spore tendrils or droplets.

Isolate CE38 had the typical morphology of *C. doradensis:* ascomata perithecial, fuscous-black base and cylindrical perithecial necks; Asci fusoid to elipsoidal, (20.0–24.5 × 4.5–6.5 μm). Ascospores hyaline (5.0–6.5 × 2.0–2.5 μm), one septa, fusoid to oval. Conidiophores hyaline, with globose to rectangular basal cells, branched irregularly at the base, total length of conidiophore 10.0–16 × 19.0–21.5 μm, conidiogenous cells 1.5–2.0 μm. Conidia 3.5–4.5-μm long, 1.5–2.0-μm wide, aseptate, cylindrical, oblong, ovoid and occasionally allantoid (Fig. 1d).

Pathogenicity test on of *Tibouchina granulosa* and *T. heteromalla* plants

After inoculation, discoloration progressed vertically in the xylem of inoculated *T. granulosa* and *T. heteromalla* plants (Fig. 3a, c). A significant difference was found between isolates of the two *Chrysoporthe* species tested ($P < 0.0001$) (Table 2). No lesions were produced on control plants of either host (Fig. 3b, d). Both isolates, CE6 and CE38, were pathogenic when inoculated on *T.*

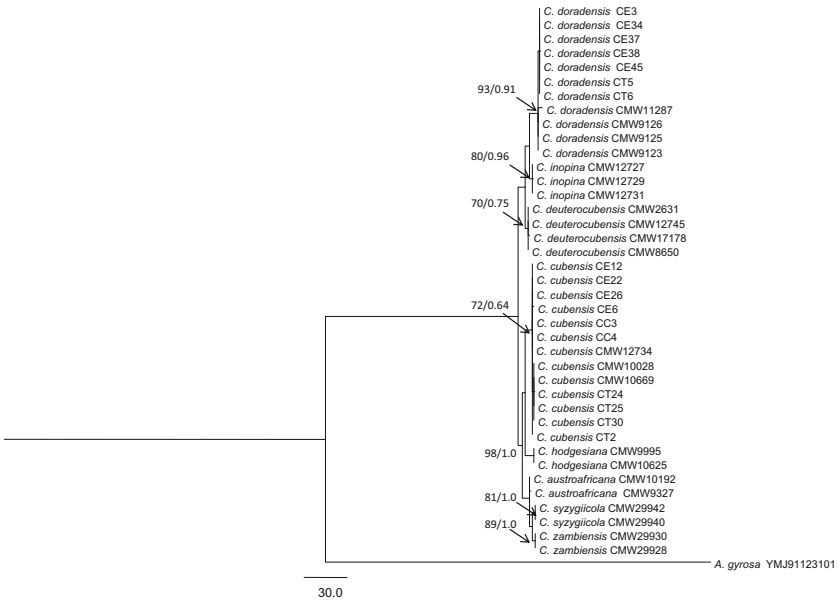

Fig. 2 One of 10 most parsimonious tree of β-tubulin and actin regions (combined dataset of ACT, BT1, and BT2). Isolates in bold were sequenced in this study. The bootstrap and posterior probability values greater than 50% and 0.5, respectively, are shown appropriate branches. *Amphilogia gyrosa* was defined as outgroup taxon

heteromalla (Table 2). However, the *C. cubensis* isolate showed longer lesion length on *T. granulosa* than *C. doradensis*.

Pathogenicity test on *Eucalyptus* plants

The inoculation of *Eucalyptus* plants showed different responses depending on the pathogen species or *Eucalyptus* clone applied, as well as the interaction between these factors ($p < 0.0001$) (Table 2). No lesions were produced on control plants of any of the clones inoculated (Fig. 3f). Clones T9 and T11 were highly susceptible to both *Chrysoporthe* species (Table 2). Clones T4 and T5 showed high resistance to both species. *Chrysoporthe cubensis* isolate CE6 caused longer lesions on the majority of the tested clones.

Discussion

Canker disease of *Eucalyptus* sp. caused by *C. cubensis* has been recognized as a serious disease in Brazil since the 1970's. The disease has been controlled primarily through the use of resistant species and clones. However, it is still considered to be a serious problem in the North and Northeast of Brazil due to expansion of plantations and lack of screening for resistant clones for these areas. The morphological and molecular analyses presented in this study demonstrated for the first time the occurrence of another species, *C. doradensis*, in Brazil on *Eucalyptus* and *Tibouchina* spp.

Chrysoporthe doradensis was described by Gryzenhout et al. (2004) from Ecuador based on phylogenetic studies using ITS and two regions of the β-tubulin genes. The origin of this species is unknown, although studies are indicating it to be native to Ecuador (Gryzenhout et al. 2005), in part because there are no reports of its occurrence in other regions of South America or elsewhere except for the new report of the fungus from Brazil. However, it is still unclear where the center of origin lies. A broader collection of isolates is needed to provide more detailed information to support evolutionary inferences.

In the current study, the fruiting bodies found on both hosts were similar to those reported for *C. doradensis* (Gryzenhout et al. 2005), except for the yellowish-brown color of the spore mass, which was similar to that reported for other *Chrysoporthe* species. Nevertheless, conidial morphology seems to be the most important characteristic to distinguish *C. doradensis* according to Gryzenhout et al. (2005). These authors observed that conidial shape and spore mass color were the most pronounced morphological characteristics, supporting phylogenetic distinction of Ecuador isolates from other species. Such morphological characteristics were observed in this study, with conidia occasionally allantoid (3.5–4.5-μm long, 1.5–2.0-μm wide) and exuded as bright luteous spore tendrils or droplets.

Host-specialization was not observed among isolates of *C. doradensis* and *C. cubensis* collected from *Eucalyptus* sp. and *T. granulosa*. The presence of *C. doradensis* in Brazil demonstrates that species is not restricted to only one country, as it was thought to occur only in Ecuador, nor to a single host. This report is highly relevant in the management of the disease and in breeding programs. Both species, *C. cubensis* and *C. doradensis* were recovered from trees with high proximity to each other, due to this, great care must be taken in the identification of these fungi, since studies have demonstrated the high genetic diversity present in populations of *C. cubensis* in Brazil (van Zyl et al. 1998). In addition, the occurrence of these two species suggests that, due to the great territorial extension that the country presents, probably other species

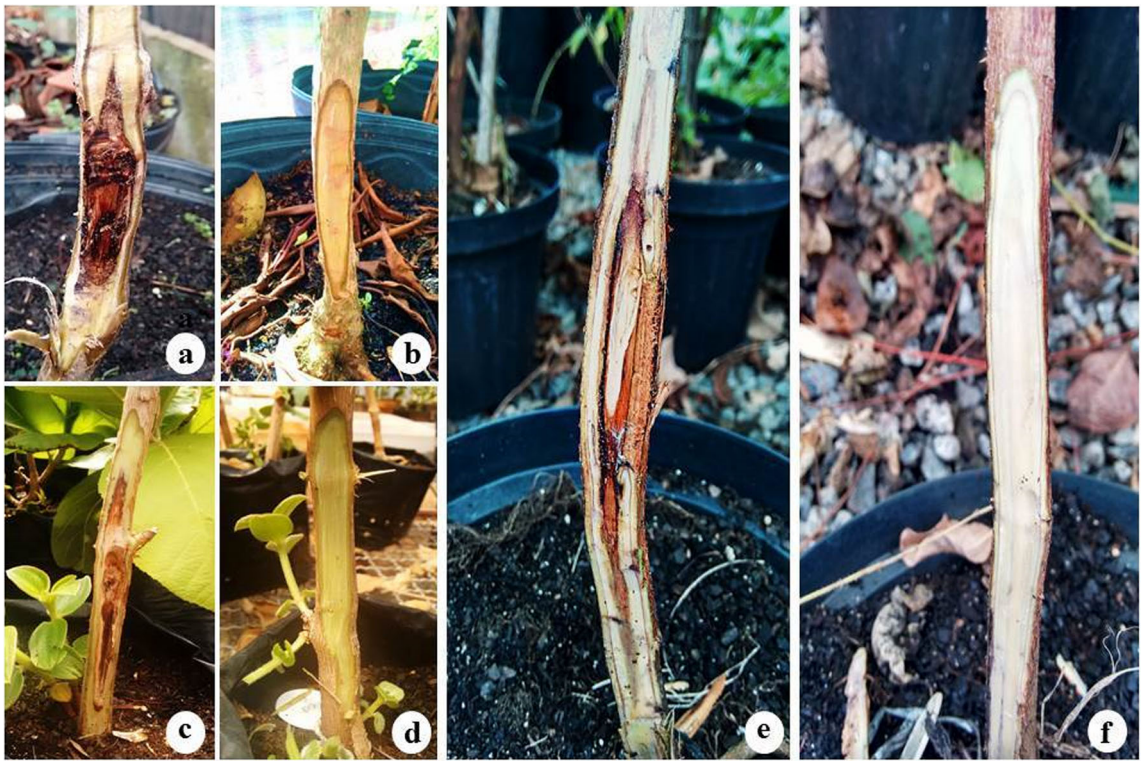

Fig. 3 Lesions on xylem of *Tibouchina granulosa, T. heteromalla* and *Eucalyptus* plants 75 days after inoculation with *Chrysoporthe doradensis*. **a**, **c**, **e** lesion length on *T. granulosa, T. heteromalla* and *Eucalyptus,* respectively. **b**, **d**, **f** control of *T. granulosa, T. heteromalla* and *Eucalyptus,* respectively

of *Chrysoporthe* may occur in native plants in Brazil, being pathogenic to *Eucalytus* and presenting a risk to the commercial plantations of the country. However, more studies with more extensive collections would be important to more accurately identify the distribution of *Chrysoporthe* in Brazil. In addition, this study proved that the diversity of hosts of *Chrysoporthe* species, implying serious disease management issues, such as the risk of commercial eucalyptus plantations being close to areas of native *Chrysoporthe* hosts. Based on the results of this study, further phylogenetic analyses, including more isolates, will be important for clarifying the origin and more accurately describing the geographic distribution of the species, especially in South America.

The pathogenicity tests demonstrated that isolates of *C. cubensis* and *C. doradensis* are able to cause canker and death in *T. granulosa* and *T. heteromalla* plants. Previous studies

Table 2 Xylem lesion length (cm) in *Tibouchina* spp. and *Eucalyptus* clones in response to inoculation with isolates of *Chrysoporthe cubensis* and *C. doradensis*

Host	CE6	SE	CE38	SE	Control
Tibouchina granulosa	4.92 b	± 0.39	3.45 a	± 0.28	–
Tibouchina heteromalla	2.50 a	± 0.69	3.66 a	± 1.72	–
Eucalyptus urophylla (T3)	1.71 Ba	± 0.52	3.55 Db	± 0.30	–
Eucalyptus grandis x E. urophylla (T4)	0.72 Aa	± 0.61	0.30 Aa	± 0.14	–
Eucalyptus grandis x E. urophylla (T5)	0.40 Aa	± 0.99	0.46 Aa	± 0.12	–
Eucalyptus grandis x E. urophylla (T6)	7.42 Fb	± 1.30	2.60 Ca	± 2.34	–
Eucalyptus grandis x E. urophylla (T7)	4.57 Ea	± 0.48	4.24 Ea	± 0.80	–
Eucalyptus grandis x E. urophylla (T8)	3.91 Db	± 0.89	1.00 Ba	± 0.10	–
Eucalyptus grandis x E. urophylla (T9)	10.40 Hb	± 0.84	7.25 Ga	± 1.13	–
Eucalyptus grandis x E. urophylla (T10)	2.87 Cb	± 0.25	0.19 Aa	± 0.80	–
Eucalyptus grandis x E. urophylla (T11)	8.25 Gb	± 0.84	5.84 Fa	± 1.63	–
Eucalyptus grandis x E. urophylla (T12)	4.00 Db	± 1.79	0.36 Ab	± 0.21	–

*Means followed by capital letters in different columns and small letters on the lines differ by Scott-Knott test ($p \leq 0.05$); the data were transformed by the function log (x + 1). T3 to T12 represent different *Eucalyptus* clones. Each pathogenicity test was performed separately. SE: standart errors

demonstrated that *Eucalyptus* sp. is relatively more susceptible than *T. granulosa* (Leppik 1970; Rodas et al. 2005; Gryzenhout et al. 2005). It is believed that pathogens are less virulent on their native hosts than to exotic species (Newhouse 1990; Liu and Milgroom 2007) which corroborate our finding that *T. granulosa* (which is native to Brazil), had smaller lesion length when inoculated with isolates from both fungal species.

Findings in the present study also corroborate other studies that have evaluated resistance in *Eucalyptus* sp. based on lesion length in the stem with relative susceptibility (Guimarães et al. 2010). The search for more efficient and accurate methods to determine the resistance level of *Eucalyptus* clones to canker diseases is quite important for its control in Brazil (Alfenas et al. 2009). The existence of genetic variability in *Eucalyptus* enables controlling the disease by selecting resistant materials (Assis and Mafia 2007; Alfenas et al. 2009). This strategy might be also applied for *T. granulosa* plants, considering the inoculation method adopted for use in this study.

In general, isolate CE6 of *C. cubensis* had the longest lesion length according to pathogenicity tests for both hosts. Isolate CE38 of *C. doradensis* resulted in shorter lesion length in *Eucalyptus* clones when compared with the *C. cubensis* isolate. By contrast, pathogenicity studies conducted by Gryzenhout (2005) showed that *C. doradensis* was highly aggressive to *E. grandis* species. However, in the present study, we evaluated clones of the hybrid *E. grandis* × *E. urophylla*, which may explain the difference between the studies.

The present study proved that *C. doradensis* and *C. cubensis* were pathogenic to *Eucalyptus* and *Tibouchina*. In addition, this study reports the occurrence of *C. doradensis* as the etiologic agent of canker disease in *Eucalyptus* and *T. granulosa* for the first time in Brazil. Nevertheless, more isolates of both species must be used in future studies to better understand the level of susceptibility in these plants.

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