

Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia

by Ahmad Muslim

Submission date: 09-Nov-2021 10:21AM (UTC+0700)

Submission ID: 1697367358

File name: host_plant_of_Ceratocystis_wilt_in_South_Sumatra,_Indonesia.pdf (1.53M)

Word count: 2842

Character count: 15497



Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia

Rahmat Pratama¹ · Ahmad Muslim² · Suwandi Suwandi² · Nurhayati Damiri² · Soleha Soleha¹

Received: 22 February 2021 / Accepted: 4 August 2021
© Australasian Plant Pathology Society Inc. 2021

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated β -tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata* sensu stricto. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords Sudden death disease · Moraceae · *Ceratocystis fimbriata* sensu stricto

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as “Nangka”, and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affecting the sapwood from the basal stem to the branches. Leaves of dying trees had yellowing symptoms, followed by wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascumal bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5 μ m (Fig. 2a). Ascumata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 μ m long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2 μ m (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4 μ m (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9 μ m (Fig. 2f). Chlamydo-spores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9 μ m (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection

✉ Ahmad Muslim
a_muslim@unsri.ac.id

¹ Agriculture Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya, Jl. Padang Selasa No. 524, Bukit Besar, Palembang, South Sumatra 30139, Indonesia

² Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra 30662, Indonesia

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus*: **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days



for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as accessions ICBB9852 and ICBB9853.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β -tubulin (β t) gene with primers β t1a and β t1b (Glass and Donaldson 1995). Amplifications were carried out in 50 μ l reactions containing 20 μ l DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase 11 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 μ l of each forward and reverse primer, 4 μ l of DNA template and 23 μ l sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial

denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β -tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410; MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β -tubulin. β -tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) (Table 1) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014), Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

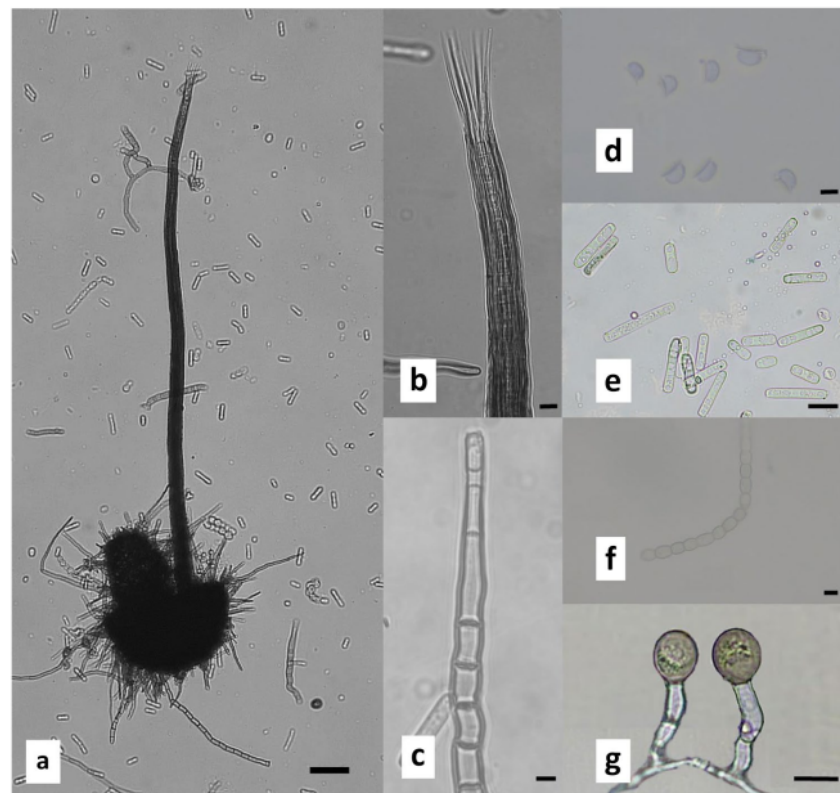
Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β -Tubulin
<i>C. fimbriata</i>	ITS1a	C1418	<i>Ipomoea batatas</i>	USA	AY157956	–
	ITS1	C1857	<i>Morus carica</i>	Brazil	HQ157542	–
	ITS1b	CMW4797	<i>Eucalyptus</i> sp.	Congo	FJ236733	–
	ITSb	CMW9998	<i>Eucalyptus</i> sp.	South Africa	FJ236721	–
	ITS2	C1655	<i>Mangifera indica</i>	Brazil	HQ157546	–
	ITS3	C1440	<i>Eucalyptus</i> sp.	Brazil	HQ157544	–
	ITS3	CMW5328	<i>E. grandis</i>	Uganda	AF395686	–
	ITS4	C1442	<i>Eucalyptus</i> sp.	Brazil	HQ157545	–
	ITS5	ICBB9852	<i>Artocarpus heterophyllus</i>	Indonesia	MT355410	MT412106
	ITS5	ICBB9853	<i>A. heterophyllus</i>	Indonesia	MT355412	MT412108
	ITS5	CAAW30817	<i>A. heterophyllus</i>	Indonesia	MT355413	MT412109
	ITS5	CMW38737	<i>E. grandis</i>	Zimbabwe	KF878326	KF878335
	ITS5	C1345	<i>Eucalyptus</i> sp.	Brazil	AY157966	–
	ITS5	A59662	<i>Camellia sinensis</i>	China	KF650948	–
	ITS5	YM061	<i>Colocasia esculenta</i>	China	AM712445	–
	ITS5	P20053	<i>Punica granatum</i>	China	AM292204	–
	ITS5	C1	<i>Acacia</i> sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	<i>A. mangium</i>	Indonesia	EU588656	EU588636
	ITS5	WRC	<i>Lansium domesticum</i>	Indonesia	MT229127	MW013766
	ITS6	C2055	<i>Mangifera</i> sp.	Brazil	HQ157548	–
	ITS6z	CMW13582	<i>Hypocryphalus mangifera</i>	Oman	KC261853	–
	ITS6z	WBC	<i>L. domesticum</i>	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	<i>M. indica</i>	Oman	AY953383	EF433308
	ITS7b	CMW23634	<i>M. indica</i>	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	<i>A. mangium</i>	Indonesia	EU588658	–
	ITS8a	CMW8856	<i>Citrus</i> sp.	Colombia	AY233867	–
	ITS8c	CMW17808	<i>Eucalyptus</i> sp.	Colombia	EF127990	–
	ITS8e	CMW22092	<i>E. deglupta</i>	Ecuador	FJ151432	–
	ITS9	C1558	<i>M. indica</i>	Brazil	AY157965	–
	ITS9	C1914	<i>C. esculenta</i>	Brazil	HQ157540	–
	ITS10	C994	<i>M. indica</i>	Brazil	AY157964	–
	ITS10a	Cf4	<i>M. indica</i>	Brazil	EF042605	–
ITS11	C1865	<i>C. esculenta</i>	Brazil	AY526286	–	
ITS12	C1926	<i>C. esculenta</i>	Brazil	HQ157541	–	
ITS14	C1688	<i>M. indica</i>	Brazil	AY526291	–	
ITS15	C925	<i>Gmelina arborea</i>	Brazil	AY157967	–	
ITS16	C924	<i>G. arborea</i>	Brazil	HQ157539	–	
<i>C. pirilliformis</i>	Asian clade (AC)	CMW6569	<i>E. nitens</i>	Australia	–	DQ371652
	AC	CMW6579	<i>E. nitens</i>	Australia	–	DQ371653
<i>C. polychroma</i>	AC	CMW11424	<i>Syzygium aromaticum</i>	Indonesia	–	AY528966
	AC	CMW11436	<i>S. aromaticum</i>	Indonesia	–	AY528967
<i>C. atrox</i>	AC	CMW19383	<i>E. grandis</i>	Australia	–	EF070430
	AC	CMW19385	<i>E. grandis</i>	Australia	–	EF070431
<i>C. neglecta</i>	Latin American clade (LAC)	CMW17808	<i>E. grandis</i>	Colombia	–	EU881898
	LAC	CMW18194	<i>E. grandis</i>	Colombia	–	EU881899
<i>C. colombiana</i>	LAC	CMW5751	<i>Coffea arabica</i>	Colombia	–	AY177225
	LAC	CMW5761	<i>C. arabica</i>	Colombia	–	AY177224
<i>C. cacaofunesta</i>	LAC	CMW14803	<i>Theobroma cacao</i>	Ecuador	–	KJ631108

Table 1 (continued)

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β -Tubulin
<i>C. papillata</i>	LAC	CMW15051	<i>T. cacao</i>	Costa Rica	–	KJ601510
	LAC	CMW8850	<i>Citrus</i> \times <i>Tangelo hybrid</i>	Colombia	–	AY233875
<i>C. fimbriata</i>	LAC	CMW8856	<i>Citrus limon</i>	Colombia	–	AY233874
	LAC	CMW14797	<i>M. indica</i>	Brazil	–	EF433307
<i>C. fimbriatomina</i>	LAC	CMW28907	<i>M. indica</i>	Brazil	–	FJ200270
	LAC	CMW1547	<i>I. batatas</i>	Papua New Guinea	–	EF070443
	LAC	C1421	<i>I. batatas</i>	USA	–	KF302689
<i>C. fimbriata</i>	LAC	CMW24174	<i>Eucalyptus hybrid</i>	Venezuela	–	EF190951
	LAC	CMW24176	<i>Eucalyptus hybrid</i>	Venezuela	–	EF190952
<i>C. fimbriata</i>	LAC	CMW21127	<i>A. crassicaarpa</i>	Indonesia	–	EU588643
	LAC	CMW24664	<i>Eucalyptus hybrid</i>	China	–	JQ862720
	LAC	CBS115173	<i>Gmelina arborea</i>	Brazil	–	KF302700
	LAC	CBS14653	<i>C. arabica</i>	Suriname	–	KF302702
<i>C. platani</i>	LAC	CMW14802	<i>Platanus occidentalis</i>	USA	–	EF070425
	LAC	CMW23450	<i>P. occidentalis</i>	Greece	–	KJ601513

Isolates from jackfruit in Indonesia are marked in bold

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a** ascomata with pirilliform base, **b** divergent ostiolar hyphae; **c** conidiophore/phialide; **d** hat-shaped ascospores; **e** cylindrical conidia; **f** Chain of barrel-shaped conidia; **g** chlamydospores of various shapes. Scale bars: a = 100 μ m; b–c, e–g = 10 μ m; d = 5 μ m



(MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replicates. The analysis involved 38 (β -tubulin) and 37 (ITS) nucleotide

sequences. All positions containing gaps and missing data were eliminated. There were 408 (β -tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was

used as the out-group. β -tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata* sensu lato (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITSS5 haplotype of *C. fimbriata* sensu stricto (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β -tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Garra et al. (1997) using five-month-old *A. heterophyllum* seedlings with stem diameters of 6–8 mm and heights < 1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of 6 seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm

diam.) taken from an actively growing colony of *C. fimbriata* on 6% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing inwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions at the inoculation site on the wood (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length of discoloration (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

This is the first report of *C. fimbriata* causing wilt and dieback in Jackfruit in Indonesia and worldwide. The symptoms

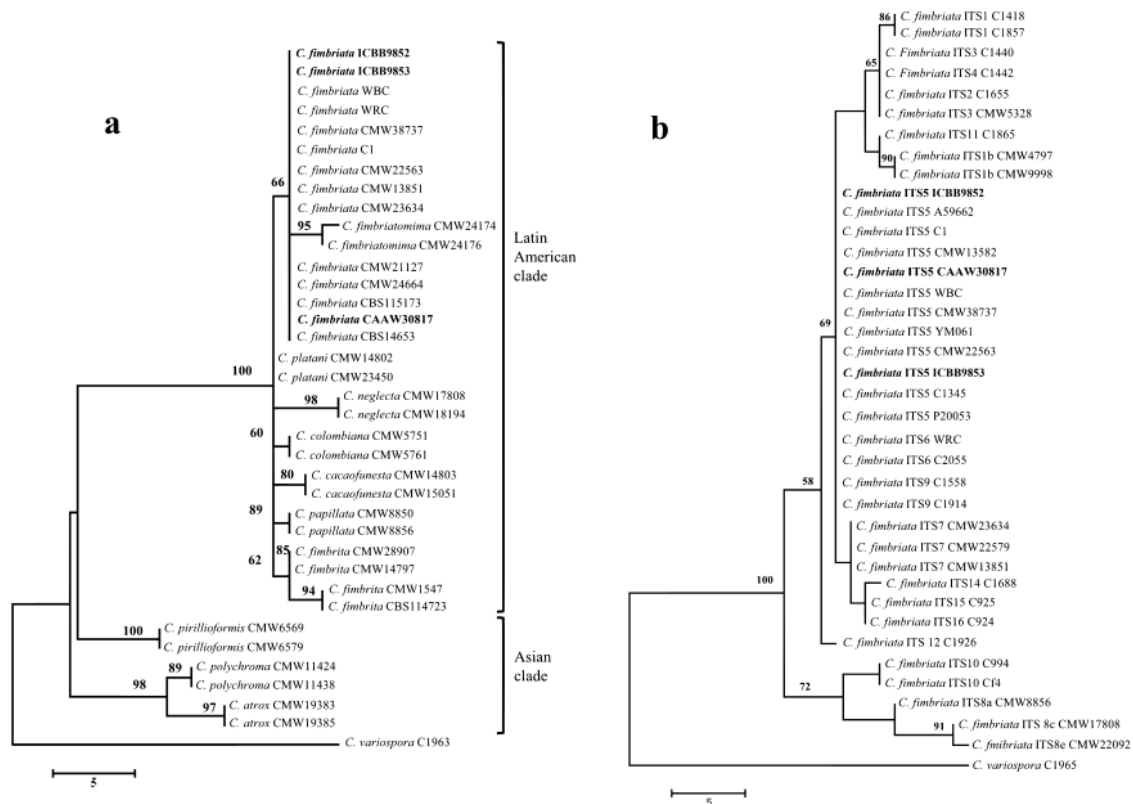
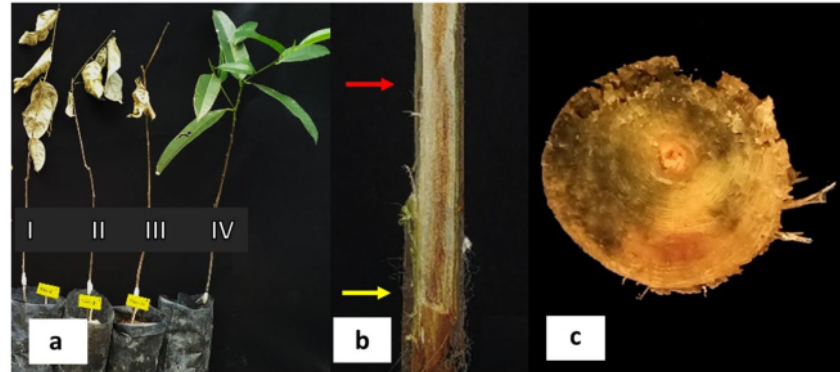


Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a** β -tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American

and Asian clade of the *C. fimbriata* species complex. **b** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata* sensu stricto

Fig. 4 Response of *Artocarpus heterophyllus* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis*. **a** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c** The discoloured wood extended to the heartwood of the basal stem of the seedling



of *C. fimbriata* wilt disease in Jackfruit include cankers on stems, with the stems becoming chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *Ceratocystis fimbriata* is a serious wilt pathogen of jackfruit, as well as of *A. mangium* and *A. crassicaipa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia.

Acknowledgements This research was funded by 10 IDSU scholarship with budget year of 2019–2021 according to the Director of Research and Community Service, Directorate 14 Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

References

- Barnes I, Fourie A, Wingfield MJ, Harrington TC, Mc-New DL, Sugiyama LS, Luiz BC, Heller WP, Keith LM (2018) New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawai'i. *Persoonia* 40:154–181
- Fourie A, Wingfield MJ, Wingfield BD, Barnes I (2015) Molecular markers delimit cryptic species in *Ceratocystis sensu stricto*. *Mycol Prog* 14:1020
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl Environ Microbiol* 61:1323–1330
- Harrington TC, Kazmi MR, Al-Sadi AM, Ismail SI (2014) Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata sensu stricto*. *Mycologia* 106:224–242
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Li Q, Harrington TC, McNew D, Li J, Huang Q, Somasekhara YM, Alfenas AC (2016) Genetic bottlenecks for two populations of *Ceratocystis fimbriata* on sweet potato and pomegranate in China. *Plant Dis* 100:2266–2274
- Moller WJ, DeVay JE (1968) Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology* 58:123–124
- O'Gara E, McComb JA, Colquhoun IL, Hardy GSJ (1997) The infection of non-wounded and wounded periderm tissue at the lower stem of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi*, in a rehabilitated bauxite mine. *Australas Plant Pathol* 26:135–141
- Oliveira LSS, Harrington TC, Ferreira MA, Damacena MB, Al-Sadi AM, Alfenas A-M (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology* 105:1229–1244
- Paul CN, Nam SS, Kachroo A, Kim HY, Yang JW (2018) Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. *Eur J Plant Pathol* 152:7–8
- Pratama R, Muslim A, Suwandi S, Damiri N, Soleha S (2021) First report of bullet wood (*Mimusops elengi*) sudden decline disease caused by *Ceratocystis manginecans* in Indonesia. *Biodiversitas* 22:2636–2645
- Ranasinghe R, Maduwanthi S, Marapana R (2019) Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): a review. *Int J Food Sci* 2019:1–12
- Suwandi S, Irsan C, Hamidson H, Umayah A, Asriyani KD (2021) Identification and characterization of *Ceratocystis fimbriata* causing lethal wilt on the Lansium tree in Indonesia. *Plant Pathol J* 37:124–136
- Tarigan M, Roux J, Van Wyk M, Tjahjono B, Wingfield MJ (2011) A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *S Afr J Bot* 77:292–304
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a sequencing guide to methods and applications*. Academic Press, San Diego, pp 315–322

Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesi

ORIGINALITY REPORT

8%

SIMILARITY INDEX

6%

INTERNET SOURCES

6%

PUBLICATIONS

3%

STUDENT PAPERS

PRIMARY SOURCES

- | | | |
|---|---|----|
| 1 | www.thermofisher.com
Internet Source | 1% |
| 2 | Gryzenhout, M.. "Pathogenicity of <i>Cryphonectria eucalypti</i> to <i>Eucalyptus</i> clones in South Africa", <i>Forest Ecology and Management</i> , 20030317
Publication | 1% |
| 3 | dspace.uevora.pt
Internet Source | 1% |
| 4 | mycokeys.pensoft.net
Internet Source | 1% |
| 5 | www.hindawi.com
Internet Source | 1% |
| 6 | Grace Nakabonge, Marieka Gryzenhout, Jolanda Roux, Brenda D. Wingfield, Michael J. Wingfield. "Celoporthe dispersa gen. et sp. nov. from native Myrtales in South Africa", <i>Studies in Mycology</i> , 2006
Publication | 1% |

7	doi.org Internet Source	1 %
8	www.kolombo.be Internet Source	1 %
9	zenodo.org Internet Source	1 %
10	Achmad Anggawirya Alimin, Bambang Heru Susanto. "Simulation of a bioavtur production process from non-edible vegetable oil", E3S Web of Conferences, 2018 Publication	1 %
11	Submitted to University of Greenwich Student Paper	1 %
12	Submitted to University of Pretoria Student Paper	1 %
13	repository.unsri.ac.id Internet Source	1 %
14	trj.stptrisakti.ac.id Internet Source	1 %

Exclude quotes On

Exclude matches < 1%

Exclude bibliography On