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A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora sp. nov.* in Indonesia

M. Tarigan [a,c], J. Roux [a,*], M. Van Wyk [b], B. Tjahjono [c], M.J. Wingfield [a]

[a] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
[b] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
[c] *PT Riau Andalan Pulp and Paper, Pekanbaru, Riau, Sumatra, Indonesia*

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Abstract

Species of *Ceratocystis* are well-known wound related pathogens of many tree species, including commercially planted *Acacia* spp. Recently, several *Ceratocystis* isolates were collected from wilting *A. mangium* in plantations in Indonesia. The aim of this study was to identify these *Ceratocystis* isolates and to investigate their ability to cause disease on two plantation-grown *Acacia* spp. using greenhouse and field inoculation experiments. For identification, morphological characteristics and comparisons of DNA sequence data for the ITS, β-tubulin and TEF 1-α gene regions, was used. *Ceratocystis* isolates were identified as *C. manginecans,* a serious pathogen of mango trees in Oman and Pakistan and a previously undescribed species, described here as *C. acaciivora sp. nov*. Both fungi produced significant lesions in inoculation experiments on *A. mangium* and *A. crassicarpa*, however, *C. acaciivora* was most pathogenic suggesting that this fungus is the primary cause of the death of trees under natural conditions.

Keywords: Plantation forestry; Quarantine threats; Tree disease

1. Introduction

Species of *Ceratocystis* have been reported to cause root rots, stem rots, vascular stains, cankers and fruit or pod rot on many plants but mainly trees in tropical parts of the world (Kile, 1993). *Ceratocystis fimbriata* Ellis & Halst *sensu lato* (s.l), for example causes disease and death of *Eucalyptus* trees in the Republic of Congo and Brazil (Roux et al., 2000), *Gmelina arborea* Roxb. and *Colocasia esculenta* (L.) Schott in Brazil (Harrington et al., 2005; Muchovej et al., 1978) and *Coffea* sp. in Colombia and Venezuela (Marin et al., 2003; Pontis, 1951). This fungus has also been reported to cause mango decline or 'seca' disease in Brazil (Ploetz, 2003; Ribeiro, 1980; Viegas, 1960) and it is considered one of the most important pathogens of agricultural and tree crops in South America.

In Indonesia *Ceratocystis* spp. were first noted when *C. fimbriata* (reported as *Rostrella coffeae* Zimm.) was reported in 1900 on *Coffea arabica* L. on the island of Java (Zimmerman, 1900). Subsequently, various species of *Ceratocystis* have been reported from other hosts on many Indonesian islands. Examples include *C. fimbriata* [reported as *Sphaeronema fimbriatum* (Ellis & Halst.) Sacc.] from *Hevea brasiliensis* Müll.Arg. in Sumatra, Kalimantan and Java (Leefmans, 1934; Tayler and Stephens, 1929; Wright, 1925), *C. polychroma* M. van Wyk, M.J. Wingf. & E.C.Y. Liew from *Syzygium aromaticum* (L.) Merrill & Perry in Sulawesi (Van Wyk et al., 2004) and *C. tribiliformis* M. van Wyk & M.J. Wingf. from *Pinus merkusii* Jungh. & de Vriese in Sumatra (Van Wyk et al., 2006a). Most recently, three previously unknown species of *Ceratocystis*, in the *C. moniliformis* s.l. group of the genus, were reported from artificially induced wounds on *Acacia mangium* Willd. trees in Indonesia (Tarigan et al., 2010). These fungi, *C. inquinans* Tarigan, M. van Wyk & M.J. Wingf., *C. sumatrana* Tarigan, M. van Wyk & M.J. Wingf. and *C. microbasis* Tarigan, M. van Wyk & M.J. Wingf., are not pathogens of *A. mangium*, similar to other fungi in the *C.*

* Corresponding author. Tel.: +27 124203938/9; fax: +27 124203960.
E-mail address: jolanda.roux@fabi.up.ac.za (J. Roux).

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moniliformis s.l. clade, which are not known as pathogens, but as mild sapstain fungi (Kile, 1993; Tarigan et al., 2010).

As a result of an increased international demand for pulp and paper products, plantation industries are proliferating rapidly in many parts of the world including Indonesia (Anonymous, 2004; Barr, 2001). *A. mangium* and *A. crassicarpa* Cunn.: Benth., native to the eastern islands of Indonesia, northern Queensland and the western Province of Papua New Guinea (Doran and Skelton, 1982; Moran et al., 1989; Turnbull, 1986), are two of the most widely planted trees in Indonesia. While these trees have displayed excellent growth in the region, they have also been negatively affected by various diseases (Old et al., 2000; Potter et al., 2006). This is typical of plantation industries based on non-native species separated from their natural enemies but that are increasingly damaged by pests and diseases (Wingfield et al., 2008).

Ceratocystis spp. have increasingly been reported as the cause of wilt and canker of plantation-grown *Acacia* spp. in many areas, world-wide (Roux and Wingfield, 2009). In Brazil, *C. fimbriata* s.l. has been reported to cause canker and wilt of *A. decurrens* Willd. (Ribeiro et al., 1988) and in South Africa, *C. albifundus* M.J. Wingf., De Beer & Morris is considered the most important pathogen of plantation-grown *A. mearnsii* De Wild. and *A. decurrens* trees (Morris et al., 1993; Roux and Wingfield, 1997; Roux et al., 1999; Wingfield et al., 1996). During the course of recent disease surveys in *A. mangium* plantations in Indonesia, significant mortality of young trees showing rapid wilt symptoms was observed. Isolates of a *Ceratocystis* sp. were collected from stained vascular tissue on these trees. The aim of this study was to identify these *Ceratocystis* isolates and to consider their ability to cause disease on two plantation-grown *Acacia* spp. using greenhouse and field inoculation experiments.

2. Materials and methods

2.1. Disease and fungal isolates

Wilt and canker symptoms were commonly observed on young *A. mangium* trees in plantations. The bark and the wood surrounding the cankers were discolored and had a black appearance due to the exudation of gum. The discolored wood typically had a streaked appearance, turning a uniform dark brown to dark blue color with age (Fig. 1). Affected trees ranged from 8 to 12 months in age. The disease was observed in several plantation areas, including Teso, Logas and Pelalawan, all in the Riau Province of Sumatra. All diseased trees had been pruned 6–12 weeks earlier and lesions appeared to originate from these wounds, suggesting the involvement of a wound colonizing pathogen.

Twenty *A. mangium* trees were selected randomly and sampled in plantations at Teso and Logas. Sections of discolored wood and bark were cut from the leading edges of cankers. These were then wrapped in newspaper to maintain moisture, and taken to the laboratory for examination.

Single spore drops, characteristic of *Ceratocystis* spp., were collected directly from fungal fruiting bodies from the diseased samples onto 2% (w/v) Malt Extract Agar (MEA) (Biolab, Midrand, South Africa). Where no fruiting structures were visible, a subset of samples was placed in plastic bags containing moistened tissue paper for 4–10 days to induce sporulation. Another subset of samples was baited for *Ceratocystis* spp. using carrot slices (Moller and De Vay, 1968). Symptomatic wood pieces were wrapped between carrot slices that had first been immersed for 10 min in a 0.001 g/vol streptomycin sulfate solution (SIGMA, Steinheim, Germany) and then placed in plastic bags and incubated for 3–5 days, or until fruiting bodies were observed.

Isolates collected in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative isolates have also been deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Delft, Netherlands and dried specimens were deposited at the National Fungal Herbarium of South Africa (PREM), Pretoria.

For the current study, we included unidentified isolates collected from a previous study (Tarigan et al., 2010) which resembled those that we found from diseased *A. mangium* in this study. These included 10 isolates from artificially wounded *A. crassicarpa* trees at Teso and 21 isolates from *A. mangium* trees wounded in Pelalawan. These 31 isolates were not identified in the study by Tarigan et al. (2010), since they did not belong to the *C. moniliformis* s.l group of fungi on which they focused in that study.

2.2. DNA extractions

Eleven isolates of the *Ceratocystis* spp. from *A. mangium* and *A. crassicarpa* were selected randomly and grown on 2% MEA at 22 °C for two weeks. The mycelium was scraped off the surface of the agar using sterilized scalpel blades and transferred to 1.5 mL Eppendorf tubes and lyophilized overnight. The lyophilized mycelium was crushed to a fine powder using a glass rod or mechanical grinder (Ball mills machine, Retsch, Haan, Germany). The DNA was extracted using the method described by Van Wyk et al. (2006b).

2.3. PCR amplification, sequencing and analyses

The Internal Transcribed Spacer regions (ITS), including the 5.8S gene of the ribosomal DNA (rDNA) operon, part of the β-tubulin (BT) and Transcription Elongation factor 1-α (TEF) gene regions were amplified using the polymerase chain reaction (PCR) and an iCycler thermocycler (Bio-Rad, Hercules, CA, USA). Amplification of the gene regions were made using the primers ITS1 and ITS4 (White et al., 1990), βt1a and βt1b (Glass and Donaldson, 1995) and EF1-728F and EF1-986R (Jacobs et al., 2004) respectively. PCR reaction mixtures consisted of 0.5 μL of each primer (10 mM), 2 μL of 10 mM dNTP mixture (2.5 mM of each dNTP), 0.5 μL DNA Fast Start Taq enzyme (Roche Molecular Biochemicals, Almeda California), 2.5 μL of a 10× concentration buffer containing $MgCl_2$ (3.5 mM), 2 μL of DNA template (2–10 ng) and 17 μL sabax water to make up 25 μL total

Fig. 1. Disease symptoms caused by *Ceratocystis acaciivora sp. nov.* and *C. manginecans* on *A. mangium* trees in Indonesia. a. Wilting and death of trees; b, c. Bark discoloration, gum exudation and cankers on stems of diseased trees; d. Wood below the cankers was discolored, often in streaked patterns, but later turning a uniform dark brown to dark blue color.

volume reactions. The PCR program, product visualization and DNA purification were carried out following methods described by Van Wyk et al. (2006b).

An ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California) was used to sequence the purified PCR amplicons in both the forward and reverse directions. Sequencing of each gene region was done using the same primers as those used for the PCR reactions. After cleaning the sequence products using the same protocol as those used for the PCR reactions, the final sequence products were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California). Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California) was used to analyze the sequence electropherograms. Using Blast analyses, the sequences obtained were compared with sequences of *Ceratocystis* spp. that are available in GenBank (The National Centre for Biological Information, http://www.ncbi.nlm.nih.gov/) (Table 1). The online version of MAFFT (Katoh et al., 2002) version 6 (http://align.bmr.kyushu-u.ac.zp/mafft/online/server/) was used to align the sequences of the Indonesian isolates with published sequences of closely related species identified using Blast and the alignments were then checked manually. A partition homogeneity test was run using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10* to determine whether the data for the three gene regions could be combined into a single data set (Swofford, 2002). Data for each data set were analyzed using PAUP version 4.0b10* (Swofford, 2002).

Table 1
Ceratocystis isolates considered in the phylogenetic analyses.

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	Collector
C. acaciivora	CMW22562	EU588655 EU588635 EU588645	*Acacia mangium*	Indonesia	M. Tarigan
C. acaciivora	CMW22563	EU588656 EU588636 EU588646	*A. mangium*	Indonesia	M. Tarigan
C. acaciivora	CMW22564	EU588657 EU588637 EU588647	*A. mangium*	Indonesia	M. Tarigan
C. acaciivora	CMW22595	EU588660 EU588639 EU588649	*A. mangium*	Indonesia	M. Tarigan
C. acaciivora	CMW22621	EU588661 EU588640 EU588650	*A. mangium*	Indonesia	M. Tarigan
C. albifundus	CMW4068	DQ520638 EF070429 EF070400	*A. mearnsii*	RSA	J. Roux
C. albifundus	CMW5329 CBS141.37	AF388947 DQ371649 EF070401 EF070442 EF070394	*A. mearnsii*	Uganda	J. Roux
C. atrox	CMW19383 CBS120517	EF070414 EF070430 EF070402	*Eucalyptus grandis*	Australia	M.J. Wingfield
C. atrox	CMW19385 CBS120518	EF070415 EF070431 EF070403	*E. grandis*	Australia	M.J. Wingfield
C. cacaofunesta	CMW15051 CBS152.62	DQ520636 EF070427 EF070398	*Theobroma cacao*	Cost Rica	A.J. Hansen
C. cacaofunesta	CMW14809 CBS115169	DQ520637 EF070428 EF070399	*T. cacao*	Ecuador	C. Suarez
C. caryae	CMW14808 CBS115168	EF070423 EF070440 EF070411	*Carya ovata*	U.S.A	J. Johnson
C. caryae	CMW14793 CBS114716	EF070424 EF070439 EF070412	*C. cordiformis*	U.S.A	J. Johnson
C. colombiana	CMW5751 CBS121792	AY177233 AY177225 EU241490	*Coffea arabica*	Colombia	M. Marin
C. colombiana	CMW5761 CBS121791	AY177234 AY177224 EU241492	Soil in citrus orchid	Colombia	B. Castro
C. fimbriata	CMW1547	AF264904 EF070443 EF070395	*Ipomoea batatas*	Papua New Guinea	E.C. McKenzie
C. fimbriata	CMW15049	DQ520629 EF070442 EF070394	*I. batatas*	USA	F.C. Andrus
C. fimbriatomima	CMW24174 CBS121786	EF190963 EF190951 EF190957	*Eucalyptus* sp.	Venezuela	M.J. Wingfield
C. fimbriatomima	CMW24176 CBS121787	EF190964 EF190952 EF190958	*Eucalyptus* sp.	Venezuela	M.J. Wingfield
C. manginecans	CMW13851	AY953383 EF433308	*Mangifera indica*	Oman	M. Deadman

(continued on next page)

Table 1 (*continued*)

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	Collector
		EF433317			
C. manginecans	CMW13852	AY953384	*Hypocryphalus mangifera*	Oman	M. Deadman
		EF433309			
		EF433318			
C. manginecans	CMW13854	AY953385	*M. indica*	Oman	M. Deadman
		EF433310			
		EF433319			
C. manginecans	CMW22579	EU588658	*A. mangium*	Indonesia	M. Tarigan
		EU588638			
		EU588648			
C. manginecans	CMW22581	EU588659	*A. mangium*	Indonesia	M. Tarigan
		EU604671			
		EU604670			
C. manginecans	CMW21123	EU588662	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588641			
		EU588651			
C. manginecans	CMW21125	EU588663	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588642			
		EU588652			
C. manginecans	CMW21127	EU588664	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588643			
		EU588653			
C. manginecans	CMW21132	EU588665	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588644			
		EU588654			
C. manginecans	CMW23628	EF433303	*Hypocryphalus mangifera*	Pakistan	A. Al-Adawi
		EF433312			
		EF433321			
C. manginecans	CMW23634	EF433302	*M. indica*	Pakistan	A. Al-Adawi
		EF433311			
		EF433320			
C. manginecans	CMW23641	EF433305	*M. indica*	Pakistan	A. Al-Adawi
		EF433314			
		EF433323			
C. manginecans	CMW23643	EF433304	*M. indica*	Pakistan	A. Al-Adawi
		EF433313			
		EF433322			
C. neglecta	CMW11284	EF127988	*Eucalyptus* sp.	Colombia	M.J. Wingfield
	CBS121789	EU881898			
		EU881904			
C. neglecta	CMW11285	EF127989	*Eucalyptus* sp.	Colombia	M.J. Wingfield
	CBS121017	EU881899			
		EU881905			
C. obpyriformis	CMW23807	EU245004	*A. mearnsii*	South Africa	R.N. Heath
		EU244976			
		EU244936			
C. obpyriformis	CMW23808	EU245003	*A. mearnsii*	South Africa	R.N. Heath
		EU244975			
		EU244935			
C. papilata	CMW8857	AY233868	*Annana muriata*	Colombia	M.J. Wingfield
		AY233878			
		EU241483			
C. papilata	CMW8856	AY233867	*Citrus* sp.	Colombia	B. Castro
	CBS121793	AY233874			
		EU241484			
C. platani	CMW14802	DQ520630	*Platanus occidentalis*	U.S.A	T.C. Harrington
	CBS115162	EF070425			
		EF070396			
C. platani	CMW23918	EF426554	*Platanus* sp.	Greece	M.J. Wingfield
		EF070426			
		EF070397			
C. pirilliformis	CMW6569	AF427105	*E. nitens*	Australia	M.J.Wingfield
		DQ371652			
		AY528982			

Table 1 (*continued*)

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	Collector
C. pirilliformis	CMW6579	AF427105 DQ371653 AY528983	*E. nitens*	Australia	M.J. Wingfield
C. polyconidia	CMW23809	EU245006 EU244978 EU244938	*A. mearnsii*	South Africa	R.N. Heath
C. polyconidia	CMW23818	EU245007 EU244979 EU244939	*A. mearnsii*	South Africa	R.N. Heath
C. polycroma	CMW11424 CBS115778	AY528970 AY528966 AY528978	*Syzygium aromaticum*	Indonesia	M.J. Wingfield
C. polycroma	CMW11436 CBS115777	AY528971 AY528967 AY528979	*S. aromaticum*	Indonesia	M.J. Wingfield
C. populicola	CMW14789 CBS119.78	EF070418 EF070434 EF070406	*Populus* sp.	Poland	J. Gremmen
C. populicola	CMW14819 CBS114725	EF070419 EF070435 EF070407	*Populus* sp.	U.S.A	T. Hinds
C. smalleyi	CMW14800 CBS114724	EF070420 EF070436 EF070408	*C. cordiformis*	U.S.A	G. Smalley
C. smalleyi	CMW26383 CBS114724	EU426553 EU426555 EU426556	*C. cordiformis*	USA	G. Smalley
C. tanganyicensis	CMW15992	EU244999 EU244971 EU244931	*A. mearnsii*	Tanzania	R.N. Heath & J. Roux
C. tanganyicensis	CMW15999	EU244998 EU244970 EU244939	*A. mearnsii*	Tanzania	R.N. Heath & J. Roux
C. tsitsikammensis	CMW14276	EF408555 EF408569 EF408576	*Rapanea melanophloeos*	South Africa	G. Kamgan Nkuekam & J. Roux
C. tsitsikammensis	CMW14278	EF408556 EF408570 EF408577	*R. melanophloeos*	South Africa	G. Kamgan Nkuekam & J. Roux
C. variospora	CMW20935 CBS114715	EF070421 EF070437 EF070410	*Quercus alba*	U.S.A	J. Johnson
C. variospora	CMW20936 CBS114714	EF070422 EF070438 EF070410	*Q. robur*	U.S.A	J. Johnson
C. virescens	CMW3276	AY528984 AY528990 AY5289991	*Quercus* sp.	USA	T. Hinds
C. virescens	CMW11164	DQ520639 EF070441 EF070413	*Fagus americanum*	USA	D. Houston
C. zombamontana	CMW15235	EU245002 EU244974 EU244934	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux
	CMW15236	EU245000 EU244972 EU244932	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux

Gaps were treated as "fifth base" and trees were obtained via stepwise addition of 1000 replicates with the Mulpar option in effect. Bootstrap confidence intervals, using 1000 replicates, were calculated. Two *Ceratocystis virescens* isolates were used as the out-group taxa. MrBayes version 3.1.1. (Ronquist and Huelsenbeck, 2003) was used to run Bayesian analyses on the combined data set. The Markov Chain Monte Carlo (MCMC) algorithm was used to calculate support of the nodes of the phylogenetic trees based on Bayesian posterior probabilities. The model of nucleotide substitution for each gene region was

determined using MrModeltest2 (Nylander, 2004), and the models obtained were used in the Bayesian analysis. One million random trees were generated with four chains and sampled every 100th generation following the MCMC procedure. Samples were taken only from trees after convergence and trees outside the point of convergence were discarded.

2.4. Culture characteristics and morphology

Three isolates from each of the groups identified based on DNA sequence comparisons were grown on 2% MEA for two weeks at 22 °C for growth studies. A five-millimeter diameter cork borer was used to cut discs of mycelium from the margins of actively growing cultures. These were placed at the centers of 90 mm diameter Petri dishes containing 2% MEA. Discs for each isolate were placed at the centers of five plates and incubated at 4 °C and from 10 °C to 35 °C at 5 °C intervals. Two measurements of colony diameter, at right angles to each other, were taken two days after incubation and averages for all measurements were computed. The entire experiment was repeated once. Data from both studies were combined and analyzed statistically using analysis of variance (ANOVA).

Two-week-old cultures grown on 2% MEA were used to describe the morphological characteristics of the isolates. Fruiting structures were mounted in lactic acid (85%) for observation and measurements. Fifty measurements of all characteristic structures from each representative isolate (extype) and 10 measurements for one additional isolate in each group, were made with a Carl Zeiss microscope and a Zeiss Axiovision camera system (Oberkochen, Germany). The average (mean), standard deviation (stdv), maximum (max) and minimum (min) measurements were calculated and they are presented as follows: (min−) mean minus stdv − mean plus stdv (−max). The color of the cultures was described using the color charts of Rayner (1970).

2.5. Pathogenicity tests

2.5.1. Greenhouse inoculations

Pathogenicity tests were carried out on one-year-old *A. mangium* and *A. crassicarpa* seedlings (~15 mm diam.) in a greenhouse. Trees were grown in 20 cm diameter plastic bags containing a mixture of topsoil and compost. The temperature and humidity of the greenhouse was adjusted to ~30 °C and 65% respectively for optimum growth of the seedlings. Five isolates (CMW22563, CMW22564, CMW22581, CMW22595, and CMW22621) representing Group 1 and four isolates representing Group 2 (CMW21123, CMW21125, CMW21127, and CMW21132) identified using DNA sequence data were used for the inoculations. Five seedlings of each *Acacia* sp. were used for each isolate. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.) and an agar disc taken from an actively growing colony on 2% MEA, with the mycelium facing downwards, was placed in the wound. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Five plants of each tree species were inoculated with sterile MEA plugs to serve as controls.

The Parafilm was removed from the stems of treated plants 10 days after inoculation and the lengths of the lesions produced in the xylem were measured. Pieces of symptomatic stem tissue were taken from the inoculated trees and these were placed into moist chambers and also baited using carrots as described previously, to induce the production of fruiting structures. Spores from these structures were then transferred to 2% MEA to verify their identity. Analysis of variance (ANOVA) was calculated for all data obtained using SAS statistical analyses (SAS, 2001).

2.5.2. Field inoculations

One-year-old *A. mangium* and *A. crassicarpa* trees growing in plantations in Riau Province of Sumatra, ranging in diameter from 70 mm to 90 mm were used in field pathogenicity tests. Inoculations were made on the stems, 1.3 m from the ground. Three isolates from each of the two *Ceratocystis* groups, found to be the most pathogenic in the greenhouse tests were used for field inoculations. Twenty trees of each *Acacia* sp. were used for each isolate and an equal number of trees were inoculated as controls. Prior to inoculation, a wound was made on the tree stems using a sterilized cork borer (10 mm diam.) and inoculations were carried out using the same technique as described for the greenhouse trials. Control inoculations were made using sterile 2% MEA plugs. After inoculation, the wounds were sealed with masking tape to reduce contamination and desiccation of the inoculum and wounds.

Six weeks after inoculation, the tree diameter at the inoculation point and the length of lesions produced on the stems were measured. Pieces of symptomatic tissue from the area associated with the inoculation points for a representative set of trees and isolates were taken and placed in moist chambers to induce sporulation and to confirm that the lesions were associated with the inoculated fungus. The entire field inoculation trial was repeated once. Data obtained were analyzed with analysis of variance (ANOVA) using SAS statistical analyses (SAS, 2001).

3. Results

3.1. Fungal isolates

A total of 46 *Ceratocystis* isolates were obtained from diseased *A. mangium* trees at Teso and Logas (Fig. 1). Within two weeks, they produced mature ascomata containing hat-shaped ascospores and a *Thielaviopsis* anamorph when grown on 2% MEA. These isolates resembled the 31 isolates obtained from the culture collection (CMW) and collected during a previous study by Tarigan et al. (2010).

3.2. PCR amplification, sequencing and analyses

For both the ITS and BT gene regions, PCR amplification resulted in fragments of ~500 base pairs (bp) in size, while for the TEF gene region, amplification resulted in fragments of ~800 bp

in size. Blast searches in GenBank indicated that isolates from Indonesia grouped within the *C. fimbriata.* s.l species complex and were most closely related to *C. manginecans* M. van Wyk, A Al-Adawi, & M.J. Wingf. The partition homogeneity test for the three data sets from ITS, BT and TEF resulted in a P value of 0.01, which is an acceptable level (Barker and Lutzoni, 2002; Cunningham, 1997) to combine the data. The combined data set consisted of a total of 1946 characters, including gaps. The dataset contained 1139 constant characters, 56 parsimony-uninformative characters and 751 parsimony-informative characters. Twenty most parsimonious trees were obtained of which one (Fig. 2) was selected for presentation. This tree had a length of 1855 steps, a consistency index (CI)=0.66, a homoplasy index (HI)=0.34, a retention index (RI)=0.86 and a rescaled consistency index (RC)=0.58.

Model test analysis resulted a HKY+G model [Prset statefreqpr=dirichlet (1, 1, 1, 1); Lset nst=2 rates=gamma] for the ITS gene region, GTR+G model [Prset statefreqpr=dirichlet (1, 1, 1, 1); Lset nst=6 rates=gamma] for the BT gene region, while for TEF it produced a HKY+I+G model [Prset

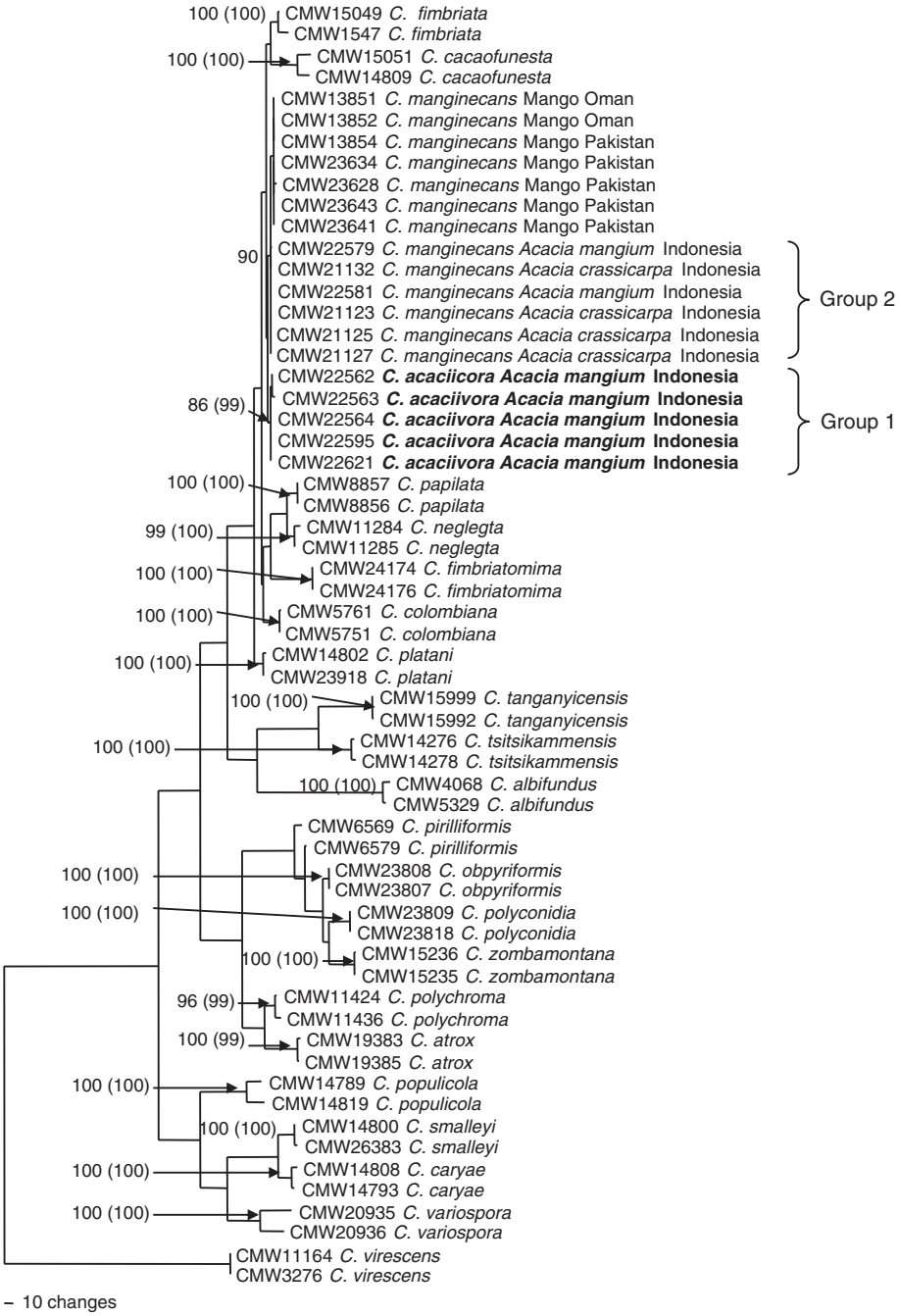

Fig. 2. A phylogenetic tree based on the combined sequence data of three gene regions; ITS, βt and TEF1-α, showing relationships between *C. fimbriata* s.l. used in this study. Isolates in bold were isolated from *A. crassicarpa* and *A. mangium* in Indonesia and sequenced as part of this study. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated at the branches and Bayesian values follow in the brackets. Two isolates of *C. virescens* were selected as the out-group.

statefreqpr=dirichlet (1, 1, 1, 1); Lset nst=2 rates=invgamma]. These models were used in the Bayesian analyses. The first 2000 trees obtained with Bayesian analyses were discarded following the burn-in procedure. Both Bootstrap and Bayesian values were attached to the posterior probability of the branch nodes of the combined dataset (Fig. 2).

The isolates from *Acacia* in Indonesia resided within the larger *C. fimbriata* s.l. species complex, forming two sub-clades, designated Group 1 and Group 2 (Fig. 2), and most closely related to *C. manginecans.* The Group 1 (CMW22562, CMW22563, CMW22564, CMW22595, and CMW22621) isolates formed a well resolved and distinct group. They had posterior probability support for the branch nodes of 86% and 99% Bootstrap and Bayesian values respectively. Group 2 (CMW21123, CMW21125, CMW21127, CMW21132, CMW22579, and CMW22581) had no Bootstrap or Bayesian support and this sub-clade grouped with *C. manginecans* with a Bootstrap support of 90%.

3.3. Culture characteristics and morphology

Ceratocystis isolates from *A. mangium* and *A. crassicarpa* in Indonesia were similar in culture characteristics and morphology, however some differences were also found. Group 1 isolates grew slightly faster than those representing Group 2. Isolates of both groups showed similar responses to temperature, all having optimal growth at 25 °C. Group 1 isolates reached a diameter of 34 mm and Group 2 isolates reached 29 mm in seven days. For all isolates, growth was limited at 15 °C and no growth occurred at 4 °C, 10 °C or 35 °C. Both of the Indonesian groups and *C. manginecans* have similar responses to temperature, all having optimal growth at 25 °C, however *C. manginecans* has a lighter culture color. Cultures representing both groups of isolates were olive green (23 m) and produced both teleomorph and anamorph structures within 1–2 weeks. When the cultures were placed in sealed plastic boxes, they produced a weak fruity aroma which disappeared when the boxes had been opened for some time.

Ceratocystis isolates from Indonesia were typical of *Ceratocystis* spp. They had black ascomatal bases that were globose to sub globose in shape. All isolates produced ascomatal necks with divergent ostiolar hyphae at their tips, from which hat-shaped ascospores exuded. Group 1 isolates had smaller ascomatal bases and shorter necks than those in Group 2 and *C. manginecans*. Group 2 isolates had ascomatal bases and ascomatal necks within the same size ranges when compared to *C. manginecans*, supporting DNA sequence data, which suggested that these isolates represent *C. manginecans*.

3.4. Pathogenicity tests

3.4.1. Greenhouse inoculations

Ten days after inoculation, all *Ceratocystis* isolates used in the pathogenicity trials produced distinct lesions on *A. crassicarpa* and *A. mangium* seedlings. The lesion lengths ranged from 17 to 345 mm on *A. crassicarpa* and 14 to 151 mm on *A. mangium*. Isolates representing Group 1 produced longer lesions on both *A. crassicarpa* and *A. mangium* compared to those of Group 2 (*C. manginecans*). Two isolates (CMW21123 and CMW21132) representing Group 2 consistently produced only small lesions that were not significantly different to the controls. *Ceratocystis* isolates were consistently re-isolated from the treated plants but they were not retrieved from the controls. Three of the most pathogenic isolates from Group 1 (CWM22563, CMW22564, and CMW22621) and Group 2 (CMW21125, CMW21127, and CMW21132) were selected for field inoculation studies.

3.4.2. Field inoculations

Within six weeks of inoculation, all *Ceratocystis* isolates produced lesions both on *A. mangium* and *A. crassicarpa* trees. The lesion lengths ranged from 100 to 660 mm on *A. crassicarpa* and 130 to 680 mm on *A. mangium* in the first trial (Fig. 3). In the second trial the lesions ranged from 80 to 580 mm on *A. crassicarpa* and 70 to 290 mm on *A. mangium* (Fig. 4). Although lesions in the second trial were smaller than those in the first trial, both trials produced similar trends where all isolates in Group 1, except CMW22621, consistently produced longer lesions, both on *A. crassicarpa* and *A. mangium*, than isolates in Group 2 (Figs. 3, 4). Isolate CMW21125 (Group 2) consistently produced significantly longer lesions compared to the controls on *A. crassicarpa* and *A. mangium* in both trials. Other isolates (CMW21127 and CMW21132) residing in Group 2 (*C. manginecans*) consistently produced lesions that were significantly larger than those of the controls when inoculated into *A. crassicarpa* in both trials, but they produced lesions that were not significantly different to the controls when they were inoculated on *A. mangium* trees. Re-isolation from lesions on inoculated trees consistently yielded *Ceratocystis* isolates that were morphologically similar to the test fungi. No *Ceratocystis* isolates were isolated from the control trees.

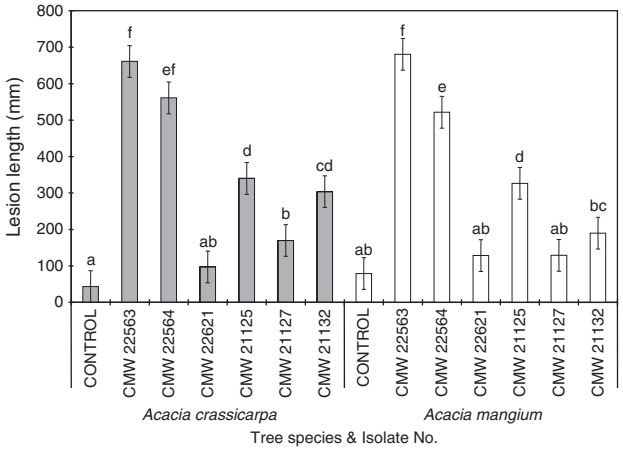

Fig. 3. Lesion lengths associated with the first set of inoculations using *C. acaciivora prov. nom.* (CMW22563, CMW22564, and CMW22621) and *C. manginecans* (CMW21125, CMW21127, and CMW21132) on one-year-old *A. mangium* and *A. crassicarpa* trees in an Indonesian plantation, six weeks after inoculation. Bars on the graph indicated with the same letter are not significantly different from each other (P value=0.05).

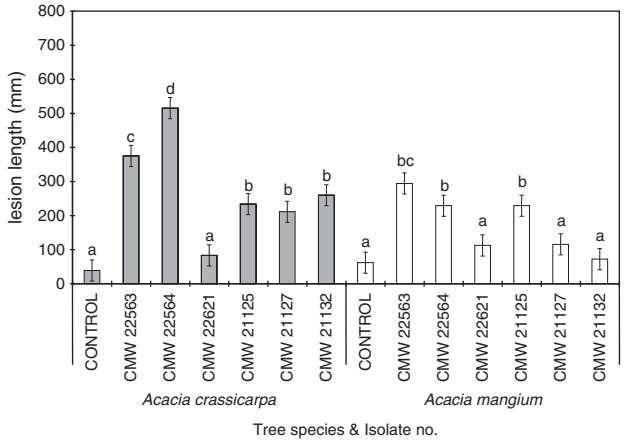

Fig. 4. Lesion lengths associated with the second set of inoculations using *C. acaciivora prov. nom.* (CMW22563, CMW22564, and CMW22621) and *C. manginecans* (CMW21125, CMW21127, and CMW21132) on one-year-old *A. mangium* and *A. crassicarpa* trees in an Indonesian plantation, six weeks after inoculation. Bars on the graph indicated with the same letter are not significantly different from each other (P value = 0.05).

4. Taxonomy

Based on morphology and DNA sequence comparisons, it was clear that *Ceratocystis* isolates in Group 1 from diseased *A. mangium* in Indonesia represent an undescribed taxon. It is consequently described as follows:

Ceratocystis acaciivora Tarigan & M. van Wyk s*p. nov.*

MB 513076 (Fig. 5)

Etymology: The name refers to the ability of this fungus to infect *Acacia* sp.

Stat.conid.: *Thielaviopsis*

Coloniae olivaceae, *mycelium* aerium. *Hyphae* leaves, septis non constrictis. *Bases ascomatum* pallide brunneae, atrobrunneae vel nigrae, globosae vel subglobosae (105−) 131–175 (−206) μm longae, (107−) 125–167 (−188) μm latae, hyphis ornatae. *Colla ascomatum* atrobrunnea vel nigra, apicem versus pallentia (301−) 348–448 (−522) μm longa, basi (25−) 33–45 (−53) μm, apice (11−) 13–17 (−20) μm lata, basi discoidea. *Hyphae ostiolares* divergentes hyalinae (30−) 35–49 (−60) μm longae. *Asci* non visi. *Ascosporae* in massis fulvo-flavescentibus mucosis in apicibus collorum ascomatum. Ascosporae lateraliter visae cucullatae hyalinae, non septatae, vaginis inclusae, 5–7 × 3–4 μm cum vagina, 4–6 × 3–4 μm sine illa.

Anamorpha Thielaviopsis: *conidiophorae primariae* in mycelio singulae, hyalinae, basi incrassatae, apicem versus angustatae, (53−) 61–95 (−127) μm longae, basi 4–6 μm, medio (2−) 5–7 (−8) μm, apice 3–6 μm latae. *Conidiophorae secondariae* in mycelio singulae hyalinae (52−) 57–69 (−68) μm longae, basi (3−) 2–4 (−5) μm, apice 4–6 μm latae. *Conidia* biformia: primaria hyalina cylindrica non septata (11−) 14–22 (−29) × 3–5 μm; secondaria hyalina doliiformia non septata (8−) 9–11 (−13) × 4–6 μm. *Chlamydosporae* ovales, parietibus crassis, laeves, brunneae, (10−) 12–14 (−15) × (7−) 8–12 (−14) μm, in agaro inclusae, terminales, singulae vel in catenis.

Typus: Indonesia: Sumatra, isolated from *A. mangium*, 2005, M. Tarigan (PREM59884 — holotype, living culture-extype: CMW22563).

Colonies olive green (23 m) in color. *Mycelium* aerial. *Optimal temperature* for growth 25 °C, growth reduced at 15 °C, no growth at 35 °C, 10 °C and below. *Hyphae* smooth, not constricted at septa. *Ascomatal bases* dark brown to black, globose to sub globose, (105−) 131–175 (−206) μm long, (107−) 125–167 (−188) μm in width, ornamented with hyphae. *Ascomatal necks*

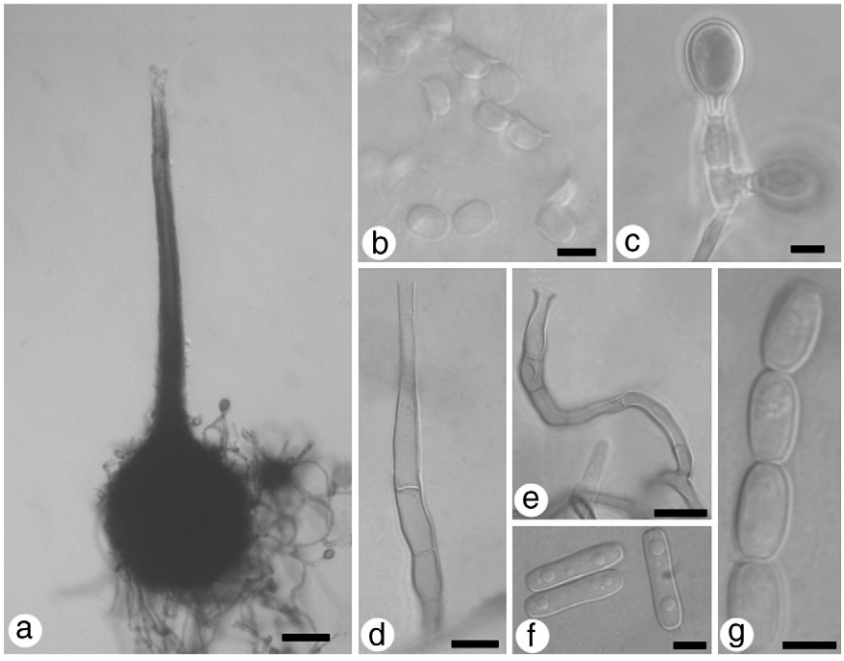

Fig. 5. Morphological characteristics of *Ceratocystis acaciivora sp.nov.* (CMW22563). (a) Globose ascomata with long neck; (b) hat-shaped ascospores in side view; (c) Chlamydospores; (d) primary phialides; (e) secondary phialides; (f) cylindrical conidia; (g) barrel-shaped conidia. Scale bars a = 50 μm; d–e = 10 μm; b, c, f, g = 5 μm.

dark brown to black becoming lighter toward the apexes, (301–) 348–448 (−522) μm long, (25−) 33–45 (−53) μm wide at the base, (11−) 13–17 (−20) μm wide at the apex. *Ostiolar hyphae* divergent, hyaline, (30−) 35–49 (−60) μm long. *Asci* not observed. *Ascospores* accumulating in buff-yellow (19d) mucilaginous masses at the apices of ascomatal necks. Ascospores cucullate in side view, aseptate, hyaline, invested in sheath, 5–7 × 3–4 μm with sheath, 4–6 × 3–4 μm without sheath.

Thielaviopsis anamorph: *primary conidiophores* occurring singly on mycelium, hyaline, swollen at the bases, tapering toward the apexes, (53−) 61–95 (−127) μm long, 4–6 μm wide at the bases, (2−) 5–7 (−8) μm wide at the middle, 3–6 μm wide at the apices. *Secondary conidiophores* occurring singly on mycelium, hyaline (52−) 57–69 (−68) μm long, (3−) 2–4 (−5) μm wide at the bases, 4–6 μm wide at the apexes. *Conidia* of two types: primary conidia hyaline, aseptate, cylindrical (11−) 14–22 (−29) × 3–5 μm, secondary conidia hyaline, aseptate, barrel-shaped (8−) 9–11 (−13) × 4–6 μm. *Chlamydospores* oval, thick walled, smooth, argus brown (13 m), (10−) 12–14 (−15) × (7−) 8–12 (−14) μm, embedded in agar, formed singly or in chains, terminally.

Additional specimens examined: Indonesia: Sumatra, isolated from *A. mangium,* 2005, M. Tarigan (culture CMW22564, PREM59885; CMW22621, PREM59886).

5. Discussion

This study presents the first report of *Ceratocystis* spp. associated with a serious disease of young *A. mangium* trees, which develop after pruning of these trees in the Riau area, Sumatra. Two species were consistently associated with diseased trees of which one was described as *C. acaciivora sp. nov.*, and the other was indistinguishable from *C. manginecans*. Both these fungi were shown to be pathogenic on inoculated trees in greenhouse and field inoculations.

Based on DNA sequence comparisons and morphological differences, one group of the *Ceratocystis* isolates collected from dying *A. mangium* trees, as well as from artificially wounded *A. mangium*, was shown to represent a distinct and previously undescribed species. Its closest phylogenetic neighbour was shown to be *C. manginecans* based on ITS, BT and TEF sequence data. When compared with *C. manginecans*, *C. acaciivora* isolates differed in having smaller ascomata and shorter ascomatal necks. The primary and secondary conidiophores of *C. acaciivora* were also found to be shorter than those of *C. manginecans* and the secondary conidiophores of *C. acaciivora* were much narrower at their bases than those of *C. manginecans*.

One group of isolates (Group 2) from *A. mangium* and *A. crassicarpa* was indistinguishable from *C. manginecans*, a serious pathogen of mango trees in Oman and Pakistan (Van Wyk et al., 2007). There were no significant morphological differences between the *Acacia* isolates and *C. manginecans* and only minor sequence differences (3 bp different in ITS and 2 bp different in TEF) were found between them.

Stem wounds such as those associated with pruning appear to be an important component of the disease of *A. mangium* found in this study. Pruning of these trees during the first year of growth to reduce branching and improve stem straightness is a routine procedure (Beadle et al., 2007). These wounds are visited by insects such as nitidulid beetles that are known to carry *Ceratocystis* spp. (Appel et al., 1990; Hayslett et al., 2005; Heath et al., 2009) and it is probable that *C. manginecans* and *C. acaciivora* are carried to fresh wounds after pruning. *A. mangium* is known to be highly sensitive to wounding and infection by *C. manginecans* and *C. acaciivora* appear to lead to rapid tree death. The fact that a similar disease is not seen in *A. crassicarpa*, which based on inoculation studies are equally susceptible, might be explained by the fact that this tree has a greater capacity to sustain wounding than does *A. mangium*.

All dying trees from which *C. manginecans* and *C. acaciivora* were isolated were those of *A. mangium* and the disease was not seen on *A. crassicarpa*. Because both species are grown in plantations in close proximity, it was considered interesting to determine their relative susceptibility to infection by *C. manginecans* and *C. acaciivora* in artificial inoculations. These tests showed that both species are equally susceptible to infection. In a recent survey, *C. acaciivora* was found on dying *A. mangium* in the absence of pruning wounds, but where wood boring beetles had infested stems (Wingfield, unpublished). It is possible that these insects are more closely attracted to *A. mangium* than *A. crassicarpa* and that this difference could be an additional reason for the absence of disease on the former species.

The discovery of a fungus that has been identified as *C. manginecans* on *Acacia* trees in Sumatra is interesting. This fungus is known as a serious pathogen of mango in Oman and Pakistan (Van Wyk et al., 2007) and this is the first report of the fungus on a host other than mango. It remains possible that the fungus from *Acacia* represents a distinct, but closely related species to *C. manginecans*, although it is not currently possible to resolve this question. Further studies are needed to resolve the taxonomic placement of isolates from *A. mangium* in Indonesia and to determine the origin of both the *Ceratocystis* spp. found in this study.

The disease of *A. mangium* that gave rise to this study is serious and management options to reduce its incidence are required. Clearly, pruning wounds are an important factor in disease development and the negative impact of pruning will require careful consideration. The fact that *C. acaciivora* has also recently been found on dying trees that have not been pruned (Wingfield, unpublished) requires further investigation. Here, the role of wood boring insects as vectors of this pathogen will need to be understood. Yet *C. manginecans* and *C. acaciivora* are an aggressive pathogens and a deeper understanding of their role in tree death will be important in the future.

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References

Anonymous, 2004. Executive Summary. Current Condition of Forestry Sector Development. Statement on Progress Towards Implementing Sustainable Forest Management. The Mid-Term CGI Meeting held in Jakarta. 2 June 2004. Ministry of Forestry of Indonesia, Jakarta, Indonesia.

Appel, D.N., Kurdyla, T., Lewis Jr., R., 1990. Nitidulids as vectors of the oak wilt fungus and other *Ceratocystis* spp. in Texas. Forest Pathology 20, 412–417.

Barker, F.K., Lutzoni, F.M., 2002. The utility of the incongruence length difference test. Systematic Biology 51, 625–637.

Barr, C., 2001. Banking on Sustainability: Structural Adjustment and Forestry Reform in post-Suharto Indonesia. Center for International Forestry Research (CIFOR) & WWF Macroeconomics for Sustainable Development Program Office, Bogor, Indonesia.

Beadle, C., Barry, K., Hardiyanto, E., Irianto, R.J., Mohammed, C., Rimbawanto, A., 2007. Effect of pruning *Acacia mangium* on growth, form and heart rot. Forest Ecology and Management 238, 261–267.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14, 733–740.

Doran, J.C., Skelton, D.J., 1982. *Acacia mangium* seed collections for international provenance trials. Food and Agriculture Organization, Rome, Italy. Forest Genetics Resource Information 11, 47–53.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. Applied and Environmental Microbiology 61, 1323–1330.

Harrington, T.C., Thorpe, D.J., Marinho, V.L.A., Furtado, E.L., 2005. First report of black rot of *Colocasia esculenta* caused by *Ceratocystis fimbriata* in Brazil. Fitopatologia Brasileira 30, 88–89.

Hayslett, M., Juzwik, J., Camilli, K., Appel, D., 2005. Frequencies of *Ceratocystis fagacearum* contaminated nitidulid beetle species in wounds on live and red oaks in Texas. Phytopathology 95 (Supplement no.6).

Heath, R.N., Van Wyk, M., Wingfield, M.J., Roux, J., 2009. Insect associates of *Ceratocystis albifundus* in South Africa and patterns of association in a native savannae ecosystem. Environmental Entomology 38, 356–364.

Jacobs, K., Bergdahl, D.R., Wingfield, M.J., Halik, S., Seifert, K.A., Bright, D.E., Wingfield, B.D., 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycological Research 108, 411–418.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059–3066.

Kile, G.A., 1993. Plant diseases caused by species of *Ceratocystis* sensu stricto and *Chalara*. In: Wingfield, M.J., Seifert, K.A., Webber, J.F. (Eds.), *Ceratocystis* and *Ophiostoma:* Taxonomy, Ecology and Pathogenicity. The American Phytopathology Society, St. Paul, Minnesota, pp. 173–183.

Leefmans, S., 1934. Diseases and pests of cultivated crops in the Dutch East Indies in 1931. Mededelingen van het Institut voor Plantziekten Onderzoek 82.

Marin, M., Castro, B., Gaitan, A., Preisig, O., Wingfield, B.D., Wingfield, M.J., 2003. Relationship of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. Phytopathology 151, 395–405.

Moller, W.J., De Vay, J.E., 1968. Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. Phytopathology 58, 1499–1508.

Moran, G.F., Muona, O., Bell, J.C., 1989. Breeding systems and genetic diversity in *Acacia auriculiformis* and *A. crassicarpa*. Biotropica 23, 250–256.

Morris, M.J., Wingfield, M.J., De Beer, C., 1993. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathology 42, 814–817.

Muchovej, J.J., Albuquerque, F.C., Ribeiro, G.T., 1978. *Gmelina arborea* — a new host of *Ceratocystis fimbriata*. Plant Disease Reporter 62, 717–719.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Old, K.M., Lee, S.S., Sharma, J.K., Yuan, Z.Q., 2000. A manual of diseases of tropical Acacias in Australia, South-East Asia and India. Center for International Forestry Research, Bogor, Indonesia, p. 104.

Potter, K., Rimbawanto, A., Beadle, C., 2006. Heart rot and root rot in tropical *Acacia* plantations. Proceedings of a Workshop Held in Yogyakarta, Indonesia. 7–9 February 2006. Canberra, ACIAR Proceedings No. 124.

Ploetz, R.C., 2003. Diseases of mango. In: Ploetz, R.C. (Ed.), Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, Oxford, pp. 327–363.

Pontis, R.E., 1951. A canker disease of the coffee tree in Columbia and Venezuela. Phytopathology 41, 178–184.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Rayner, R.W., 1970. A mycological colour chart. Commonwealth Mycological Institute Kew, Surrey & British Mycological Society.

Ribeiro, I.J.A., 1980. Seca de manguera. Agentes causais e studio da molesta. Anais do I Simposio Brasiliero Sobre a Cultura de Mangeura, November 24 – 28. Sociedad Brasileira de Fruticultura, Jacoticobal, pp. 123–130.

Ribeiro, I.J.A., Ito, M.F., Filho, O.P., De Castro, J.P., 1988. Gomose da *Acacia*-negra causada por *Ceratocystis fimbriata* Ell. & Halst. Bragantia Campinas 47, 71–74.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Roux, J., Wingfield, M.J., 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management 99, 327–336.

Roux, J., Dunlop, R., Wingfield, M.J., 1999. Susceptibility of elite *Acacia mearnsii* families to *Ceratocystis* wilt in South Africa. Journal of Forestry Research 4, 187–190.

Roux, J., Wingfield, M.J., Bouillett, J.P., Wingfield, B.D., Alfenas, A.C., 2000. A serious new disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. Forest Pathology 30, 175–184.

Roux, J., Wingfield, M.J., 2009. *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. Southern Forests: a Journal of Forest Science 71, 115–120.

SAS Institute, 2001. SAS Statistical Software, SAS/STAT Users Guide, Version 8.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.

Tarigan, M., Van Wyk, M., Roux, J., Tjahjono, B., Wingfield, M.J., 2010. Three new *Ceratocystis* spp. in the *Ceratocysis moniliformis* complex from wounds on *Acacia mangium* and *A. crassicarpa*. Mycoscience 51, 53–67.

Tayler, V.A., Stephens, J., 1929. Native Rubber in the Dutch East Indies. In Report to the Rubber Growers' Association. Rubber Growers Association, London, UK.

Turnbull, J.W., 1986. Multipurpose Australian trees and shrubs. Australian Centre for International Agricultural Research, Canberra, Australia.

Van Wyk, M., Roux, J., Barnes, I., Wingfield, B.D., Liew, E.C.W., Assa, B., Summerell, B.A., Wingfield, M.J., 2004. *Ceratocystis polychroma* sp. nov., a new species from *Syzygium aromaticum* in Sulawesi. Studies in Mycology 50, 273–282.

Van Wyk, M., Roux, J., Barnes, I., Wingfield, B.D., Wingfield, M.J., 2006a. Molecular phylogeny of the *Ceratocystis moniliformis* complex and description of *C. tribiliformis* sp. nov. Fungal Diversity 21, 181–201.

Van Wyk, M., Van der Merwe, N.A., Roux, J., Wingfield, B.D., Kamgan, G.N., Wingfield, M.J., 2006b. Population genetic analyses suggest that the *Eucalyptus* fungal pathogen *Ceratocystis fimbriata* has been introduced into South Africa. South African Journal of Science 102, 259–263.

Van Wyk, M., Al Adawi, A.O., Khan, I.A., Deadman, M.L., Al Jahwari, A.A., Wingfield, B.D., Ploetz, R., Wingfield, M.J., 2007. *Ceratocystis mangine-cans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fungal Diversity 27, 213–230.

Viegas, A.P., 1960. Mango blight. Bragantia 19, 163 – 182 (abstracted in Revue of Applied Mycology 42, 696.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and Direct Sequencing Of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A sequencing guide to methods and applications. Academic Press, San Diego, CA, pp. 315–322.

Wingfield, M.J., De Beer, C., Visser, C., Wingfield, B.D., 1996. A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. Systematic and Applied Microbiology 19, 191–202.

Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T.A., Wingfield, B.D., Roux, J., 2008. Eucalypt pests and diseases: growing threats to plantation productivity. Southern Forests: a Journal of Forest Science 70, 139–144.

Wright, W.H., 1925. The modern aspects of disease control. Indian Rubber Journal 628–631.

Zimmerman, A., 1900. Ueber den krebs von *Coffea arabica*, verursacht durch *Rostrella coffeae* gen. et sp. n. Mededelengin uit's Lands Plantetuin 37, 24–62.

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