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Pathogenicity of *Chrysoporthe deuterocubensis* on eucalypts in Indonesia

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Several economically important pathogens, including species of *Chrysoporthe*, pose a threat to the rapidly expanding eucalypt plantation industry in Southeast Asia. During 2019 disease surveys in Riau and Kalimantan (Indonesia), cankers were observed on eucalypt trees and a collection of fungal isolates was obtained from them. The aim of this study was to confirm the identity of the isolates and to evaluate their relative pathogenicity on different eucalypt clones. Using the DNA sequence data based on the internal transcribed spacer (ITS) region of the ribosomal DNA and two regions of the β-tubulin gene (*TUB1* and *TUB2*), 31 fungal isolates were identified as *C. deuterocubensis*. Pathogenicity trials showed that *C. deuterocubensis* isolates differed in their pathogenicity and that different eucalypt genotypes differed in their susceptibility to the pathogen. These results will provide valuable information to reduce the threat of stem canker to future eucalypt plantation development.

Keywords: Cryphonectriaceae, fungal pathogen, planted forest, stem cankers, susceptibility

Introduction

The Cryphonectriaceae (Diaporthales, Ascomycota) is a globally distributed group of mostly pathogenic fungi of woody plants (Gryzenhout et al. 2006a; 2009). These pathogens cause cankers on stems and branches, which can lead to tree death (Hodges et al. 1976, 1979; Sharma et al. 1985; Gryzenhout et al. 2009, 2010; Begoude et al. 2010; Chen et al. 2013, 2018; Fan et al. 2013; Rodas et al. 2005; Jiang et al. 2019; Wang et al. 2020). The Cryphonectriaceae include at least 28 genera (Gryzenhout et al. 2009; Chen et al. 2018; Ali et al. 2018; Ferreira et al. 2019; Rauf et al. 2020; Wang et al. 2020; Huang et al. 2022). Amongst the most important pathogen in this group is *Cryphonectria parasitica,* which causes the devastating disease chestnut blight on *Castanea* spp. in Europe and North America (Anagnostakis 1987; Rigling and Prospero 2018).

The Cryphonectriaceae have undergone substantial taxonomic revision over the last three decades (Gryzenhout et al. 2004, 2006a, 2009; van der Merwe et al. 2010). This is the result of extensive surveys, particularly in the tropics and the southern hemisphere, and the application of DNA sequence-based phylogenetic inference to define species boundaries (Gryzenhout et al. 2006a; van der Merwe et al. 2010; Hyde et al. 2020; Jiang et al. 2020; Wang et al. 2020). One of the most important of these changes was the recognition that the eucalypt pathogen previously known as *Cryphonectria cubensis* resides in a distinct genus, *Chrysoporthe,* distantly related to *Cryphonectria parasitica* and its relatives (Gryzenhout et al. 2004, 2009). Species of *Chrysoporthe* are found specifically on trees in the Myrtales order, including Myrtaceae, Melastomaceae and Lythraceae families (Wingfield 2003; Rodas et al. 2005; Gryzenhout et al. 2005, 2009; Oliveira et al. 2021).

Nine species of *Chrysoporthe* have been described. Those from South and Central America include *C. cubensis* (Hodges et al. 1976, 1979; Rodas et al. 2005), *C. doradensis* (Gryzenhout et al. 2005), *C. inopina* (Gryzenhout et al. 2006b), *C. hodgesiana* (Gryzenhout et al. 2004) and *C. puriensis* (Oliveira et al. 2021). Species occurring in central and southern Africa include *C. zambiensis*, *C. syzygiicola* (Chungu et al. 2010) and *C. austroafricana* (Wingfield et al. 1989; Gryzenhout et al. 2004). The other species is *C. deuterocubensis,* which is mainly found in Southeast Asia (Hodges et al. 1986; Myburg et al. 2003; van der Merwe et al. 2010; Gryzenhout et al. 2006b; Rauf et al. 2022; Suzuki et al. 2022). *Chrysoporthe deuterocubensis* was originally treated as a single taxon together with *C. cubensis* (Hodges 1980). Subsequently, based on multigene phylogenetic analyses, it was shown to represent a cryptic species and was separated from its close relatives, *C. cubensis* and *C. austroafricana* (Gryzenhout et al. 2004; van der Merwe et al. 2010). *Chrysoporthe deuterocubensis* has been reported not only from Southeast Asia (Gryzenhout et al. 2006b; van der Merwe et al. 2010), but also from other parts of the world, including Kenya, Malawi, Mozambique (Nakabonge et al. 2006), Republic of Congo (Roux et al. 2003), Australia (Davison and Coates 1991; van der Merwe et al. 2010), Hawaii (Gryzenhout et al. 2006b; van der Merwe et al. 2010; Roux et al. 2020), India (Sharma et al. 1985) and China (Zhou et al. 2008;

Chen et al. 2010; Wang et al. 2020). In Indonesia, it has been reported from woody plants in the Myrtales, including *Syzygium aromaticum* on the island of Sulawesi (Hodges et al. 1986; Myburg et al. 2003; van der Merwe et al. 2010), as well as *Melastoma malabathricum* (Gryzenhout et al. 2006b) and *Eucalyptus* spp. on the island of Sumatra (van der Merwe et al. 2010).

During disease surveys in 2019, symptoms resembling infection by a *Chrysoporthe* species, including bark cracking and the presence of characteristic fruiting structures, were observed on eucalypts in the clonal trials located in Riau and Kalimantan. The objectives of this study were to verify the identity of these isolates and to evaluate their relative pathogenicity on different eucalypt genotypes.

Materials and methods

Field incidence, sample collection and fungal isolation

Surveys were conducted in eucalypt clonal trials situated in Riau and Kalimantan, Indonesia (Figure 1). These trial plots consisted of 20 different eucalypt genotypes (10 *Eucalyptus pellita* and *E. grandis* × *E. pellita*) with a 7 × 7 plant plot established for each clone. The occurrence of cankers resembling infection by a *Chrysoporthe* sp. (Figure 2) on individual trees in each plot was recorded and calculated as a percentage.

Stem and bark samples were collected from cankers (Figure 2) on infected trees in eight eucalypt clonal trials, including seven sites in Riau (Kuansing East = 4, Kuansing West = 1, Kuansing North = 1, and Kuansing South = 1) and one site in East Kalimantan province. Samples were placed in separate brown paper bags and then transferred to the laboratory for isolation. Fruiting structures with conidial masses were observed on the samples under a dissecting microscope and these spore masses were lifted from the structures using a sterile needle and transferred to potato dextrose agar (PDA Acumedia®: 40 g l^{-1}) in Petri-dishes and incubated at 25 °C for 12–14 days. Pure cultures were obtained by transferring single hyphal tips to clean PDA. All the isolates were then deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1).

DNA extraction, PCR amplification and sequencing

DNA was extracted from mycelium of 14-day-old pure cultures using a Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA). DNA sequences of all isolates were generated for three loci, including the internal transcribed spacer (ITS) region of the ribosomal RNA using primers ITS-1 and ITS-4 (White et al. 1990), and two regions of the β-tubulin gene (*TUB1* and *TUB2*) using primers Bt1a/Bt1b and Bt2a/Bt2b, respectively (Glass and Donaldson 1995).

Polymerase chain reaction (PCR) amplification was performed in 13 µl reactions containing 2 µl of 5× MyTaq buffer (Bioline, London, UK), 0.1 µl MyTaq DNA polymerase (Bioline), 1 µl DNA, 0.5 µl of each primer (10 µm), and sterile deionised water. The PCR protocol used was as follows; initial denaturation (96 °C, 3 min), 30 cycles of 30 s at 95 °C, 45 s at 55 °C, 1 min at 72 °C, and a final extension (72 °C, 7 min). PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and sequenced using the BigDye Terminator Sequencing Kit 3.1 (Applied Biosystems, Forster City, CA, USA) in both the forward and reverse directions. Sequencing was performed on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Forster City, CA, USA). CLC Main Workbench V20.1 (Qiagen, Hilden, Germany) was used to assemble and edit the raw sequences. All sequences resulting from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) (Table 1).

Phylogenetic analyses

Reference sequences for species closely related to those found in this study were downloaded from the GenBank database (Table 1). All sequences were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and, where necessary, manually confirmed using MEGA v. 7 (Kumar et al. 2016). Maximum likelihood (ML) analyses were performed on the combined datasets of the three sequenced regions, using RaxML v. 8.2.4 on the CIPRES Science Gateway v. 3.3 (Stamatakis 2014) with the default GTR substitution matrix and 1 000 rapid bootstraps. Sequences for *Amphilogia gyrosa* (CMW 10469 and CMW 10470) were used as outgroups. The resulting trees were viewed using MEGA v. 7 (Kumar et al. 2016).

Pathogenicity tests

Relative aggressiveness of isolates

Four isolates (CMW 55421, CMW 55433, CMW 55438 and CMW 55446) were selected for inoculation and specifically chosen to represent the range of geographical locations. Inoculations were carried out on 3-year-old *E. grandis* x *E. pellita* hybrid clones (ECL105) with a stem diameter at breast height (DBH) of 130 mm to 140 mm. Twenty trees were used for each isolate and the same number of trees were inoculated as controls. A sterilised cork-borer (10 mm) was used to make a wound in the stems at approximately 1.3 m above ground level, and inoculation was carried out by placing a plug of agar taken from the edges of three-week-old actively growing cultures with the mycelial surface facing the cambium. A sterile 2% PDA plug was used for control inoculations. Inoculation points were sealed with masking tape to reduce desiccation of the agar plugs and wounds.

The length of lesions produced on the stems was measured 12 weeks after inoculation. Re-isolations were made from the lesions to verify the presence of the inoculated fungus. The data were analysed using Kruskal–Wallis tests to determine whether there were statistically significant differences between the treatments. The Wilcoxon rank-sum test with continuity correction was then used for pairwise comparisons. R statistical software, version 3.2.0 (R Core Team 2020) was used for all statistical analyses.

Relative tolerance of eucalypt clones

Five eucalypt clones, including two of *Eucalyptus pellita* (ECL101, ECL102) and three of *E. grandis* × *E. pellita* hybrids (ECL103, ECL104, ECL105), were selected to test for susceptibility to the two most aggressive isolates arising from the initial inoculation trial. Inoculation was carried out on 3-year-old eucalypt trees with stem diameters ranging

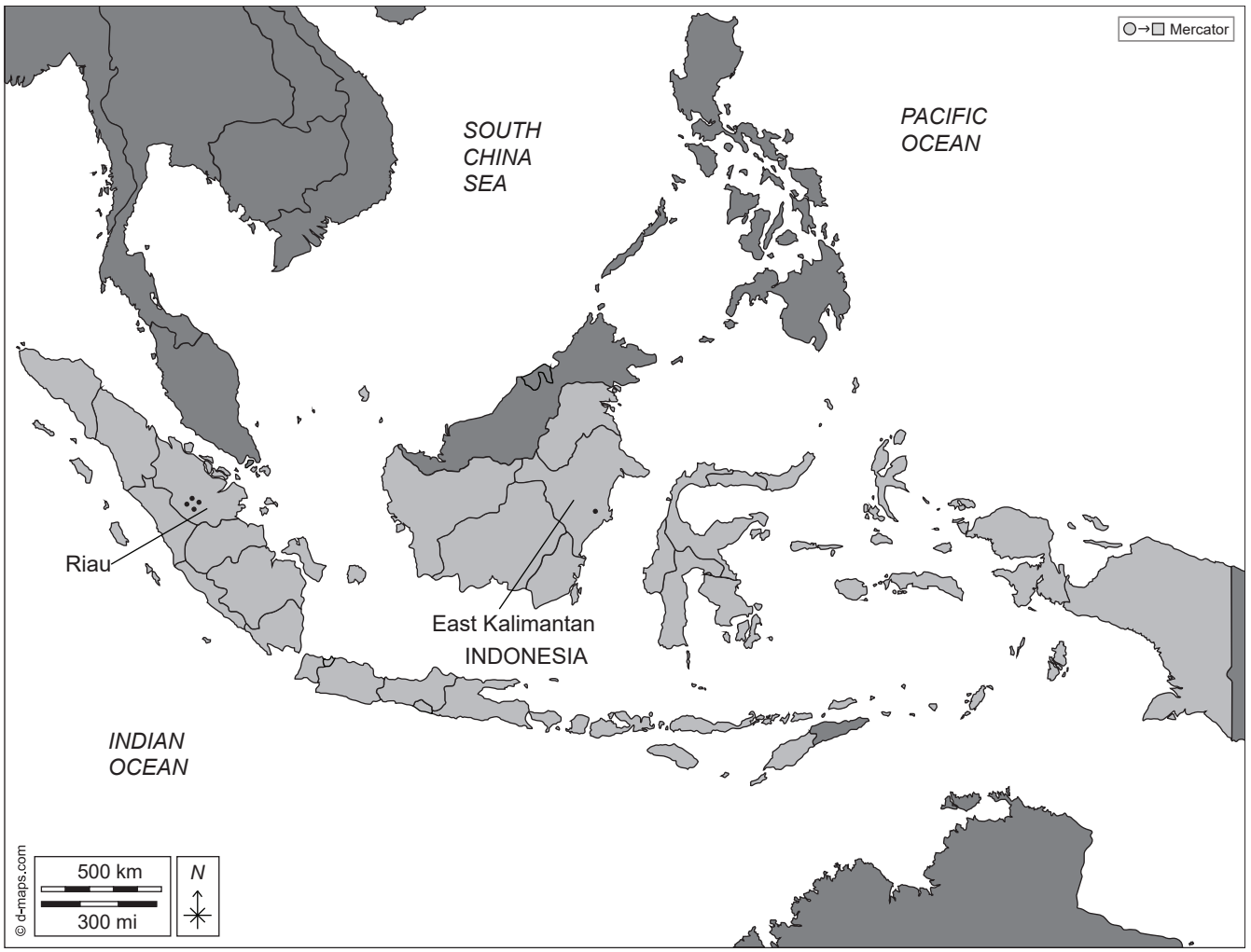

Figure 1: Geographic location of the surveys conducted in Sumatra and Kalimantan, Indonesia. Black dots on the map represent the sampling sites in each region

from 130 mm to 140 mm, at a height of approximately 1.3 m above the ground. Twenty trees of each eucalypt clone were used for each isolate and the same number of trees were inoculated as controls. Inoculations, lesion length measurements and re-isolations were carried out using the same protocols as for the trial comparing the relative aggressiveness of the isolates. Data were also analysed in the same way as that for the first trial.

Results

Field incidence, sample collection and fungal isolation

Cankers reminiscent of those caused by *Chrysoporthe* species occurred on only two *E. grandis* × *E. pellita* hybrid clones with incidence levels of 30% and 10%, respectively, on clone ECL105 and clone ECL106. This showed that not all trees in clonal trials, even those of the same clone, were affected by this disease. A further eight *E. grandis* × *E. pellita* clones and 10 *E. pellita* clones were free of infection.

Thirty-one isolates were obtained from diseased tissues associated with cankers on the stems of trees. Of these, eight isolates were obtained from *E. grandis* × *E. pellita* clone ECL106 in Sumatra (seven isolates) and East Kalimantan (one isolate), and 23 isolates were obtained from *E. grandis* × *E. pellita* clone ECL105 grown in Kuantan Singingi Regency,

Figure 2: Symptoms of *Chrysoporthe* infection: (a) on stem of *Eucalyptus* tree, and (b) fruiting structures observed on the cankers

Table 1: Collection details and GenBank accession numbers of isolates included in the phylogenetic analyses. Isolates obtained in this study are indicated in **bold**

Species	Isolate number	Host	Locality	ITS	*TUB1*	*TUB2*	Reference
Chrysoporthe austroafricana	CMW 2113T	*Eucalyptus grandis*	South Africa	AF046892	AF273067	AF273462	Gryzenhout et al. 2004; van der Merwe et al. 2010
	CMW 10192	*Syzygium cordatum*	South Africa	AY214299	GQ290176	GQ290187	van der Merwe et al. 2010
	CMW 9327	*Tibouchina granulosa*	South Africa	GQ290158	GQ290185	AF273455	Gryzenhout et al. 2004; van der Merwe et al. 2010
Chrysoporthe cubensis	CMW 10639	*Eucalyptus grandis*	Colombia	AY263421	AY263419	AY263420	Rodas et al. 2005
	CMW 10028	*Miconia rubiginosa*	Colombia	GQ290153	GQ290175	GQ290186	van der Merwe et al. 2010
	CMW 10669	*Eucalyptus sp.*	Republic of Congo	GQ290154	GQ290177	AF535126	Gryzenhout et al. 2004; van der Merwe et al. 2010
	CMW 10778	*Syzygium aromaticum*	Brazil	GQ290155	GQ290178	GQ290189	van der Merwe et al. 2010
Chrysoporthe deuterocubensis	CMW 12745	*Tibouchina urvilleana*	Singapore	DQ368764	GQ290183	DQ368781	Gryzenhout et al. 2006b; van der Merwe et al. 2010
	CMW 12746	*Eucalyptus sp.*	China	HM142105	HM142121	HM142137	Chen et al. 2010
	CMW 17178	*Tibouchina urvilleana*	Thailand	DQ368766	AH015649	AH015649	Gryzenhout et al. 2006b; van der Merwe et al. 2010
	CMW 2631	*Eucalyptus marginata*	Australia	GQ290157	GQ290184	AF543825	Gryzenhout et al. 2004; van der Merwe et al. 2010
	CMW 8650	*Syzygium aromaticum*	Indonesia	AY084001	AY084024	GQ290193	van der Merwe et al. 2010
	CMW 55417	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723734	OR729450	OR729481	This study
	CMW 55418	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723735	OR729451	OR729482	This study
	CMW 55419	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723736	OR729452	OR729483	This study
	CMW 55420	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723737	OR729453	OR729484	This study
	CMW 55421	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723738	OR729454	OR729485	This study
	CMW 55422	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723739	OR729455	OR729486	This study
	CMW 55423	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723740	OR729456	OR729487	This study
	CMW 55424	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723741	OR729457	OR729488	This study
	CMW 55425	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723742	OR729458	OR729489	This study
	CMW 55426	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723743	OR729459	OR729490	This study
	CMW 55427	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723744	OR729460	OR729491	This study
	CMW 55428	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723745	OR729461	OR729492	This study

Table 1: Collection details and GenBank accession numbers of isolates included in the phylogenetic analyses. Isolates obtained in this study are indicated in **bold** *(continued)*

Species	Isolate number	Host	Locality	ITS	*TUB1*	*TUB2*	Reference
Chrysoporthe deuterocubensis (contd)	CMW 55429	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723746	OR729462	OR729493	This study
	CMW 55430	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723747	OR729463	OR729494	This study
	CMW 55431	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723748	OR729464	OR729495	This study
	CMW 55432	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723749	OR729465	OR729496	This study
	CMW 55433	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723750	OR729466	OR729497	This study
	CMW 55434	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723751	OR729467	OR729498	This study
	CMW 55435	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723752	OR729468	OR729499	This study
	CMW 55436	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723753	OR729469	OR729500	This study
	CMW 55437	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723754	OR729470	OR729501	This study
	CMW 55438	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723755	OR729471	OR729502	This study
	CMW 55439	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723756	OR729472	OR729503	This study
	CMW 55440	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723757	OR729473	OR729504	This study
	CMW 55441	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723758	OR729474	OR729505	This study
	CMW 55442	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723759	OR729475	OR729506	This study
	CMW 55443	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723760	OR729476	OR729507	This study
	CMW 55444	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723761	OR729477	OR729508	This study
	CMW 55445	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723762	OR729478	OR729509	This study
	CMW 55446	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723763	OR729479	OR729510	This study
	CMW 55447	*Eucalyptus grandis* × *Eucalyptus pellita*	Indonesia	OR723764	OR729480	OR729511	This study
Chrysoporthe doradensis	CMW 11286	*Eucalyptus grandis*	Ecuador	AY214290	AY214218	AY214254	Gryzenhout et al. 2005
	CMW 11287T	*Eucalyptus grandis*	Ecuador	GQ290156	GQ290179	GQ290190	Gryzenhout et al. 2005
Chrysoporthe hodgesiana	CMW 9995	*Tibouchina semidecandra*	Colombia	AY956969	AH014904	AH014904	Rodas et al. 2005
	CMW 10641T	*Tibouchina semidecandra*	Colombia	AY692322	AY692326	AY692325	Gryzenhout et al. 2004

Table 1: Collection details and GenBank accession numbers of isolates included in the phylogenetic analyses. Isolates obtained in this study are indicated in **bold**

Species	Isolate number	Host	Locality	ITS	*TUB1*	*TUB2*	Reference
Chrysoporthe inopina	CMW 12727T	*Tibouchina lepidota*	Colombia	DQ368777	AH015657	AH015657	Gryzenhout et al. 2006b
	CMW 12729	*Tibouchina lepidota*	Colombia	DQ368778	AH015656	AH015656	Gryzenhout et al. 2006b
	CMW 12731	*Tibouchina lepidota*	Colombia	DQ368779	AH015655	AH015655	Gryzenhout et al. 2006b
Chrysoporthe puriensis	CT10	*Tibouchina granulosa*	Brazil	MN590028	MN590040	MN590040	Oliveira et al. 2021
	CT13	*Tibouchina granulosa*	Brazil	MN590029	MN590041	MN590041	Oliveira et al. 2021
Chrysoporthe syzygiicola	CMW 29940T	*Syzygium guineense*	Zambia	FJ655005	FJ805230	FJ805236	Chungu et al. 2010
	CMW 29942	*Syzygium guineense*	Zambia	FJ655007	FJ805232	FJ805238	Chungu et al. 2010
Chrysoporthe zambiensis	CMW 29928T	*Eucalyptus grandis*	Zambia	FJ655002	FJ858709	FJ805233	Chungu et al. 2010
	CMW 29930	*Eucalyptus grandis*	Zambia	FJ655004	FJ858711	FJ805235	Chungu et al. 2010
Amphilogia gyrosa	CMW 10469	*Elaeocarpus dentatus*	New Zealand	AF452111	AF525707	AF525714	Myburg et al. 2004
	CMW 10470	*Elaeocarpus dentatus*	New Zealand	AF452112	AF525708	AF525715	Myburg et al. 2004

ITS = internal transcribed spacer regions 1 and 2, including the 5.8S region of the rRNA
T = ex-type strain TUB1 = β-tubulin 1 TUB2 = β-tubulin 2

Riau Province, including Kuansing East (six isolates), Kuansing West (four isolates), Kuansing North (two isolates) and Kuansing South (11 isolates) (Figure 2).

Phylogenetic analyses

Amplicons of approximately 490 bp for the ITS, 450 bp for *TUB1* and 345 bp for *TUB2* were generated. The combined sequence data sets used for phylogenetic analyses included 56 ingroup taxa and contained 1292 characters. All isolates sequenced in this study grouped in a well-supported monophyletic clade (ML = 90%), comprised of two sub-clades, with the representative isolates of *C. deuterocubensis* (Figure 3). These isolates were therefore confirmed as being of *C. deuterocubensis.*

Pathogenicity tests

Relative aggressiveness of isolates

Twelve weeks after inoculation, all four *C. deuterocubensis* isolates inoculated on *E. grandis* × *E. pellita* clone (ECL105) caused bark cracking and lesions were observed under the bark (Figure 4). Aggressiveness varied between isolates, with mean lesion lengths ranging from 50 to 128 mm (Figure 5). Isolate CMW 55446 was the most aggressive with a mean lesion length of 128 mm, followed by CMW 55421, CMW 55438 and CMW 55433 with mean lesion lengths of 87 mm, 80 mm, and 50 mm respectively. The Kruskal–Wallis test gave a value of H = 46.47, df = 4 and $p < 1.9e\text{-}9$ confirming that there were significant differences in relative aggressiveness between the *Chrysoporthe* isolates. No disease symptoms were observed on the plants inoculated as the controls. *Chrysoporthe deuterocubensis* was re-isolated from lesions on the inoculated trees but never from the controls.

Relative tolerance of eucalypt clones

Isolates CMW 55421 and CMW 55446, which were the most aggressive in the prior comparison, were used in the clone screening test. Twelve weeks after inoculation, the *E. pellita* clone (ECL102) and three of the *E. grandis* x *E. pellita* hybrids (ECL103, ECL104 and ECL105) inoculated with both *C. deuterocubensis* isolates (CMW 55421 & CMW 55446) showed mean lesions ranging from 10 to 55 mm (Figure 6). However, no symptoms were observed on the *E. pellita* clone (ECL101) or the control trees. Clone ECL105 was found to be the most susceptible, followed by clones ECL102 and ECL103, while ECL101 and ECL104 were found to be more tolerant to infection. Overall, isolate CMW 55446 produced longer lesions than CMW 55421, which was similar to the results where isolates were screened for aggressiveness. Based on the results of the Kruskal–Wallis test, there were significant differences in susceptibility between the clones tested (H = 108.4, df = 14 and $p < 2.2e\text{-}16$). *Chrysoporthe deuterocubensis* was re-isolated from lesions on all clones except ECL101, and was never present in the controls.

Discussion

Chrysoporthe canker has been known to occur on eucalypts for many years (Boerboom & Maas 1970; Hodges et al. 1976; Hodges 1980; Florence et al. 1986; Wingfield et al. 1989), but

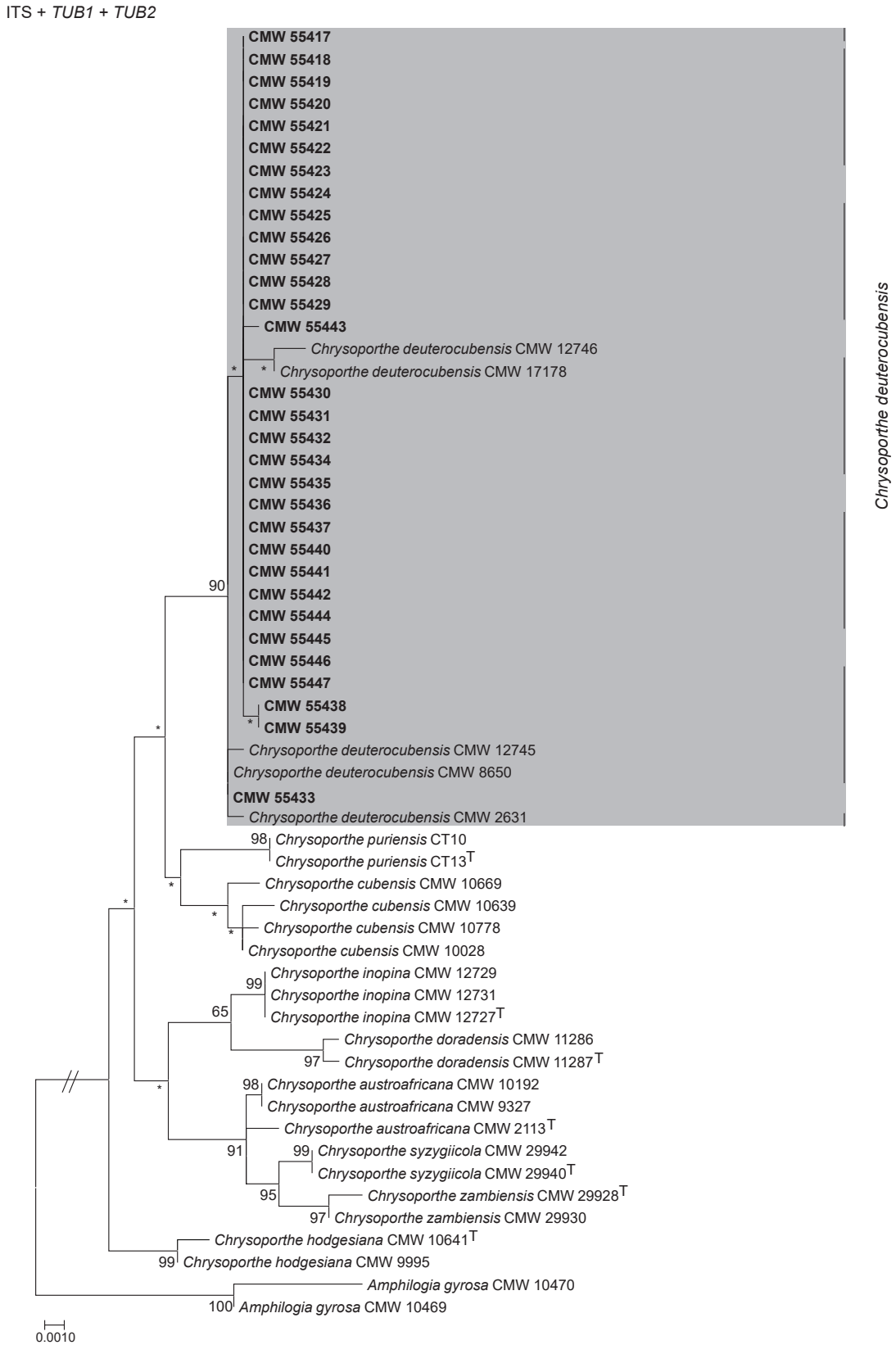

Figure 3: Phylogenetic tree based on maximum likelihood (ML) analysis of a combined data set of ITS, *TUB1* and *TUB2* sequences for *Chrysoporthe* spp. Isolates sequenced in this study are presented in **bold.** Bootstrap values of ≥ 60% for ML analyses are indicated at the nodes. Bootstrap values < 60% are marked (*). Isolates representing ex-type cultures are marked (T). Sequences for *Amphilogia gyrosa* (isolates CMW 10469 and CMW 10470) were used as the outgroup

little is known regarding its relative importance in Indonesia. The results of this study, considering plantation areas in Sumatra and Kalimantan, suggested that the disease is relatively rare. This could be due to the fact that species known to be highly susceptible to infection, such as *E. grandis* (Boeerbomm & Maas 1970; Sharma et al. 1985; Seixas et al. 2004, Mangwanda et al. 2015), have been replaced with species such as *E. urophylla* and *E. pellita* and their hybrids. In this regard, *E. urophylla* (Chen et al. 2010; Mangwanda et al. 2015; Soares et al. 2018) and *E. pellita* (Alfenas et al. 1983; Rauf et al. 2020; Chen et al. 2010) have been recorded to be relatively tolerant to infection by *Chrysoporthe* spp. This is also consistent with our field observations in the present study, where no infection was observed on *E. pellita* clones, whereas hybrids of this species with *E. grandis* were more prone to *Chrysoporthe* infection.

Figure 4: Symptoms of infection by *C. deuterocubensis* 12 weeks after inoculation: (a) bark of control plant inoculated with clean agar; (b) absence of lesion development on the control plant; (c) cracked bark of inoculated plant; (d) lesion resulting from inoculation of *C. deuterocubensis*

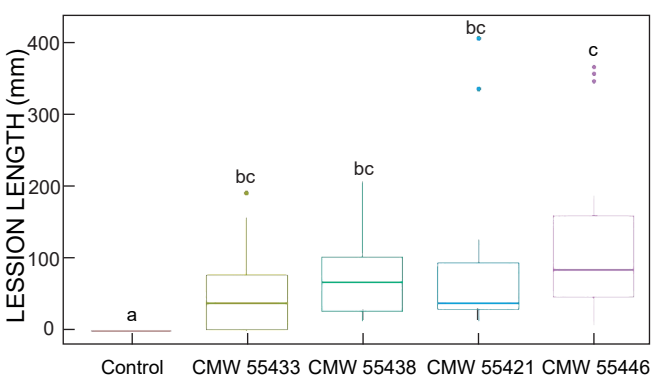

Figure 5: Lesion lengths associated with inoculation of *Eucalytpus* clone ECL105 with four different isolates of *C. deuterocubensis* and a control

An interesting observation in this study was the fact that individual trees of a clone had cankers while many remained uninfected. This situation is distinctly different to the case where highly susceptible clones are planted and where every tree would be cankered, with infections occurring through natural wounds (MJ Wingfield unpubl. data). *Chrysoporthe* spp. require wounds for infection to occur (Gryzenhout et al. 2009; Seixas et al. 2004) and it is probably that those trees observed with cankers in this study had physical wounds and were sufficiently susceptible to develop disease. It also supports the view that the clones, mainly of hybrids including *E. pellita* and *E. urophylla* are not likely to be threatened by *C. deutero-cubensis* infections.

Inoculation trials showed that isolates of *C. deutero-cubensis* differed in their relative aggressiveness. This is similar to the results of previous studies with *Chrysoporthe* spp. such as *C. cubensis* (Rodas et al. 2005), *C. austroafricana* (Roux et al. 2003; Chungu et al. 2010), *C. zambiensis*, *C. syzygiicola* (Chungu et al. 2010) and *C. deuterocubensis* (Chen et al. 2010; Rauf et al. 2020). This result is also consistent with the fact that *C. deutero-cubensis* is most likely native to Southeast Asia, where it has undergone a host shift (Slippers et al. 2005) from native woody plants in the Myrtales (Suzuki et al. 2022).

Inoculations on different eucalypt clones using selected aggressive isolates of *C. deuterocubensis* showed that planting stock being commercially utilised in Indonesia differs in susceptibility to the pathogen. The results were, however relatively variable, where in the case of *E. pellita*, clone ECL101 was more tolerant compared to clone ECL102. Likewise, in *E. grandis* × *E. pellita* hybrids, clone ECL104 was much less susceptible than clones ECL103 and ECL105. In addition, the lesions developing from the inoculations were relatively short, also suggesting that the clones being deployed for plantation development have only low levels of susceptibility.

An obvious shortcoming of this study is that it did not include clones known to be highly susceptible to infection by *C. deuterocubensis*. Such clones would likely have been of *E. grandis*, which is no longer planted in the area. Replacement of *E. grandis* with species such as *E. urophylla* and particularly *E. pellita*, which are better suited to plantation areas in the humid tropics, appears to have diminished the relative importance of canker caused by *C. deuterocubensis*. It is, however important, to recognise that this pathogen is undergoing sexual reproduction in the background environment (Suzuki et al. 2022), and that genotypes having the ability to infect and cause serious disease could easily emerge in the future.

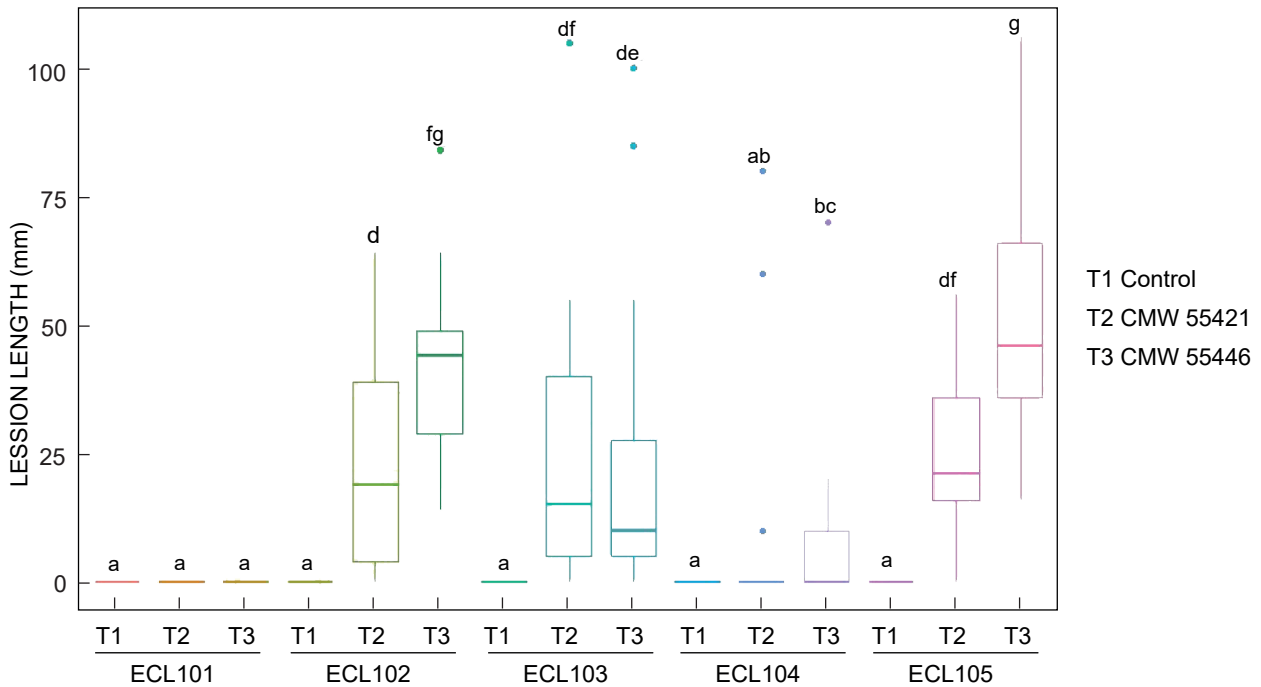

Figure 6: Lesion lengths on five different commercially deployed *Eucalyptus* clones after inoculation with two isolates (CMW 55421 and CMW 55446) of *C. deuterocubensis* and a control. Isolate CMW 55446 consistently produce longer lesion compared to CMW 55421. Clone ECL105 found to be the most susceptible, while ECL101 was the most tolerant

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