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Judul Artikel : Jackfruit (*Artocarpus heterophyllus*), a new host plant of Ceratocystis wilt in South Sumatra, Indonesia
Jurnal : Australasian Plant Disease Notes, 2021, volume 16(24), 1-6
Penulis : Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha

NO	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit	22 Februari 2021
2	Bukti konfirmasi review dan hasil review pertama	17 Mei 2021
3	Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit	24 Mei 2021
4	Bukti konfirmasi review dan hasil review kedua	12 Juni 2021
5	Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit	14 Juni 2022
6	Bukti konfirmasi review dan hasil review ketiga	18 Juni 2021
7	Bukti konfirmasi submit revisi ketiga, respon kepada reviewer, dan artikel yang diresubmit	14 Juli 2021
8	Bukti konfirmasi review dan hasil review keempat	25 Juli 2021
9	Bukti konfirmasi submit revisi keempat, respon kepada reviewer, dan artikel yang diresubmit	31 Juli 2021
10	Bukti konfirmasi accepted	5 Agustus 2021
11	Bukti konfirmasi dan hasil proof corrections	27 Agustus 2021
12	Bukti konfirmasi artikel published online	11 September 2021

1.Bukti konfirmasi submit artikel dan artikel yang disubmit pertama (22 Februari 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Thank you for your approval - [EMID:040bb87487b633bd]

APDN <em@editorialmanager.com> Mon, Feb 22, 2021 at 8:26 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

Thank you for approving the changes that we made to your submission entitled "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia".

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is https://www.editorialmanager.com/apdn/.

Thank you for submitting your work to this journal.

Kind regards,

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Submission Confirmation

APDN <em@editorialmanager.com> Thu, Mar 18, 2021 at 5:56 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia", submitted to Australasian Plant Disease Notes

The manuscript number is APDN-D-21-00015R1.

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a. muslim unsri <a_muslim@unsri.ac.id>

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APDN <em@editorialmanager.com> Thu, Mar 18, 2021 at 5:56 AM
Reply-To: APDN <jude.estrera@springernature.com>
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2. Bukti konfirmasi review dan hasil review pertama (17 Mei 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Major Revisions requested APDN-D-21-00015R1

2 messages

APDN <em@editorialmanager.com> Mon, May 17, 2021 at 11:45 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisers on your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" (APDN-D-21-00015R1), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
the Editorial Manager system which can be accessed at:

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Please submit you revised manuscript before 14 Jun 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Reviewer: In this Note, the authors describe for the first time the Ceratocystis wilt on Artocarpus heterophyllus (Jackfruit) in Indonesia. The note needs a major revision for publication. Please, find below my comments:

1. There are currently different approaches on defining the boundaries of species identification on Ceratocystis fimbriata complex. However, many descriptions were based on ITS variation alone and some of them have already been synonymized based on solid studies (Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244). According to the Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244, C. manginecans reported on mango are in fact genotypes of C. fimbriata.
2. The phylogenetic analysis conducted in the current study derived from a poor dataset that did not consider the Ceratocystis species diversity. Therefore, to provide a better characterization of Ceratocystis on Jackfruits, isolates from Latin American Clade (LAC) and Asian-Australian clade (AAC) must be taken into consideration.
3. Regardless the approach used by the authors, relevant publications should not be ignored. References provided do not represent fully the current context considered on Ceratocystis research.
4. In which substrate the plants for inoculation were grown? In which condition the inoculated plants were kept? How was the climate variation during the 45 days after inoculation? How was the plant response in the inoculation point? Did the authors measure the lesion size of inoculated plants? Please provide this information and compare with control plants.
5. Line 10: Avoid repetition of keywords in the title.

6. Line 19-20: Please, provide a better description from symptoms on the woods.
7. Line 49-50: Which primers were used?
8. The writing requires minor revision.

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a. muslim unsri <a_muslim@unsri.ac.id> Mon, May 24, 2021 at 7:33 AM
To: APDN <jude.estrera@springernature.com>

Dear Prof. Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

We are really appreciating the corrections.

We have revised and made some modified corrections as suggested by the reviewer(s).
We will submit revised our manuscript through process review in the Springer System.

We hope we can send you our revision today.

Thank you very much for your kindness and excellent cooperation.

Best regard

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
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3. Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (24 Mei 2021)

Author(s) details

Rahmat Pratama[1] · Ahmad Muslim[2*] · Suwandi Suwandi[2] · Nurhayati Damiri[2] · Soleha Soleha[1]

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. 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*. *C. fimbriata* causing sudden death disease in *A. heterophyllus* is being reported for the first time in Indonesia and worldwide.

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

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown widely in Indonesia and 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 a recent study of jackfruit in 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. Jackfruit trees were reported to die within a period from July to September 2019. 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 affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the 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. Ascomatal bases dark brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7×101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4 to 6.8×2.1 to 6.2 µm (length/width) (Fig. 2d). Bacilliform conidia 11.1 to 36.1×2.1 to 7.4 µm (length/width) (Fig. 2e). Barrel conidia 4.4 to 16.1×2.7 to 6.9 µm (length/width) (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7 to 16.5×5.9 to 12.9 µm (length/width) (Fig. 2g). Based on morphological characters, the fungus was identified as *C. fimbriata.*

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from 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) and part of the β-tubulin (βt) gene. Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl 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 CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) 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) 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 (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 bootstrap replications. 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 ITS5 haplotype of *C. fimbriata sensu stricto* (Fig. 3b).

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. 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 Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *C. fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of 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 chaired by Ahmad Muslim.

References

Al Adawi AO, Barnes I, Khan IA, Al Subhi AM, Al Jahwari AA, Deadman ML, Wingfield BD, Wingfield MJ (2013) *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. Australas Plant Pathol 42:179–193

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

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. Molecular Biology and Evolution 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, Al-Mahmooli HIS Alfenas AC (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. Phytopathology 105:1229-1244

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

Paul CN, Nam SS, Kachroo A, Kim HY and Yang JW (2018) Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. Eur J Plant Pathol: 7-8

Ranasinghe R, Maduwanthi S, Marapana R (2019) Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review. International Journal of Food Science 2019: 1-12

147 Suwandi S, Irsan C, Hamidson H, Umayah A, Asriyani KD (2021) Identification and
148 Characterization of *Ceratocystis fimbriata* Causing Lethal Wilt on the Lansium Tree in
149 Indonesia. Plant Pathol J 37:124-136
150 Tarigan M, Roux J, Van Wyk M, Tjahjono B, Wingfield MJ (2011) A new wilt and die-back
151 disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora*
152 sp. nov. in Indonesia. S Afr J Bot 77:292–304
153 Van Wyk M, Al Adawi AO, Khan IA, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz
154 R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive
155 mango wilt disease in Oman and Pakistan. Fung Div 27: 213–230

Table

156 **Table 1** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	ITS5	**CAAW30268**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

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.

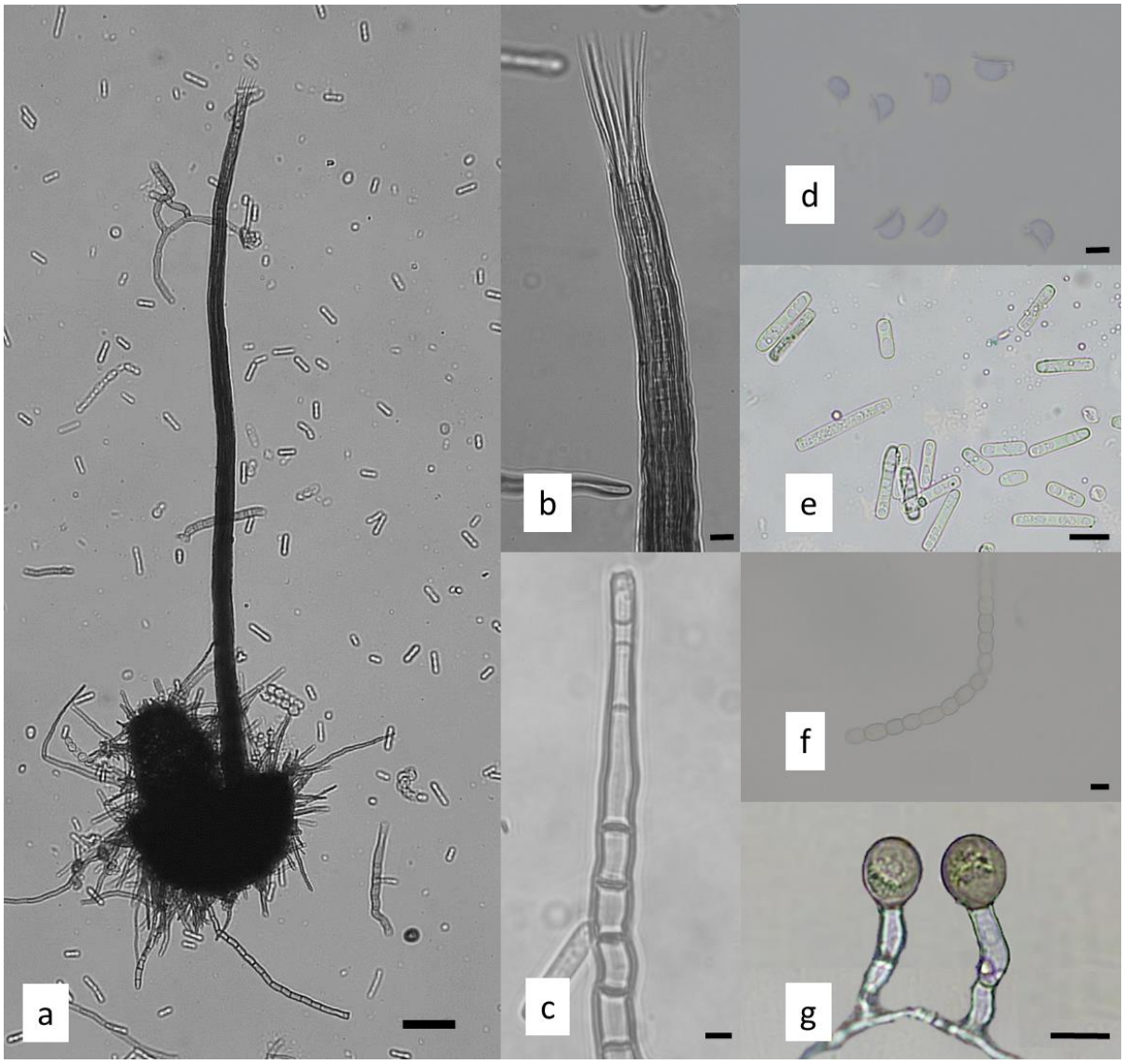

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped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

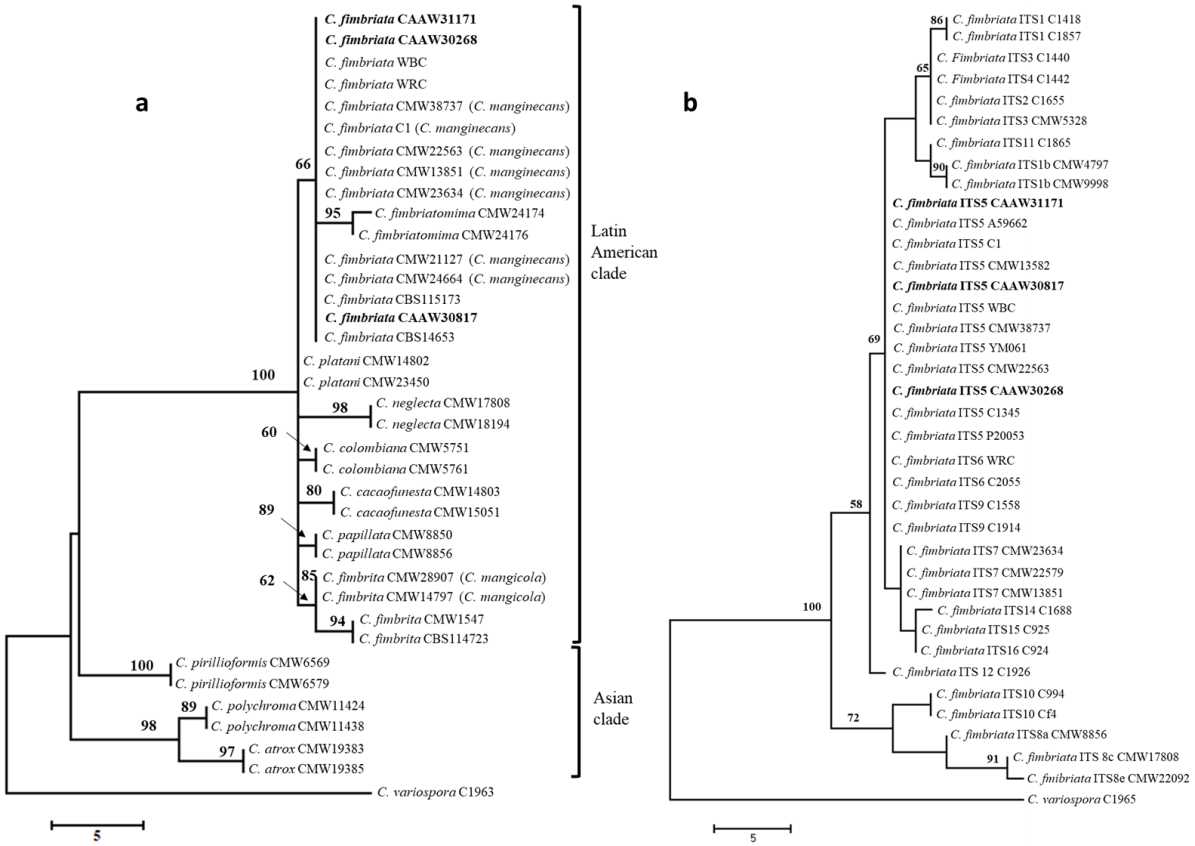

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis of the **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. Species names considered to be synonyms of *C. fimbriata sensu stricto* are in parentheses (Harrington et al. 2014; Oliveira et al. 2015). **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). 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 percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. 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.

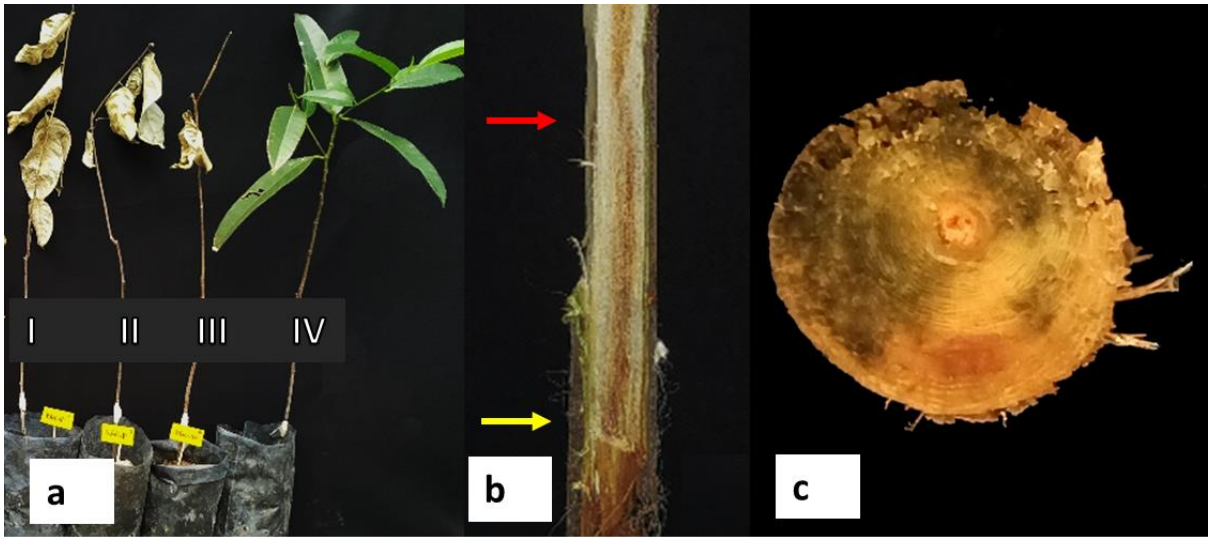

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis*. **a.** total wilting of plant inoculated with CAAW31171 (I), CAAW30817 (II), CAAW30268 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured seedlings wood extended to the heartwood of the basal stem.

Unsri Google Apps

a. muslim unsri <a_muslim@unsri.ac.id>

Submission Confirmation

1 message

APDN <em@editorialmanager.com> Mon, May 24, 2021 at 2:04 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia", submitted to Australasian Plant Disease Notes

The manuscript number is APDN-D-21-00015R2.

You may check the status of your manuscript at any time by accessing the following web site:

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We will inform you of the Editor's decision as soon as possible.

With best regards,
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Springer
P.O. Box 990
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The Netherlands

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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4. Bukti konfirmasi review dan hasil review kedua (12 Juni 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R2

1 message

APDN <em@editorialmanager.com> Sat, Jun 12, 2021 at 12:49 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R2), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
the Editorial Manager system which can be accessed at:

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Please submit you revised manuscript before 09 Jul 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Dear authors
Thank you for uploading the revised manuscript. It seems to me that it is quite better than on its first version. However, I believe that there are still corrections to be done.

line 8-9. Never start a sentence with an abreviation. So, please correct “C. fimbriata” to “Ceratocystis fimbriata”. Alternatively, you can change the last sentence to be read as follows: "This is the first report of C. fimbiriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide.
line 12-13. The first time that a scientific name is presented in the main text, it should not be abbreviated. Please change the phrase "Jackfruit (A. heterophyllus) belongs to the family Moraceae, and it is known in Indonesian as “Nangka”. Jackfruit is grown..." to read as follows: "Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated ..."
line 13. remove "Indonesia and"
line 16. The first sentence mention a recent study of 2019, but fails to present the reference. Please present the literature at the end of the sentence (at line 18).
line 38. replace "subglobes to globes" by "subglobose to globose"
lines 39-45 . Range measurements should be done using en-dashes.
line 39-45. remove "(length/width)"

line 46. The first time that a scientific name is presented in the main text, it should not be abbreviated.
line 48. Replace "Mycelial mat" by "Mycelium"
line 52. As requested by the reviewer, please provide the primers names used on the study. Also provide the references of each primer
line 99. Never start a sentence with an abbreviation
line 175. Replace "b,c,e,f,g" by "b-c, e-g" using en-dashes
line 186. The caption is too long. Lot of the information presented here should be at the main text, and not on the figure caption. Please make it short.
line 186, 187. Replace "of the..." by "of:..."

In accordance with APDN Submission guidelines, it is mandatory the deposition of a pure culture of the pathogen in a culture collection registered by the World Federation for Culture Collections (http://www.wfcc.info/collections). Please provide the culture collections codes, the collection name, and where it is housed.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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5. Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit (14 Juni 2021)

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R3
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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Order of Authors Secondary Information:	
Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. 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.
Response to Reviewers:	June 14, 2021 Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

Comment [1]: line 8-9. Never start a sentence with an abreviation. So, please correct “C. fimbriata” to “Ceratocystis fimbriata”. Alternatively, you can change the last sentence to be read as follows: "This is the first report of C. fimbriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide.
Our response: We agree and change sentence to be "This is the first report of C. fimbriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide”.

Comment [2]: line 12-13. The first time that a scientific name is presented in the main text, it should not be abbreviated. Please change the phrase "Jackfruit (A. heterophyllus) belongs to the family Moraceae, and it is known in Indonesian as “Nangka”. Jackfruit is grown..." to read as follows: "Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated ..."
Our response: Thank you very much. We agree and change sentence to be “Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated”.

Comment [3]: line 13. remove "Indonesia and"
Our response: We agree and removed "Indonesia and" in sentence

Comment [4]: line 16. The first sentence mention a recent study of 2019, but fails to present the reference. Please present the literature at the end of the sentence (at line 18).
Our response:
We agree and change sentence to be “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.”

Comment [5]: line 38. replace "subglobes to globes" by "subglobose to globose"
Our response:
We agree and change sentence to be "subglobose to globose"

Comment [6]: lines 39-45 . Range measurements should be done using en-dashes.
Our response:
Thank you very much. We agree and change sentence to be “measured (n=100), 131.5-250.7×101.6-236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7-579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4-6.8×2.1-6.2 µm (Fig. 2d). Bacilliform conidia 11.1-36.1×2.1-7.4 µm (Fig. 2e). Barrel conidia 4.4-16.1×2.7-6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7-16.5×5.9-12.9 µm (Fig. 2g)”.

Comment [7]: line 39-45. remove "(length/width)"
Our response:
Thank you very much. We agree and remove "(length/width)"

Comment [8]: line 46. The first time that a scientific name is presented in the main text, it should not be abbreviated.
Our response:
We agree and change sentence to be "Ceratocystis fimbriata”

Comment [9]: line 48. Replace "Mycelial mat" by "Mycelium"
Our response:
We agree and replace "Mycelial mat" by "Mycelium"

Comment [10]: line 52. As requested by the reviewer, please provide the primers names used on the study. Also provide the references of each primer
Our response:
We agree and change sentence to be "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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995)”

Comment [11]: line 99. Never start a sentence with an abbreviation
Our response:
We agree and change sentence to be "Ceratocystis fimbriata”

Comment [12]: line 175. Replace "b,c,e,f,g" by "b-c, e-g" using en-dashes
Our response:
We agree and change sentence to be "Scale bars: a = 100 μm; b-c, e-g = 10 μm; d = 5 μm.”

Comment [13]: line 186. The caption is too long. Lot of the information presented here should be at the main text, and not on the figure caption. Please make it short.
Our response:
We agree and change sentence “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. Species names considered to be synonyms of C. fimbriata sensu stricto are in parentheses (Harrington et al. 2014; Oliveira et al. 2015). b. ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the C. fimbriata sensu stricto. The ITS haplotypes of C. fimbriata are numbered following the numerical designations of Harrington et al. (2014). 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 percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. 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.” to be "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.”

Comment [14]: line 186, 187. Replace "of the..." by "of:..."
Our response:
We agree and change sentence to be "analysis by”

Comment [15]: In accordance with APDN Submission guidelines, it is mandatory the deposition of a pure culture of the pathogen in a culture collection registered by the World Federation for Culture Collections (http://www.wfcc.info/collections). Please provide the culture collections codes, the collection name, and where it is housed.
Our response:
Specimens were deposited in the culture collection of the Phytopathology Laboratory of Sriwijaya University (Indralaya, Indonesia) as HPTUnsri-2101. Currently our isolates are being processed for deposit in the Indonesian Culture Collection (InaCC), under the management of Microbiology Division, Research Center for Biology, the Indonesian Institute of Sciences (Cibinong, Indonesia).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia

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Author(s) details

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[1]Agriculture Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia
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*Corresponding Author: a_muslim@unsri.ac.id

Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. 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 affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the 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. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 μm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 μm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 μm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 μm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 μm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 μm (Fig. 2g). Based on morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Specimens were deposited in the culture collection of the Phytopathology Laboratory of Sriwijaya University (Indralaya, Indonesia) as HPTUnsri-2101.

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 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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 μl reactions containing 20 μl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 μl of each forward and reverse primer, 4 μl of DNA template and 23μl 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 CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) 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) 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 (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. 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 ITS5 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'Gara et al. (1997) using Five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. 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 Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the

stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of 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

Al Adawi AO, Barnes I, Khan IA, Al Subhi AM, Al Jahwari AA, Deadman ML, Wingfield BD, Wingfield MJ (2013) *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. Australas Plant Pathol 42:179–193

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. Molecular Biology and Evolution 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, Al-Mahmooli HIS Alfenas AC (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. Phytopathology 105:1229-1244

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

Paul CN, Nam SS, Kachroo A, Kim HY and Yang JW (2018) Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. Eur J Plant Pathol: 7-8

Ranasinghe R, Maduwanthi S, Marapana R (2019) Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review. International Journal of Food Science 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

Van Wyk M, Al Adawi AO, Khan IA, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fung Div 27: 213–230

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

169 **Table 1** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	ITS5	**CAAW30268**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

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.

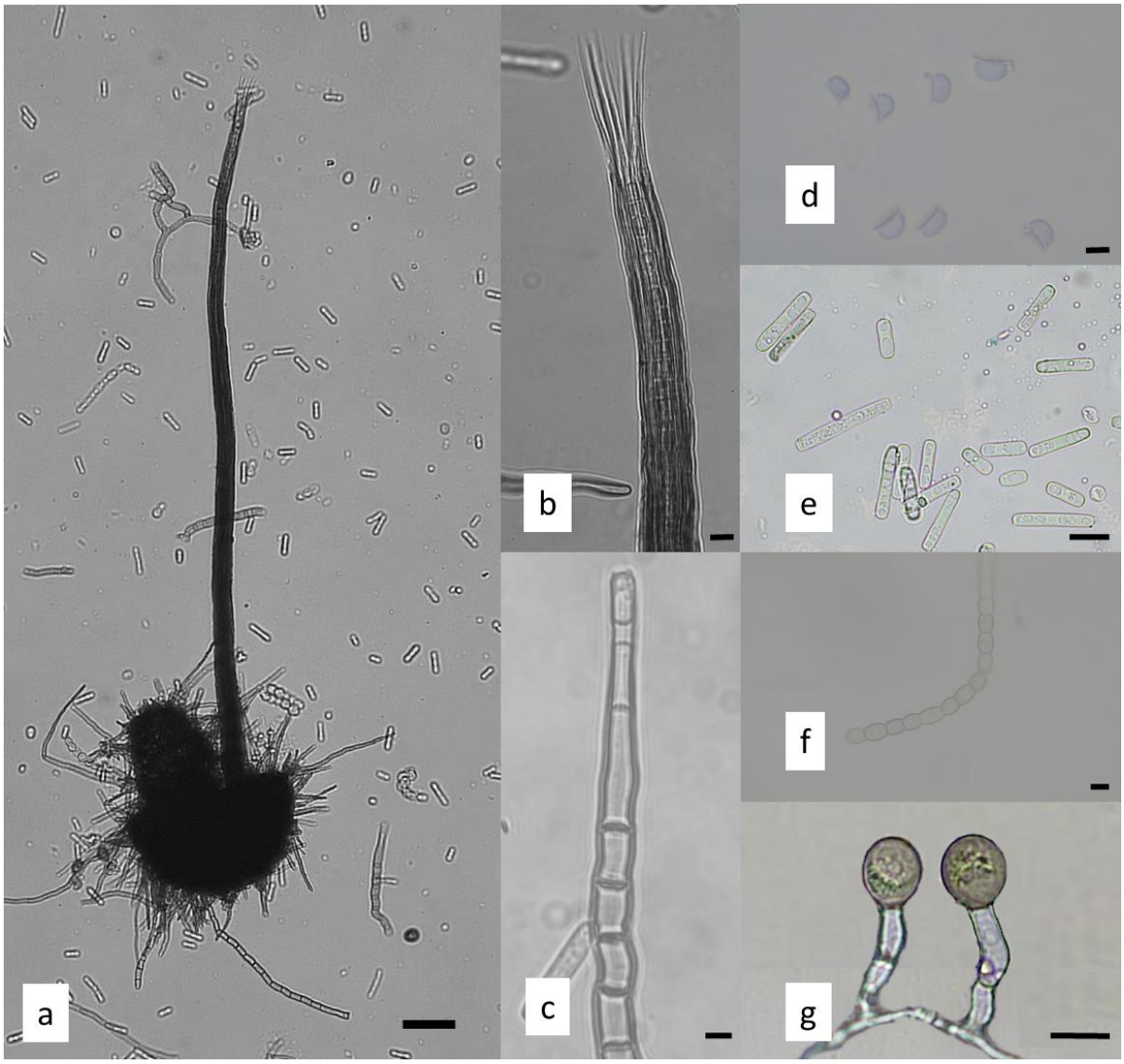

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped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

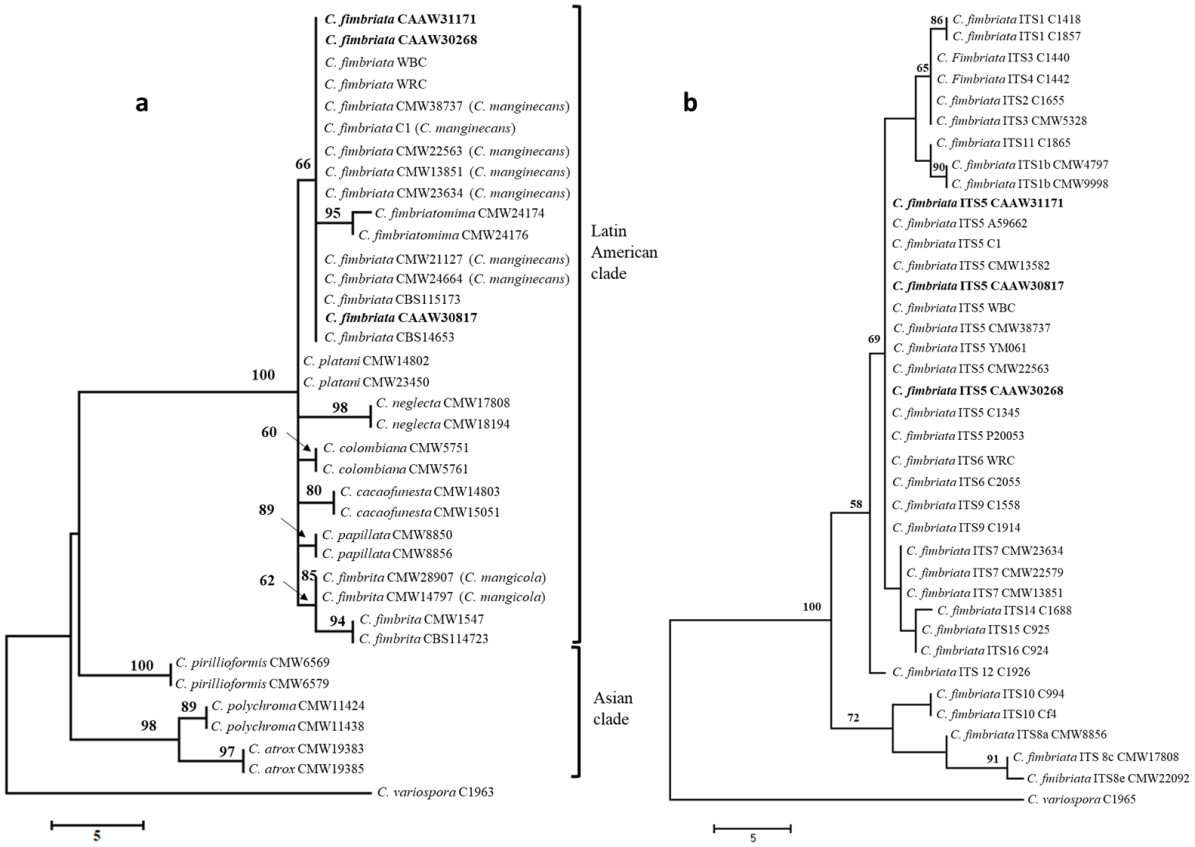

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

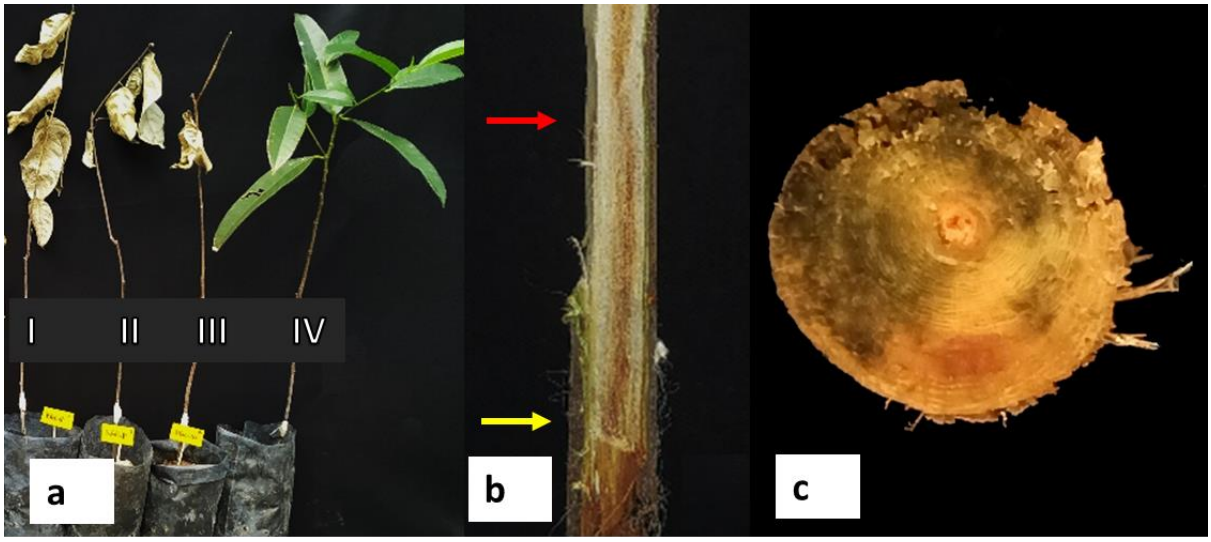

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis*. **a.** total wilting of plant inoculated with CAAW31171 (I), CAAW30817 (II), CAAW30268 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured seedlings wood extended to the heartwood of the basal stem.

a. muslim unsri <a_muslim@unsri.ac.id>

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1 message

APDN <em@editorialmanager.com> Mon, Jun 14, 2021 at 11:29 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

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6. Bukti konfirmasi review dan hasil review ketiga (18 Juni 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R3

3 messages

APDN <em@editorialmanager.com> Fri, Jun 18, 2021 at 12:59 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R3), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
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Please submit you revised manuscript before 15 Jul 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Dear authors
Thank you for submitting the reviewed manuscript.
However, I do not think you understood the question raised regarding the submission into a culture collection.
As postulated in the guidelines, authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. Without these informations, your manuscript cannot be further processed.
When you get the accession codes of InaCC, please add the name of the collection at lines 45-46, and replace the private collection codes by those of InaCC in the text, tables and figures (trees included).
Then, resubmit so we can continue to analyze.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Jun 29, 2021 at 3:38 PM
To: APDN <jude.estrera@springernature.com>

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s)

Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled "Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

Below is a summary of our response to the reviewers' comments.

Comment [1]: authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. please add the name of the collection at lines 45-46, and replace the private collection codes in the text, tables and figures (trees included).
Our response: We agree and specimens were deposited in the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853. Our private collection codes replaced in the text, tables and figures (trees included).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id
[Quoted text hidden]

---

a. muslim unsri <a_muslim@unsri.ac.id> Wed, Jul 14, 2021 at 9:26 AM
To: APDN <jude.estrera@springernature.com>
Cc: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

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Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id

On Fri, Jun 18, 2021 at 1:00 AM APDN <em@editorialmanager.com> wrote:
[Quoted text hidden]

7. Bukti konfirmasi submit revisi ketiga, respon kepada reviewer, dan artikel yang diresubmit (14 Juli 2021)

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R4
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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First Author:	Rahmat Pratama, S.Si
First Author Secondary Information:	
Order of Authors:	Rahmat Pratama, S.Si
	A. Muslim, Ph.D.
	Suwandi Suwandi, PhD
	Nurhayati Damiri, Professor
	Soleha Soleha, S.P
Order of Authors Secondary Information:	
Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. 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.
Response to Reviewers:	June 28, 2021 Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

	Comment [1]: authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. please add the name of the collection at lines 45-46, and replace the private collection codes in the text, tables and figures (trees included). Our response: We agree and specimens were deposited in the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853. Our private collection codes replaced in the text, tables and figures (trees included). We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification. Thank you very much for your consideration of the manuscript and excellent cooperation Yours sincerely, Ahmad Muslim Associate Professor Faculty of Agriculture, Sriwijaya University Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia E-mail: a_muslim@unsri.ac.id

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. 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 affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the 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. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 µm (Fig. 2g). Based on morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as 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 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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl 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) 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 (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. 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 ITS5 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'Gara et al. (1997) using Five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. 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 Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (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 die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

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References

Al Adawi AO, Barnes I, Khan IA, Al Subhi AM, Al Jahwari AA, Deadman ML, Wingfield BD, Wingfield MJ (2013) *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. Australas Plant Pathol 42:179–193

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. Molecular Biology and Evolution 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, Al-Mahmooli HIS Alfenas AC (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. Phytopathology 105:1229-1244

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

Paul CN, Nam SS, Kachroo A, Kim HY and Yang JW (2018) Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. Eur J Plant Pathol: 7-8

Ranasinghe R, Maduwanthi S, Marapana R (2019) Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review. International Journal of Food Science 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

Van Wyk M, Al Adawi AO, Khan IA, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fung Div 27: 213–230

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

Table

Table 1 *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

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.

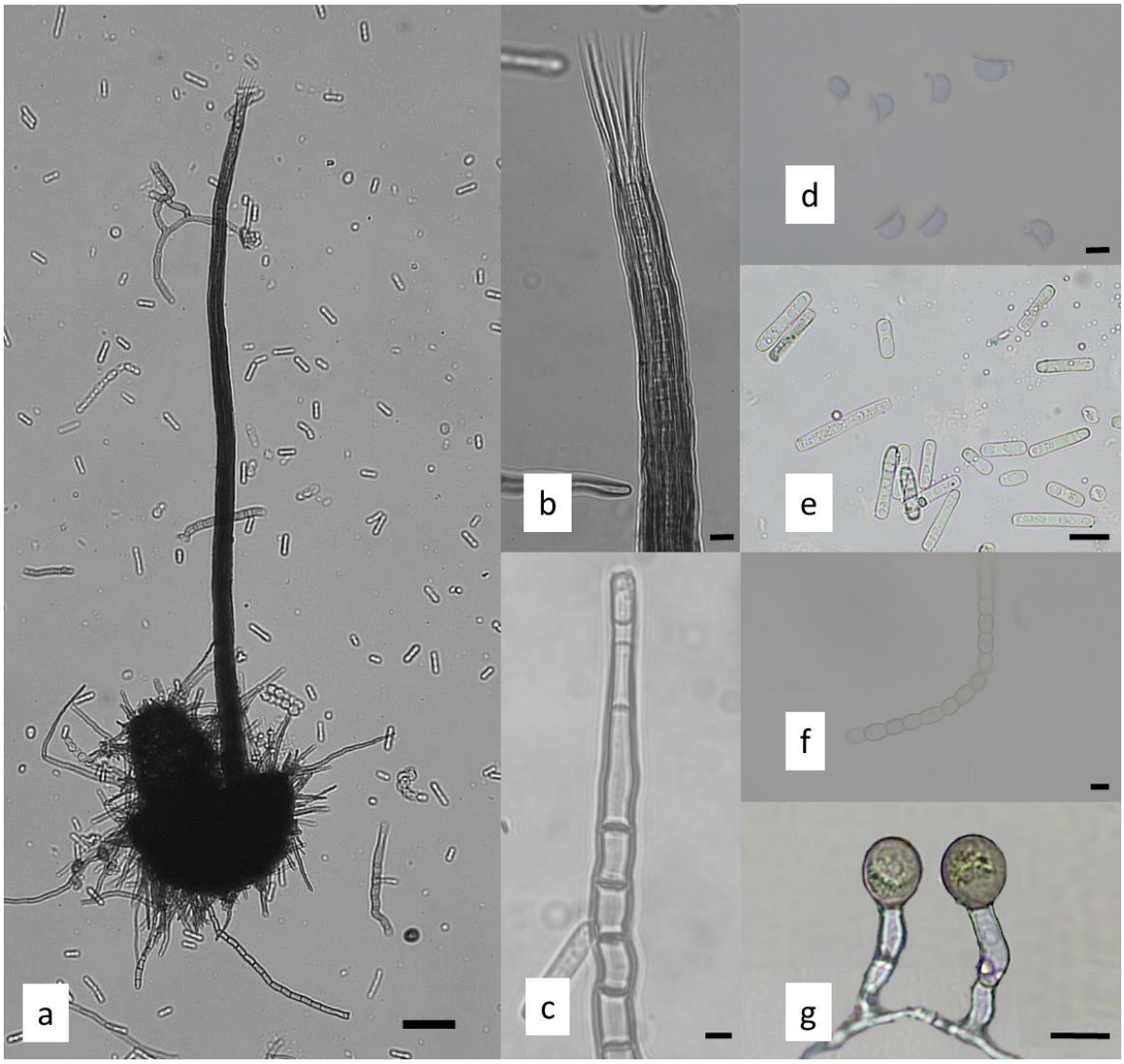

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped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

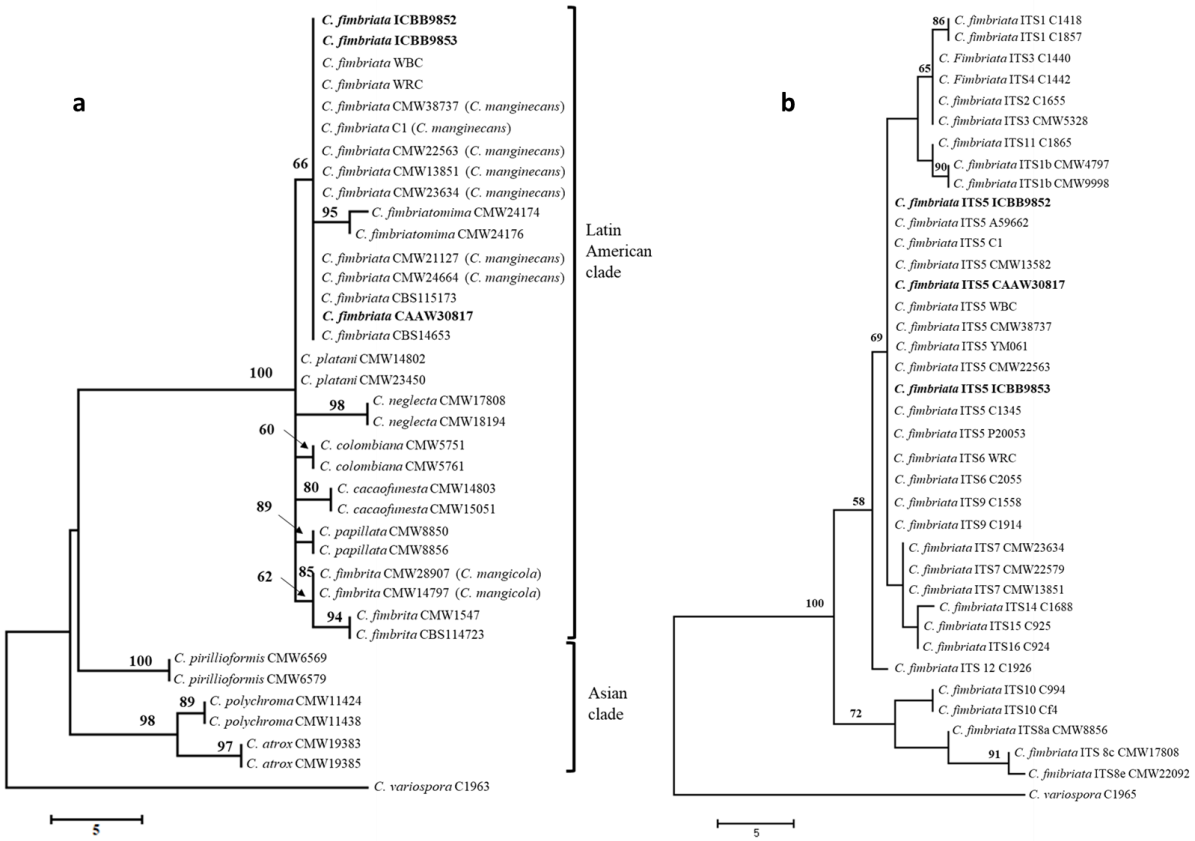

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

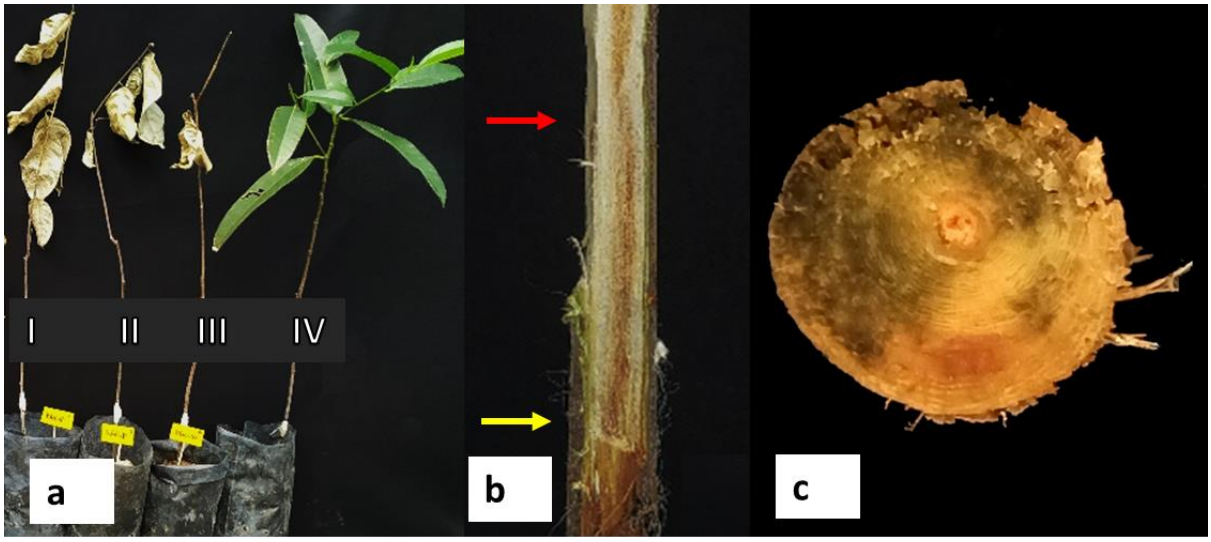

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis.* **a.** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the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

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Re: Your Submission APDN-D-21-00015R3 [APDN] [AU] [REVSUB] [R]

1 message

Jude Estrera <Jude.Estrera@springernature.com> Wed, Jul 14, 2021 at 11:47 PM
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Cc: "dagmar.hanold@adelaide.edu.au" <dagmar.hanold@adelaide.edu.au>, "dhanold@gmail.com" <dhanold@gmail.com>

Dear Dr. Muslim,

Thank you for your email.

This is to confirm that your response to review comments has been uploaded accordingly in the EM. Your paper has been assigned to the Editor and rest assured that it will be process accordingly.

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Subject: Re: Your Submission APDN-D-21-00015R3

[External - Use Caution]

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s)

Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

Below is a summary of our response to the reviewers' comments.

Comment [1]: authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. please add the name of the collection at lines 45-46, and replace the private collection codes in the text, tables and figures (trees included).
Our response: We agree and specimens were deposited in the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853. Our private collection codes replaced in the text, tables and figures (trees included).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
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On Fri, Jun 18, 2021 at 1:00 AM APDN <em@editorialmanager.com> wrote:

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R3), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

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Please submit you revised manuscript before 15 Jul 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Dear authors
Thank you for submitting the reviewed manuscript.
However, I do not think you understood the question raised regarding the submission into a culture collection.
As postulated in the guidelines, authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. Without these informations, your manuscript cannot be further processed.
When you get the accession codes of InaCC, please add the name of the collection at lines 45-46, and replace the private collection codes by those of InaCC in the text, tables and figures (trees included).
Then, resubmit so we can continue to analyze.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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8. Bukti konfirmasi review dan hasil review keempat (25 Juli 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R4

1 message

APDN <em@editorialmanager.com> Sun, Jul 25, 2021 at 4:30 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R4), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
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Please submit you revised manuscript before 20 Aug 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Kerrie Ann Davies, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Having read through your MS, please make the following changes:

Lines 5-6 : delete 'has been noted'. This simply repeats 'were observed', and is not needed.

Line 22: 'affected' should be 'affecting'; and 'until' should be replaced with 'to'

Line 23: delete 'the' between 'by' and 'wilting

Line 43: insert 'these' between 'Based on' and 'morphological'

Line 46: add the word 'accessions' before the numbers on Line 47

Line 50: insert 'the' between 'extracted from' and 'fungal ....'

Line 56: should 1.5 not 1,5

Line 57: add a space between 23 and ul

Line 83: replace F on 'Five' with a lower case 'f'

Line 90: 'downwards' should be 'inwards'

Lines 93-94: Sentence should read '....morphological characteristics for confirmation of Koch's postulates."

Line 95: should read "...lesions at the inoculation site on the wood (Fig 4b).'

line 97: should read '...and length of discolouration (downward .....'

Line 101-102: should read '...Jackfruit include cankers on stems, with the stems becoming chapped as.....'

Line 103 - 106: I do not understand why this sentence is important for the story your MS is trying to tell. Whu is it important to know that jackfruit in Indonesia also showed symptoms typical of other pathogens? Are you trying to say that C. fimbriata is not the only potentially serious pathogen for Jackfruit in Indonesia, or that the symptoms of the fungi you list are similar to those of Ceratocystis? Please amend to clarify.

Line106-108: This final sentence should read '.....infections of native trees in these countries could potentially lead to devastation of .... components of the natural biodiversity in Indonesia'.

Caption for Table 1: replace 'considered' with 'included'

Note that figure captions should all be listed at the end of the list of references - please move

The caption for Fig 4 should read: 'Response of Artocarpus heterophyllous seedlings 45 days after under-bark ..... ', and on line 211 ....(III), and the healthy control seedling (IV); and line 213 should read '..discoloured wood extended ..... of the basal stem of the seedling.'

Please make any comments in the Response to Reviewers box. Thank you.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

9. Bukti konfirmasi submit revisi keempat, respon kepada reviewer, dan artikel yang diresubmit (31 Juli 2021)

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R5
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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Order of Authors Secondary Information:	
Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. 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.
Response to Reviewers:	July 31, 2021 Dear Kerrie Ann Davies, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R4 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

Comment [1]: Lines 5-6 : delete 'has been noted'. This simply repeats 'were observed', and is not needed.
Our response: We agree and change sentence to be " In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit)".

Comment [2]: Line 22: 'affected' should be 'affecting'; and 'until' should be replaced with 'to'
Our response: Thank you very much. We agree and change 'affected' to be 'affecting' dan replaced 'until' to be 'to'.

Comment [3]: Line 23: delete 'the' between 'by' and 'wilting
Our response: We agree and delete 'the' between 'by' and 'wilting.

Comment [4]: Line 43: insert 'these' between 'Based on' and 'morphological'
Our response:
We agree and insert 'these' between 'Based on' and 'morphological'.

Comment [5]: Line 46: add the word 'accessions' before the numbers on Line 47
Our response:
We agree and added the word 'accessions' before the numbers on Line 47.

Comment [6]: Line 50: insert 'the' between 'extracted from' and 'fungal ....'
Our response:
Thank you very much. We agree and insert 'the' between 'extracted from' and 'fungal ....'

Comment [7]: Line 56: should 1.5 not 1,5
Our response:
Thank you very much. We agree and change 1,5 to be 1.5.

Comment [8]: Line 57: add a space between 23 and ul
Our response:
We agree and add a space between 23 and ul.

Comment [9]: Line 83: replace F on 'Five' with a lower case 'f'
Our response:
We agree and replace F on 'Five' with a lower case 'f'

Comment [10]: Line 90: 'downwards' should be 'inwards'
Our response:
We agree and change 'downwards' to be 'inwards'.

Comment [11]: Lines 93-94: Sentence should read '....morphological characteristics for confirmation of Koch's postulates."
Our response:
We agree and change sentence to be "…..re-identified using morphological characteristics for confirmation of Koch's postulates."

Comment [12]: Line 95: should read "...lesions at the inoculation site on the wood (Fig 4b).'
Our response:
We agree and change sentence to be "...lesions at the inoculation site on the wood (Fig 4b).'

Comment [13]: line 97: should read '...and length of discolouration (downward .....'
Our response:
We agree and change sentence '...and length of discolouration (downward .....'.

Comment [14]: Line 101-102: should read '...Jackfruit include cankers on stems, with the stems becoming chapped as.....'
Our response:
We agree and change sentence to be '...Jackfruit include cankers on stems, with the

stems becoming chapped as.....'.

Comment [15]: Line 103 - 106: I do not understand why this sentence is important for the story your MS is trying to tell. Whu is it important to know that jackfruit in Indonesia also showed symptoms typical of other pathogens? Are you trying to say that C. fimbriata is not the only potentially serious pathogen for Jackfruit in Indonesia, or that the symptoms of the fungi you list are similar to those of Ceratocystis? Please amend to clarify.
Our response:
We explain that C. fimbriata is also a serious wilt pathogen of Acacia mangium, Acacia crassicarpa, Lansium domesticum in Indonesia and Pomegranate in China. The symptoms of the fungi we list are similar of Ceratocystis at those plants. We change sentence to be “Ceratocystis fimbriata is a serious wilt pathogen of jackfruit, as well as of A. mangium and A. crassicarpa in Indonesia (Tarigan et al. 2011), Lansium domesticum in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016)”.

Comment [16]: Line106-108: This final sentence should read '.....infections of native trees in these countries could potentially lead to devastation of .... components of the natural biodiversity in Indonesia'.
Our response:
We agree and change sentence to be " Ceratocystis fimbriata infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia”.

Comment [17]: Caption for Table 1: replace 'considered' with 'included'
Our response:
We agree and change 'considered' to be 'included'

Comment [18]: Note that figure captions should all be listed at the end of the list of references - please move
Our response:
We agree and move all figure captions at the end of the list of references

Comment [19]: The caption for Fig 4 should read: 'Response of Artocarpus heterophyllous seedlings 45 days after under-bark ..... ', and on line 211 ....(III), and the healthy control seedling (IV); and line 213 should read '..discoloured wood extended ..... of the basal stem of the seedling.'
Our response:
We agree and change sentence to be " Response of Artocarpus heterophyllous 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”.

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

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. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection 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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl 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) 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 (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. 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 ITS5 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'Gara et al. (1997) using five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% 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 discolouration (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 die-back in Jackfruit in Indonesia and worldwide. The symptoms 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. crassicarpa* 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.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of 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

Al Adawi AO, Barnes I, Khan IA, Al Subhi AM, Al Jahwari AA, Deadman ML, Wingfield BD, Wingfield MJ (2013) *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. Australas Plant Pathol 42:179–193

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. Molecular Biology and Evolution 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, Al-Mahmooli HIS Alfenas AC (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. Phytopathology 105:1229-1244

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

Paul CN, Nam SS, Kachroo A, Kim HY and Yang JW (2018) Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. Eur J Plant Pathol: 7-8

Ranasinghe R, Maduwanthi S, Marapana R (2019) Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review. International Journal of Food Science 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

Van Wyk M, Al Adawi AO, Khan IA, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fung Div 27: 213–230

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

Table

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

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.

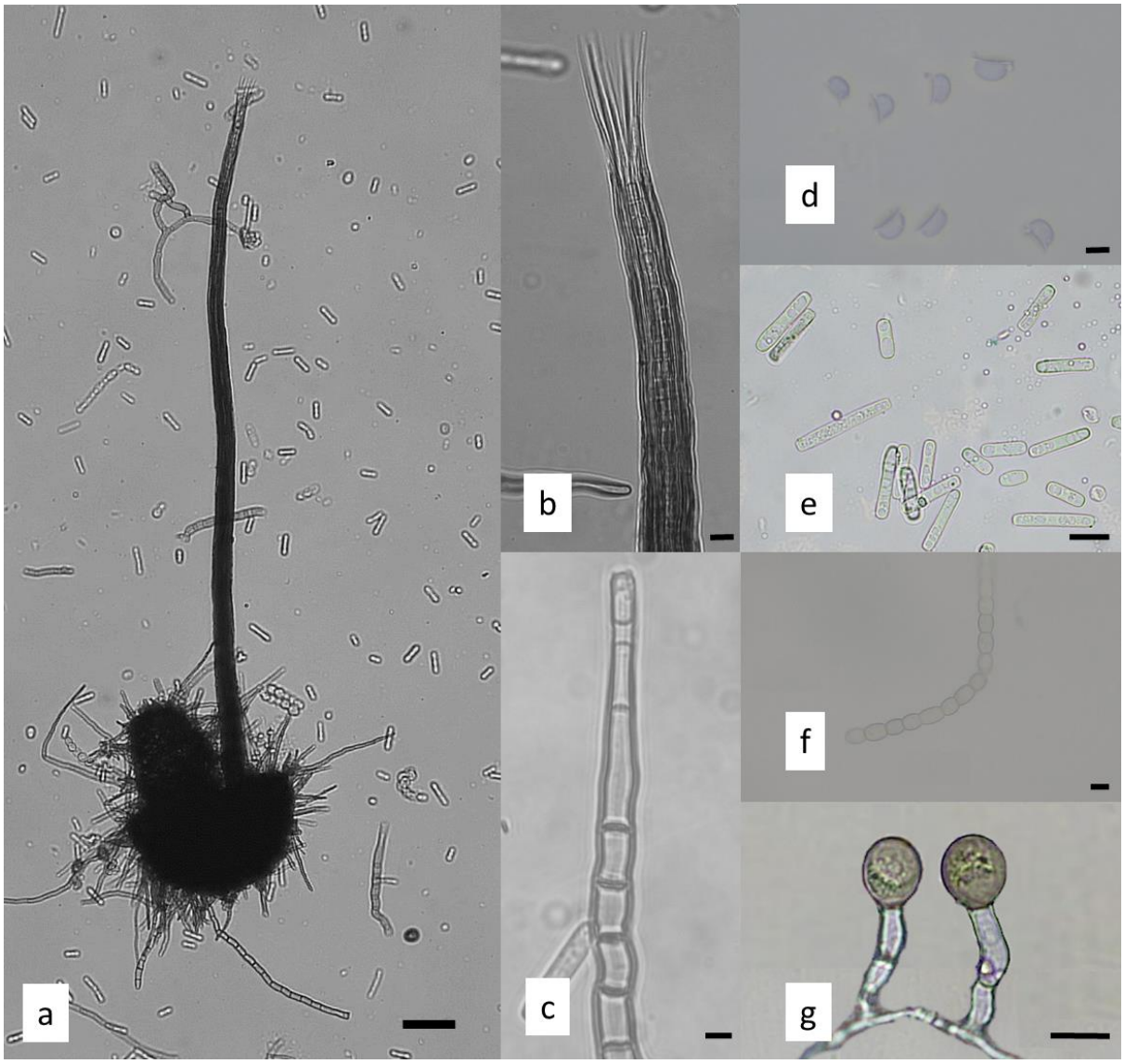

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped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

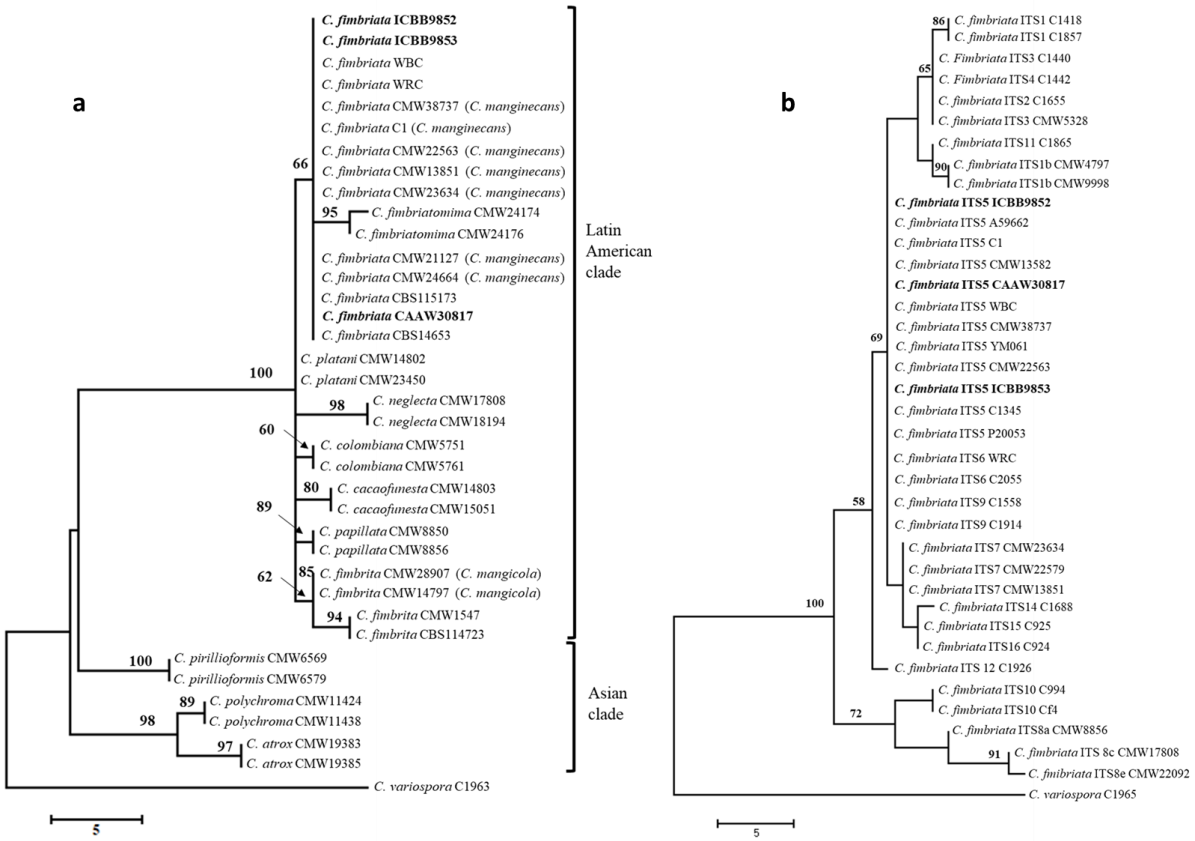

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

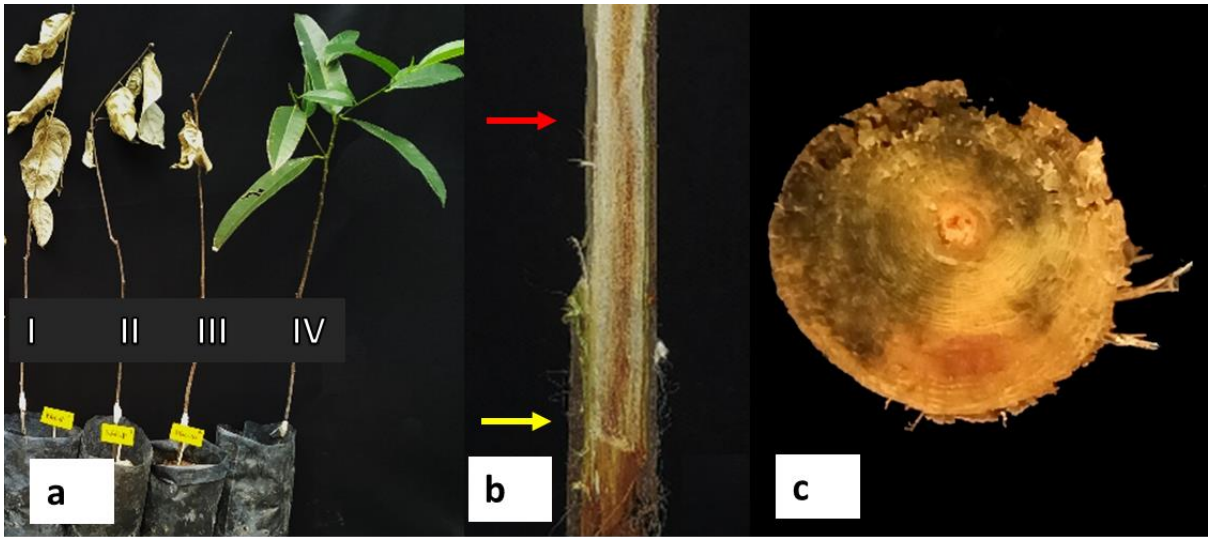

Fig. 4 Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis.* **a.** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b.** yellow arrow indicates the point of inoculation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

10. Bukti konfirmasi accepted (05 Agustus 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R5 - [EMID:4e8494bedda6eb9f]

2 messages

APDN <em@editorialmanager.com> Thu, Aug 5, 2021 at 7:49 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We are pleased to inform you that your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" (APDN-D-21-00015R5), has been accepted for publication in Australasian Plant Disease Notes.

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Editor in Chief
Australasian Plant Disease Notes

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We are really happy to hear that our paper has been accepted for publishing in your Journal " Australasian Plant Disease Notes".
We hope we can contribute to submit another paper in your journal namely, in the aspect of new diseases in plants.

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Best Regard
Ahmad Muslim
Sriwijaya University
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11. Bukti konfirmasi dan hasil proof corrections (27 Agustus 2021)

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Next steps for publishing your article (10.1007/s13314-021-00435-x) - Your publishing agreement

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DOI : 10.1007/s13314-021-00435-x
APDN-D-21-00015R5

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JACKFRUIT (ARTOCARPUS HETEROPHYLLUS), A NEW HOST PLANT OF CERATOCYSTIS WILT FROM SOUTH SUMATRA, INDONESIA

Article DOI: 10.1007/s13314-021-00435-x

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12. Bukti konfirmasi artikel published online (11 September 2021)

- Published: 11 September 2021

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

- Rahmat Pratama,
- Ahmad Muslim✉,
- Suwandi Suwandi,
- Nurhayati Damiri &
- Soleha Soleha

Australasian Plant Disease Notes **16**,
Article number: 24 (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.

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).

Fig. 1

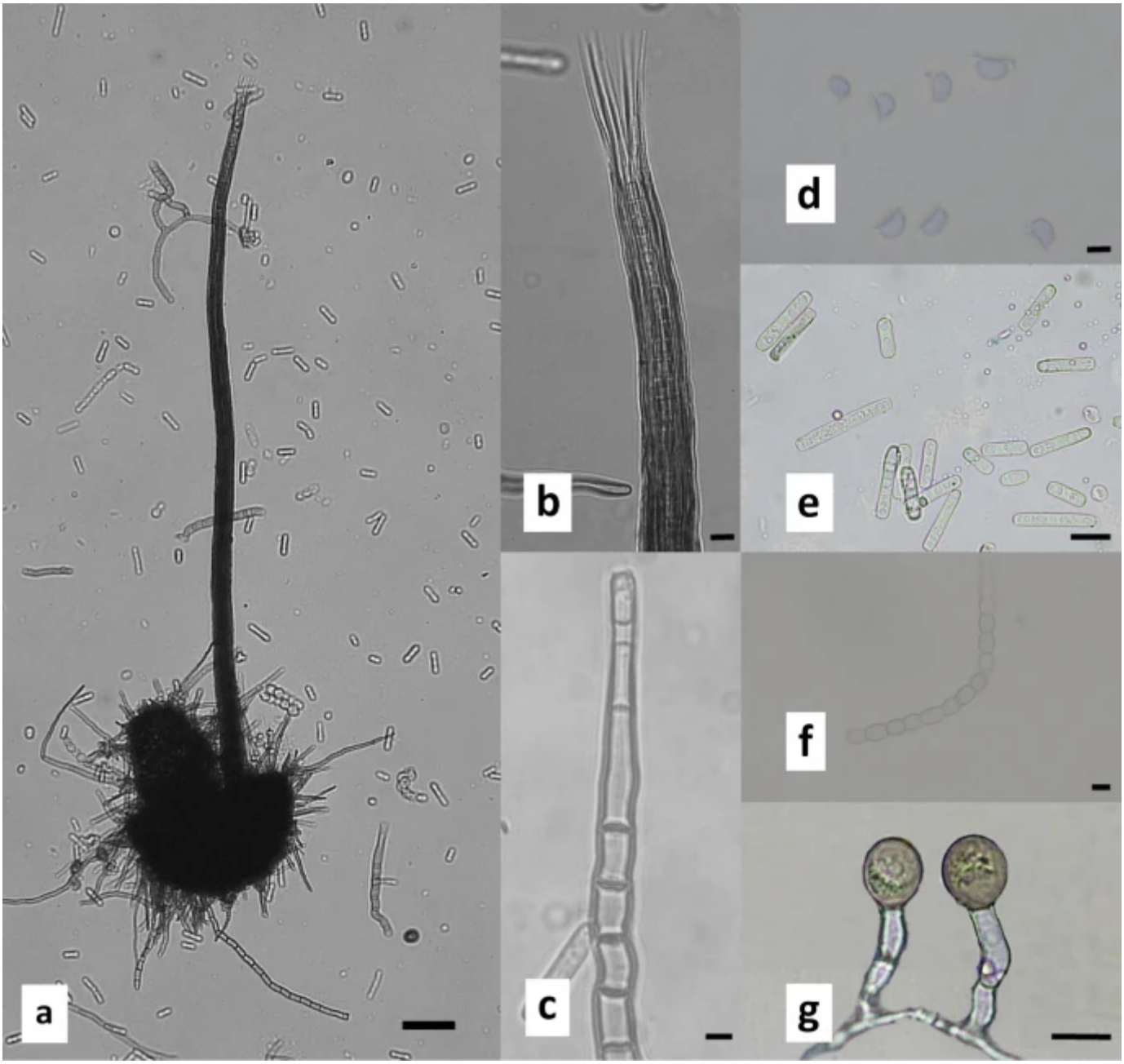
Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus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

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. Ascomatal bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the

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

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 µm; b–c, e–g = 10 µm; d = 5 µm

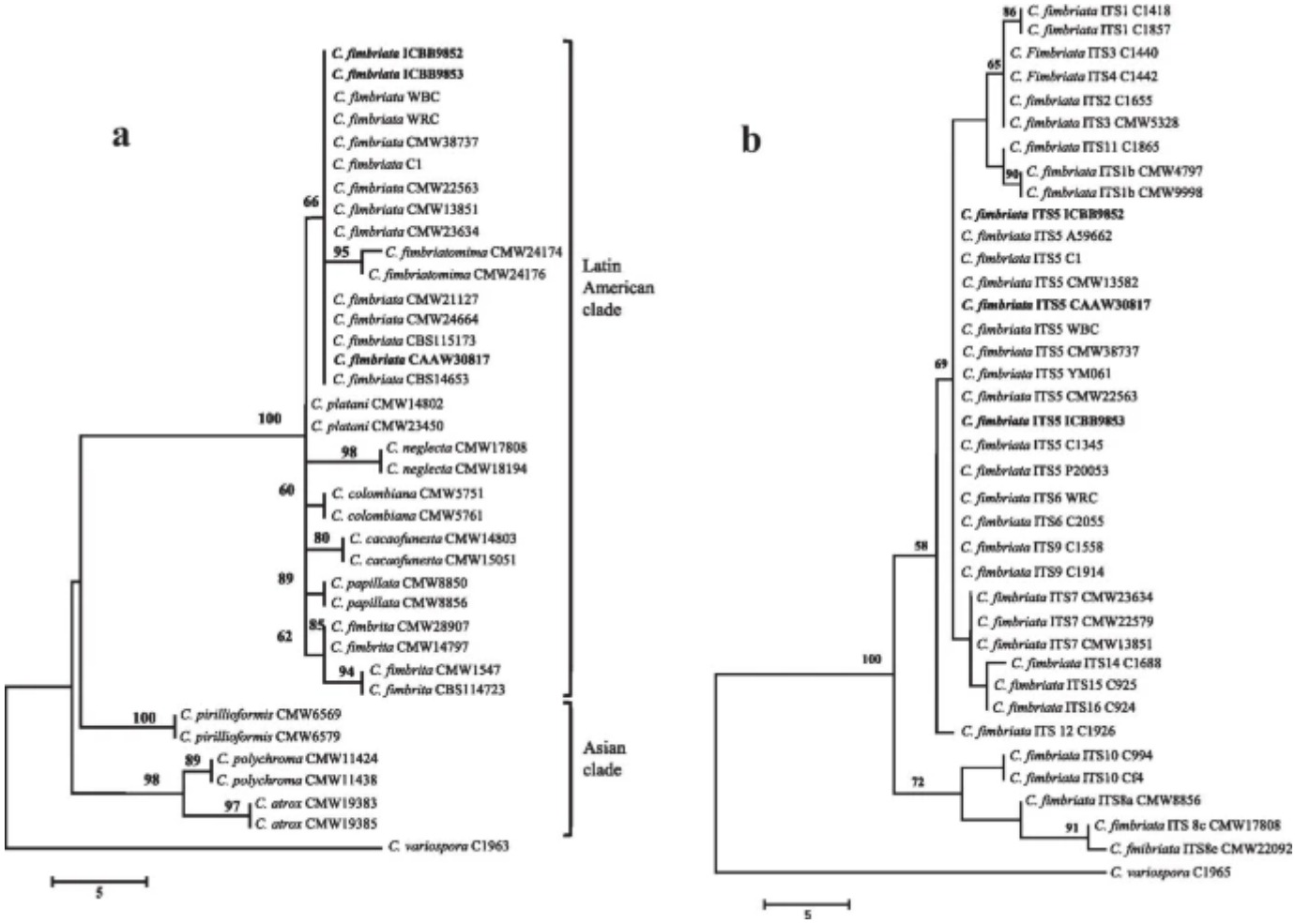
To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, 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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl 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 (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. 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 ITS5 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.

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

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

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% 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 discolouration (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).

Fig. 4

Response of *Artocarpus heterophyllous* 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

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms 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. crassicarpa* 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.

References

1. 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

2. Fourie A, Wingfield MJ, Wingfield BD, Barnes I (2015) Molecular markers delimit cryptic species in *Ceratocystis* sensu stricto. Mycol Prog 14:1020

3. 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

4. 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

5. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

6. 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

7. Moller WJ, DeVay JE (1968) Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. Phytopathology 58:123–124

8. 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

9. 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

10. 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

11. 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

12. 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

13. 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

14. 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

15. 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

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- Moraceae
- *Ceratocystis fimbriata* sensu stricto

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

Rahmat Pratama[1] · Ahmad Muslim[2] · Suwandi Suwandi[2] · Nurhayati Damiri[2] · Soleha Soleha[1]

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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).

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

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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 (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl 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
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	–
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	–
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	–
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	–
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	–
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	–
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	–
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	–
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	–
	ITS5	A59662	*Camellia sinensis*	China	KF650948	–
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	–
	ITS5	P20053	*Punica granatum*	China	AM292204	–
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	–
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	–
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	–
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	–
	ITS8c	CMW17808	*Eucalyptus* sp.	Colombia	EF127990	–
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	–
	ITS9	C1558	*M. indica*	Brazil	AY157965	–
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	–
	ITS10	C994	*M. indica*	Brazil	AY157964	–
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	–
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	–
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	–
	ITS14	C1688	*M. indica*	Brazil	AY526291	–
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	–
	ITS16	C924	*G. arborea*	Brazil	HQ157539	–
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	–	DQ371652
	AC	CMW6579	*E. nitens*	Australia	–	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	–	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	–	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	–	EF070430
	AC	CMW19385	*E. grandis*	Australia	–	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	–	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	–	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	–	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	–	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	–	KJ631108

Table 1 (continued)

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

Isolates from jackfruit in Indonesia are marked in bold

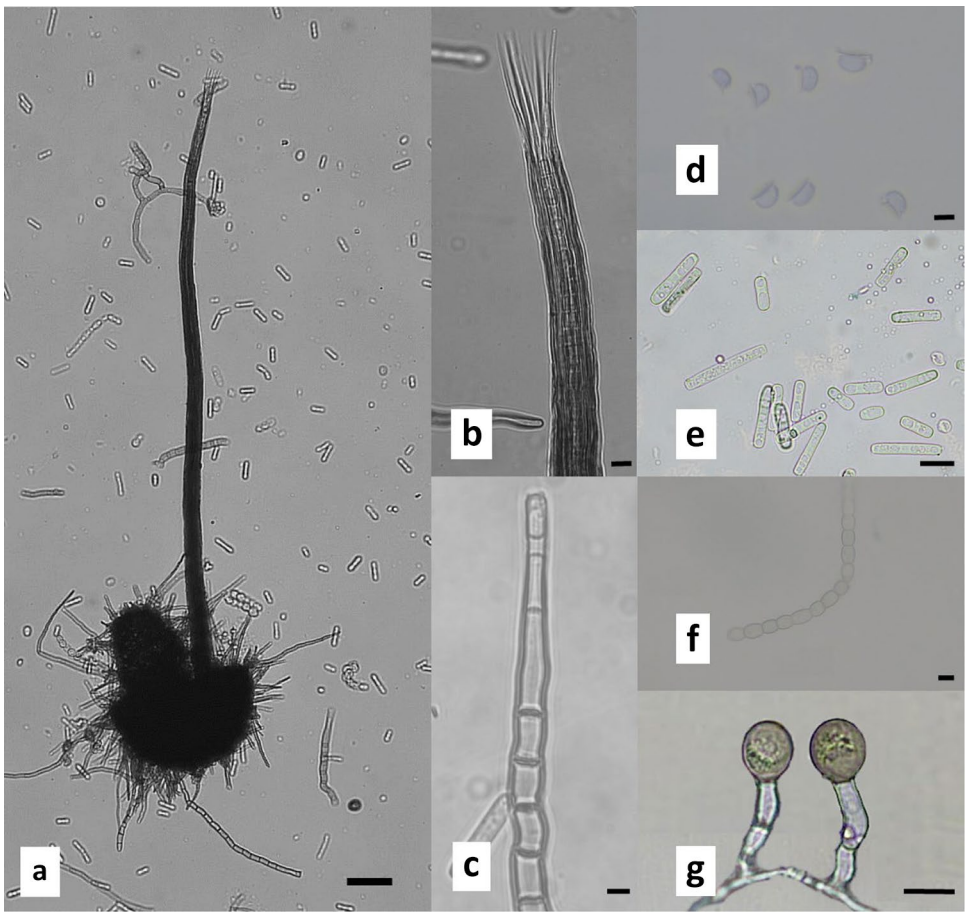

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a** ascomata with pirilliform base, **b** divergent ostiolar hyphae; **c** conidiophore/phialide; **d** hat-shaped ascospores; **e** cylindrical conidia; **f** Chain of barrel-shaped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

(MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. 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 ITS5 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'Gara et al. (1997) using five-month-old *A. heterophyllus* 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 the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% 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 discolouration (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

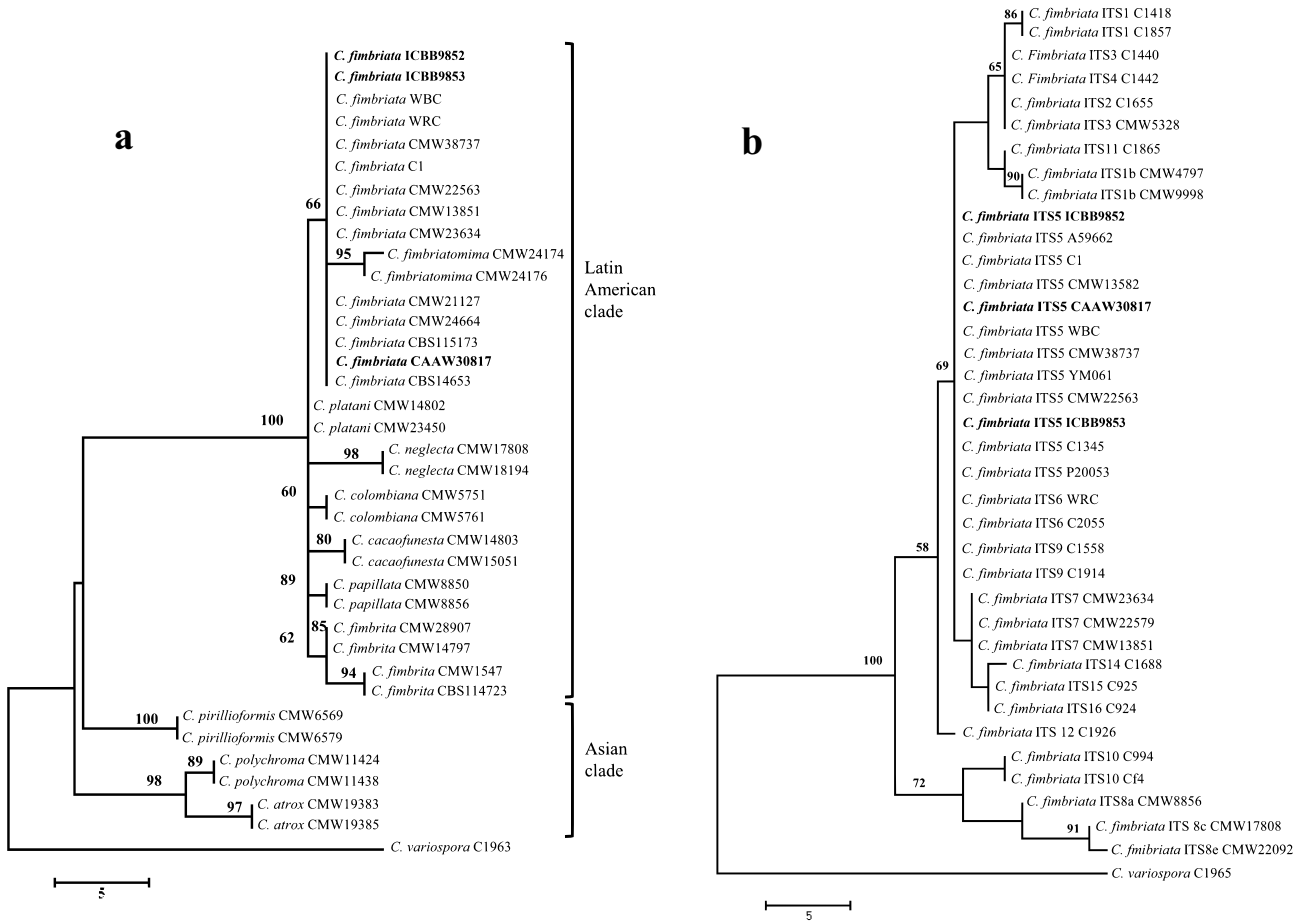

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a** β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata* sensu stricto

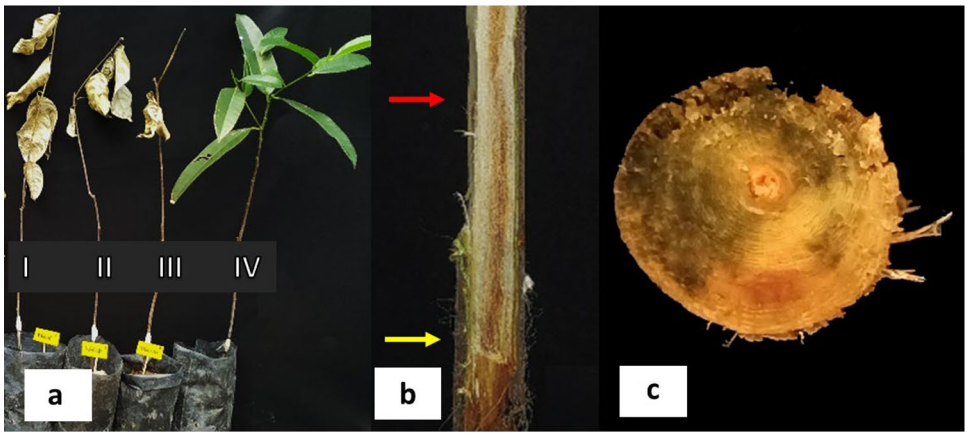

Fig. 4 Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis*. **a** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b** yellow arrow indicates the point of inoculation 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. crassicarpa* 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.

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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