Author Dashboard

3 Manuscripts with Decisions

Start New Submission

Legacy Instructions

5 Most Recent E-mails

Manuscripts with Decisions

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	ADM: Kwak, Youn-Sig • Accept (25-Jan-2021) • Awaiting Production Checklist view decision letter Contact Journal	PPJ-OA-08-2020-0147.R2	Identification and characterization of *Ceratocystis fimbriata* causing a lethal wilt on *Lansium* tree in Indonesia View Submission	25-Jan-2021	25-Jan-2021

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
a revision has been submitted (PPJ-OA-08-2020-0147.R2)	ADM: Kim, Yoonjin • Minor Revision (20-Dec-2020) • a revision has been submitted view decision letter Contact Journal	PPJ-OA-08-2020-0147.R1	Identification and characterization of *Ceratocystis fimbriata* causing a lethal wilt on *Lansium* tree in Indonesia View Submission	01-Dec-2020	20-Dec-2020
a revision has been submitted (PPJ-OA-08-2020-0147.R1)	ADM: Kim, Yoonjin • Major Revision (11-Sep-2020) • a revision has been submitted view decision letter Contact Journal	PPJ-OA-08-2020-0147	Identification and characterization of *Ceratocystis fimbriata* causing a lethal wilt on *Lansium* tree in Indonesia View Submission	05-Aug-2020	11-Sep-2020

The Plant Pathology Journal - Manuscript ID PPJ-OA-08-2020-0147

1 message

The Plant Pathology Journal <onbehalfof@manuscriptcentral.com> Tue, Aug 4, 2020 at 2:05 PM
Reply-To: paper@kspp.org
To: suwandi@fp.unsri.ac.id, suwandi.saleh@gmail.com

04-Aug-2020

Dear Dr. Suwandi:

Your manuscript entitled "Identification and characterization of Ceratocystis fimbriata causing a lethal wilt on Lansium tree in Indonesia" has been successfully submitted online and is presently being given full consideration for publication in The Plant Pathology Journal.

Your manuscript ID is PPJ-OA-08-2020-0147.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at https://mc.manuscriptcentral.com/ppj and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://mc.manuscriptcentral.com/ppj.

Thank you for submitting your manuscript to The Plant Pathology Journal.

Sincerely,
Yoonjin Kim
Administrative Editor
The Plant Pathology Journal
paper@kspp.org

The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-08-2020-0147

1 message

The Plant Pathology Journal <onbehalfof@manuscriptcentral.com> Fri, Sep 11, 2020 at 6:14 PM
Reply-To: kiwoo@knu.ac.kr
To: suwandi@fp.unsri.ac.id, suwandi.saleh@gmail.com

11-Sep-2020

Dear Dr. Suwandi Suwandi:

Manuscript ID PPJ-OA-08-2020-0147 entitled "Identification and characterization of Ceratocystis fimbriata causing a lethal wilt on Lansium tree in Indonesia" which you submitted to The Plant Pathology Journal, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have requested major revisions before a final decision. Failure to carefully consider the reviewer comments may lead to your revised manuscript being rejected without further review. Therefore, I invite you to respond to the reviewer comments and revise your manuscript.

To revise your manuscript, log into https://mc.manuscriptcentral.com/ppj and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using colored text to indicate the altered portion.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to The Plant Pathology Journal, your revised manuscript should be uploaded as soon as possible. If it is not possible for you to submit your revision in a reasonable amount of time, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to The Plant Pathology Journal and I look forward to receiving your revision.

Sincerely,
Prof. Ki Woo Kim
Editor
The Plant Pathology Journal
kiwoo@knu.ac.kr

Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author
This report of a new host, Lansium domesticum (duku), for Ceratocystis fimbriata in Indonesia is noteworthy. The manuscript is generally well-written, but it could use light editing, and there are a few muddled sections that need clarification.

This important information could be presented as a disease note rather than a full manuscript. But even as a much

shorter disease note, the isolates should be deposited in a referenced culture collection, the ITS and Btub sequences need to be deposited in GenBank, and the alignment for the phylogenetic tree (Fig. 3) should be available to reviewers. I think there is a major problem with the tree as none of the isolates from the Wingfield collection (CMW isolates) match any of the isolates from the Harrington collection (C isolates). Alignments of such sequences are difficult because of indels. Also, the Harrington isolates have been trimmed to exclude the 16S and 28S regions, leaving only ITS1, 5.8S and ITS2, but the CMW accessions in GenBank are longer and also need to be trimmed to compare. Perhaps this explains the misleading tree.

Without the tree and dropping Table 3, I think it would be sufficient to present the accession numbers of the duku isolates and say how many base differences there are with the ITS5, ITS6 and ITS7 sequences, which represent the Asian introductions of C. fimbriata from Brazil, as described in several papers listed in the literature cited (but not fully discussed). Another paper more fully discusses the ITS5 strain in China, which is also in India and Southeast Asia (Li, et al. 2016. Plant Dis. 100:2266-2274).

Page Lines
6 9 Uninoculated controls or control experiments? How much discoloration was seen in the controls of each host species (a few mm?), and was there always more discoloration in the inoculated seedlings?

6 10 If each of the experiments was repeated, the results of both experiments should be presented (combined if possible), not just the second experiment.

7 2 The holes that are illustrated were made by ambrosia beetles, which are well-known to attack trees killed by Ceratocystis. They are not vectors, but they facilitate spread of the fungus by expelling frass containing the aleurioconidia of the pathogen. The fungus is then windborne and rain-splashed, and it is soilborne to infect roots. Are the authors sure the infections are not starting in the roots and moving up the stem?

7 13-14 Besides roots, squirrel and monkey wounds would be suitable infection courts for this inoculum. But it seems the squirrel wounds come later?

8 1-3 Table 3 is not very useful because there are no differences in the measurements among the isolates. The two duku isolates do not differ, yet it is stated that WRC was similar to the description of C. acaciivora, implying that WBC is different.

8 4-12 It would be better just to say how many base substitutions are needed to distinguish the two new sequences from each other and ITS5, 6 and 7, or other sequences that may be seen in the Harrington et al. 2014 or Oliveira et al. papers. The tree is not helpful, probably because of improper alignment of the sequences.

8 13-25 If a longer report is published with the table, perhaps comparisons of the means could be included, along with the controls.

11 15-19 The epidemiology of the disease is still uncertain, but insect transmission is not very likely important, and the infectious ambrosia beetle frass would be wind and rain-dispersed in substantial amounts.

11 24-25 I am not clear what they mean by “without evidence of massive mortality” because they describe very high levels of mortality at three sites. Do they mean that there are scattered areas of mortality with limited mortality in between?

12 1-2 This would suggest that squirrels are not important in spreading the disease.

12 10-14 It is hard to envision that pruning would eliminate a systemic pathogen, so this statement should be backed up with more detailed data. It does not seem relevant to the study in any case.

Table 2 Is “n=” referring to the total number of trees in the three orchards at that location?

Reviewer: 2

Comments to the Author
This paper is reporting a fungal disease caused by Ceratocystis fimbriata on Lansium tree in Indonesia. The research focused identification and characterization of the pathogen. In general, all the mycological and pathological study performed was scientifically sound. It is new disease report on the host plant. The blow points need to be answered.

1. Typo in abstract. Cancer needs to be changed with canker
2. Although DNA sequence data support the causal agent is Ceratocystis, it would be useful to biologically confirm with other method. Cycloheximide is used for differentiation of Ceratocystis vs Ophiostoma (one of structurally similar

fungal group). Ophiostoma is not sensitive to this antimicrobial compound but Ceratocystis is sensitive. Does the authors tried to grow cycloheximide-contained PDA to confirm it is Ceratocystis?

3. The ITS sequence based phylogenetic analysis need to include other Ceratocystis species which have similar morphology and nucleotide sequences.

4. Phylogram tree based on TUB gene sequence is also needed with other Ceratocystis species which have similar morphology and nucleotide sequences.

5. The cited reference is not enough to confirm what species is the vector of the pathogen on Lansium tree. Information on the insect species that transmit the fungal pathogen is not clearly defined.

December 1, 2020

Dear Prof. Ki Woo Kim,
Editor of The Plant Pathology Journal

Enclosed you will find a first revised version with tracked or highlighted changes of the manuscript ID PPJ-OA-08-2020-0147 entitled " Identification and characterisation of Ceratocystis fimbriata causing lethal wilt on the Lansium tree in Indonesia" by S. Suwandi, C. Irsan, H. Hamidson, A. Umayah, and K.D. Asriyani which we would like to re-submit for publication in The Plant Pathology Journal.

Reviewers recommended some revisions that we have made corrections accordingly. We would like to thank for all reviewers' suggestions and corrections.

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

Reviewer's #1 comment [1]: This important information could be presented as a disease note rather than a full manuscript. But even as a much shorter disease note.
Our response: In this paper, we do not only describe the disease and its causal agent, but we also describe the disease progress and spread for 5 years. The paper also describes role of partial flooding as factors on the disease progress and discusses the genetic relationship of the pathogen with other aggressive populations of *Ceratocystis fimbriata*. The information could be sufficient for a full manuscript submission.

Reviewer's #1 comment [2]: The isolates should be deposited in a referenced culture collection, the ITS and Btub sequences need to be deposited in GenBank.
Our response: A reputable institution that formally preserved the fungal plant pathogen in Indonesia was not available yet, and therefore the isolates used in the study has been preserved in our laboratory collection. The isolates could be freely and openly used within and outside Sriwijaya University. The ITS and β tubulin sequences have been deposited in GenBank as MT229127 and MW013766 for isolate WRC and MT229128 and MW013767. The GenBank accession number of isolates are listed in Table 1 and in the result section.

Reviewer's #1 comment [3]: the alignment for the phylogenetic tree (Fig. 3) should be available to reviewers. I think there is a major problem with the tree as none of the isolates from the Wingfield collection (CMW isolates) match any of the isolates from the Harrington collection (C isolates). Alignments of such sequences are difficult because of indels. Also, the Harrington isolates have been trimmed to exclude the 16S and 28S regions, leaving only ITS1, 5.8S and ITS2, but the CMW accessions in GenBank are longer and also need to be trimmed to compare. Perhaps this explains the misleading tree.
Our response: We agree and are grateful for this suggestion. Following trimming sequence to exclude the 16S and 28S regions, manual sequence alignment against representative ITS haplotypes of *C. fimbriata* as designated by Harrington et al. (2014) suggested that the WBC isolate and CMW13582 from *Hypocryphalus*, Oman are grouped as the ITS6z. The alignments of trimmed CMW isolates are provided to reviewers and phylogenetic tree (Fig.3) has been revised accordingly.

Reviewer's #1 comment [4]: Without the tree and dropping Table 3, I think it would be sufficient to present the accession numbers of the duku isolates and say how many base differences there are with the ITS5, ITS6 and ITS7 sequences, which represent the Asian introductions of C. fimbriata from Brazil, as described in several papers listed in the literature cited (but not fully discussed). Another paper more fully discusses the ITS5 strain in China, which is also in India and Southeast Asia (Li, et al. 2016. Plant Dis. 100:2266-2274).

Our response: We are very appreciating and agreeing for this comment. The duku isolates are belong to known ITS haplotypes (ITS5 for WRC and ITS6z for WBC) based on manual sequence alignment against representative ITS haplotypes of *C. fimbriata* as designated by Harrington et al. (2014). We agree to drop the Maximum parsimony tree as failure to separate the ITS5, ITS6 and ITS7 even after trimming the Wingfield collection (CMW isolates) and replacing the tree with UPGMA tree. We agree to include statement that the duku isolate of *C. fimbriata* in Indonesia belong to the ITS5 haplotype, which represent the Asian introductions of *C. fimbriata* from Brazil. We included in the discussion section that the pathogen belongs to the ITS5 haplotype, the aggressive and widely distributed ITS genotype of *C. fimbriata*.

***Reviewer's #1 comment [5]*:** Page 6 Line 9. Uninoculated controls or control experiments? How much discoloration was seen in the controls of each host species (a few mm?), and was there always more discoloration in the inoculated seedlings?.

Our response: We agree to change "control experiments" to be "Uninoculated controls". We added the lesion size of control plant in the result section to be "The control plants, inoculated with malt extract agar, remained asymptomatic with small lesion (less than 5 mm) and had only a trace of xylem discolouration at the wound site." and the detailed size was listed in Table 3.

***Reviewer's #1 comment [6]*:** Page 6 Line 10. If each of the experiments was repeated, the results of both experiments should be presented (combined if possible), not just the second experiment.

Our response: We agree to combined the result from two experiments after verifying the variance homogeneity using the Levene test. All measurements of lesion size and plant mortality were changed and the detailed changes were listed in Table 3.

***Reviewer's #1 comment [7]*:** 7 2 The holes that are illustrated were made by ambrosia beetles, which are well-known to attack trees killed by Ceratocystis. They are not vectors, but they facilitate spread of the fungus by expelling frass containing the aleurioconidia of the pathogen. The fungus is then windborne and rain-splashed, and it is soilborne to infect roots. Are the authors sure the infections are not starting in the roots and moving up the stem?.

Our response: We agree with those mentioned reviewers' command. The fungus is known as soilborne and therefore the infection might start from the roots and moving up the stem. However, during disease surveys, all cut plants with initial symptoms were free from wood discoloration at the basal or main stem suggesting the initial infection starting from the top branches or twigs.

***Reviewer's #1 comment [6]*:** 7 13-14 Besides roots, squirrel and monkey wounds would be suitable infection courts for this inoculum. But it seems the squirrel wounds come later?.

Our response: It was likely that the squirrel attacks was come earlier as some healthy trees had also squirrel wounds.

***Reviewer's #1 comment [6]*:** 8 1-3 Table 3 is not very useful because there are no differences in the measurements among the isolates. The two duku isolates do not differ, yet it is stated that WRC was similar to the description of C. acaciivora, implying that WBC is different.
Our response: We agree with the reviewer suggestion and deleted the Table 3 for size comparison between isolates from *Lansium* and the reference isolate for *C. fimbriata* s.s., neotype BPI 595863.

***Reviewer's #1 comment [6]*:** 8 4-12 It would be better just to say how many base substitutions are needed to distinguish the two new sequences from each other and ITS5, 6 and 7, or other sequences that may be seen in the Harrington et al. 2014 or Oliveira et al. papers. The tree is not helpful, probably because of improper alignment of the sequences.
Our response: We are very appreciating this comment. The WRC showed 100% similarity with other ITS5 haplotype of *C. fimbriata* isolated from tea tree (KF650948), taro (AM712445), pomegranate (AM292204) in China; from eucalyptus (KF878326) in Zimbabwe; from acacia (MF033455) in Vietnam; and from acacia (EU588656) in Indonesia. WBC had 100% similarity with member of ITS6z haplotype of *C. fimbriata* isolated from *Hypocryphalus mangifera* (KC261853) in Oman. We agree to drop the Maximum parsimony tree, trimming the sequences and replacing the tree with UPGMA tree.

***Reviewer's #1 comment [6]*:** 8 13-25 If a longer report is published with the table, perhaps comparisons of the means could be included, along with the controls.
Our response: We agree with the reviewer suggestion, combining two data sets from different experiments, and made an appropriate statistical comparison between control and inoculated measurement or counts.

***Reviewer's #1 comment [6]*:** 11 15-19 The epidemiology of the disease is still uncertain, but insect transmission is not very likely important, and the infectious ambrosia beetle frass would be wind and rain-dispersed in substantial amounts.
Our response: We are very appreciating and agreeing for this comment. The change has been made accordingly.

***Reviewer's #1 comment [6]*:** 11 24-25 I am not clear what they mean by "without evidence of massive mortality" because they describe very high levels of mortality at three sites. Do they mean that there are scattered areas of mortality with limited mortality in between?.
Our response: We are very appreciating for this comment and "without evidence of massive mortality" had been changed to be "with limited mortality".

***Reviewer's #1 comment [6]*:** 12 1-2 This would suggest that squirrels are not important in spreading the disease.
Our response: Squirrel attacks were not found on recently infected trees with limited mortality, but the attack was extensive on either diseased or healthy trees during the disease outbreaks in 2013-2014.

***Reviewer's #1 comment [6]*:** 12 10-14 It is hard to envision that pruning would eliminate a systemic pathogen, so this statement should be backed up with more detailed data. It does not seem relevant to the study in any case.

Our response: We agree with the reviewer suggestion and delete all these statements. Pruning could not eliminate a systemic pathogen when the infection started from roots, but could be applied when the infection starting from shoot or branches. During the disease surveys, we cut the stem of 3 trees showing the initial symptoms (wood discoloration was limited on branches and without any wood discoloration on the stem). The trees started to produce new shoots within 6 months and remained healthy for 5 years. Next study with more samples in needed to support this control technique.

***Reviewer's #1 comment [6]*:** Table 2 Is "n=" referring to the total number of trees in the three orchards at that location?.

Our response: Yes it was, n refers the total number of trees in the three orchards at that location. trees/location

***Reviewer's #2 comment [1]*:** Typo in abstract. Cancer needs to be changed with canker.

Our response: Thank you for reviewer correction, the change has been made accordingly.

***Reviewer's #2 comment [2]*:** Although DNA sequence data support the causal agent is Ceratocystis, it would be useful to biologically confirm with other method. Cycloheximide is used for differentiation of Ceratocystis vs Ophiostoma (one of structurally similar fungal group). Ophiostoma is not sensitive to this antimicrobial compound but Ceratocystis is sensitive. Does the authors tried to grow cycloheximide-contained PDA to confirm it is Ceratocystis?

Our response: We are very appreciating for this comment and agree that differences in cycloheximide sensitiveness could be used to differentiate *Ceratocystis* and *Ophiostoma*. In this study, we prefer to identify the isolate based on the data of DNA sequences. We did not use cycloheximide-contained PDA to confirm the species identity.

***Reviewer's #2 comment [3]*:** The ITS sequence based phylogenetic analysis need to include other Ceratocystis species which have similar morphology and nucleotide sequences.

Our response: We thank the reviewer for these suggestions. Identification of Ceratocystis species in this study was mainly based on BLAST searches on the GenBank. BLAST searches of the ITS region of WRC (MT229127) and WBC (MT229128) identified both sequences with the GenBank deposits for *Ceratocystis fimbriata* with 100% of similarity and query coverage (the BLAST hits no. 98 out of 104 for WRC and 91 out of 101 for WBC). A similar BLAST result was obtained with the TUB sequence (MW013766 and MW013767 for WBC and WBC, respectively) and confirmed the assignment to *Ceratocystis fimbriata* with 100% of similarity and query coverage (the BLAST hits no. 101 out of 101 for both WRC and WBC). The WRC (MT229127) has an identical ITS sequence (with 100% of similarity and query coverage) to the isolates from taro (AM712445) and pomegranate (AM292204) in China that had been identified based on ITS, mating type genes, microsatellite alleles, and fertility with tester strains of *C. fimbriata* sensu stricto.

We did not identify the *Ceratocystis* species based on the ITS sequence based phylogenetic analysis because of intragenomic ITS variation is common in introduced population of *Ceratocystis fimbriata* sp. complex (Harrington et al., 2014; Oliveira et al., 2015). Therefore, we prefer to use

the phylogenetic UPGMA tree to describe relationship between the duku isolates with known genotype (ITS haplotype) of *C. fimbriata* sensu stricto.

***Reviewer's #2 comment [4]*:** Phylogram tree based on TUB gene sequence is also needed with other Ceratocystis species which have similar morphology and nucleotide sequences.
Our response: We thank the reviewer for these suggestions. The TUB gene sequence was used in our study to complement the identification based on the ITS sequence. The BLAST results with the TUB sequence (MW013766 and MW013767 for WBC and WBC, respectively) confirmed the assignment to *Ceratocystis fimbriata* with 100% of similarity and query coverage (the BLAST hits no. 101 out of 101 for both WRC and WBC). The TUB sequence (MW013766 and MW013767 for WBC and WBC, respectively) has an identical sequence (with 100% of similarity and query coverage) to the reference sequence of *Ceratocystis fimbriata* (MK161091).

***Reviewer's #2 comment [5]*:** The cited reference is not enough to confirm what species is the vector of the pathogen on Lansium tree. Information on the insect species that transmit the fungal pathogen is not clearly defined.
Our response: We are very appreciating and agreeing for this comment. The cite reference has been changed accordingly.

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in the The Plant Pathology Journal. Please feel free to contact me if you need any additional information or clarification.

Sincerely,
Suwandi Suwandi
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: suwandi@fp.unsri.ac.id; suwandi.saleh@gmail.com

Identification and characterization of *Ceratocystis fimbriata* causing a lethal wilt on *Lansium* tree in Indonesia

Journal:	*The Plant Pathology Journal*
Manuscript ID	PPJ-OA-08-2020-0147.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	01-Dec-2020
Complete List of Authors:	Suwandi, Suwandi; Sriwijaya University Faculty of Agriculture, Plant Protection Irsan, Chandra; Sriwijaya University Faculty of Agriculture, Plant Protection Hamidson, Harman; Sriwijaya University Faculty of Agriculture, Plant Protection Umayah, Abu; Sriwijaya University Faculty of Agriculture, Plant Protection Asriyani, Khoirotun ; Sriwijaya University Faculty of Agriculture, Plant Protection
Keyword:	A. Plant Pathogens

Identification and characterisation of *Ceratocystis fimbriata* causing lethal wilt on the *Lansium* tree in Indonesia

Running text: *Ceratocystis fimbriata*, wilt pathogen of *Lansium* tree

Suwandi Suwandi*, Chandra Irsan, Harman Hamidson, Abu Umayah, Khoirotun Dwi Asriyani

Department of Plant Protection, Faculty of Agriculture, Sriwijaya University

Jl. Palembang-Prabumulih Km.32 Indralaya, Palembang, Indonesia

Corresponding author: S. Suwandi, E-mail: suwandi@fp.unsri.ac.id, Tel./fax. +62-711-580059, ORCID ID: 0000-0003-3096-5797

Abstract

Bark ~~cancer~~canker, wood discolouration, and wilting~~, and massive mortality~~ of the duku tree (*Lansium domesticum* Corr.) along the watershed of Komering River~~in Ogan Komering Ulu (OKU)~~, South Sumatra Province, Indonesia first appeared in 2013~~ and caused total losses in affected orchards~~. The incidence of tree mortality was 100% within three years in badly infected orchards. ~~Bark cancer and wood discolouration were observed along infected stems and branches.~~ A *Ceratocystis* species was consistently isolated from the diseased tissue and identified by morphological and sequence analyses of the ITS and TUB regions. ~~discoloured wood of diseased trees.~~ Pathogenicity tests were conducted and Koch's postulates were confirmed. The fungus was also pathogenic on *Acacia mangium*, but was less pathogenic on mango. Partial flooding was unfavourable for disease development. Two described isolates

(WRC and WBC) had minor variation in morphology and DNA sequences, but the former exhibited a more pathogenic on both duku and acacia. The ITS phylogenies grouped the most pathogenic isolate (WRC) causing wilting of the duku tree within the aggressive and widely distributed ITS5 haplotype of *C. fimbriata*. ~~Stem inoculation with a fungal culture on one-year-old duku seedlings caused substantial wood discolouration, wilting and plant death similar to symptoms in the field, confirming Koch's postulates. The fungus also caused extensive wood discolouration and wilting on *Acacia mangium* seedlings and induced slight wood discolouration without wilting on mango seedlings. Teleomorph and anamorph characteristics were similar to those of *C. acaciivora*, a conspecific of *C. fimbriata*. BLAST searches of ITS and TUB regions in GenBank indicated that two described isolates (WRC and WBC) have a 99.7-100% similarity with sequences of *C. fimbriata*. The ITS phylogenies and manual alignment with ITS haplotypes grouped the pathogen causing wilting of the duku tree within the ITS5 haplotype of *C. fimbriata*.~~

Keywords: *Ceratocystis* canker and wilt, *Ceratocystis fimbriata*, *Lansium* tree, *Acacia mangium*

Introduction

The duku (*Lansium domesticum Corr.*), also known as the langsat and the kokosan is a tropical lowland fruit tree native to western Southeast Asia, from Borneo in the east (Indonesia) to peninsular Thailand in the west. It occurs wild and cultivated in its native countries and is one of the most widely cultivated fruits (Techavuthiporn, 2018; Yaacob and Bamroongrugsa, 1991). Duku is among the most popular local fruits in Indonesia. In 2017, the total number of harvested duku trees in Indonesia was 2.4 million trees, with a total yield of 138.4 metric tons (BPS-Statistics Indonesia, 2018). The most famous cultivars are grown in

South Sumatra (duku Palembang and duku Komering) due to their sweet flavour combined with a subacid taste and having few seeds, or even being seedless. In South Sumatra, duku is mainly grown as a backyard or garden tree in combination with other native fruit trees along the watershed of the Musi, Komering, Ogan, Lematang and Rawas Rivers.

Lethal disease has rarely been evident on duku trees growing in the wild or cultivated orchard areas. Anthracnose caused by *Colletotrichum gloeosporioides*, appearing as brownish spots on the fruit bunch and often resulting in premature fruit drop and post-harvest losses, is commonly evidenced throughout the tropics (Yaacob and Bamroongrugsa, 1991). Corky bark disease, which makes the bark become rough and corky and flake off, often resulting in little to no fruit production has been reported on dukus in tropical USA (Keith et al., 2013; Whitman, 1980). In Hawaii, a corky bark canker is associated with an Ascomycete fungus, *Dolabra nepheliae*, and insect larvae of *Araecerus* sp. (Coleoptera: Anthribidae) and *Corticeus* sp. (Coleoptera: Tenebrionidae) feeding under the loosened bark (Keith et al., 2013).

During early January 2014, massive mortality of duku trees along the watershed of the Komering River in OKU District was reported by most local and some national newspapers. In total, more than 2,000 trees of the most popular cultivar, duku Komering, died. The symptoms first appeared during the early rainy season of October 2013. Most of the trees that died were predisposed due to partial flooding to a depth of about 20 cm for about one month from the end of December 2013 to January 2014. However, some affected trees were found growing on non-flooded sites, indicating an infectious disease. In this study, we describe a new bark ~~cancer~~ canker and wilting associated with massive mortality of duku trees in Indonesia, illustrate morphological and molecular-based identification of the pathogen, and describe the pathogenicity of the causal fungus on duku trees and other hosts. Disease progress and spread for five years is also discussed.

Materials and Methods

Disease incidence and isolation of the causal agent. Incidence of diseased trees was assessed in 2014 and 2017 at eight duku orchards in Ogan Komering Ulu (OKU) District of South Sumatra. In each orchard, five 10 × 10 m plots starting from the centre of the diseased trees were selected. The trees were recorded as infected if any part of the shoot or stem showed disease symptoms. Twenty diseased duku trees were randomly selected from the affected orchards. Sections of the discoloured wood from the stem were cut, wrapped in a paper towel and transported to the laboratory for examination. Isolation of the fungal pathogen was performed from discoloured wood that had been surface-sterilized with 70% ethanol for 30 s and 1% NaOCl for 2 min. Small sections (5 × 5 mm) from the margin of discolouration were placed on a malt extract agar (MEA) amended with 50 µg/ml streptomycin in Petri dishes. Another subset of surface-sterilized wood sections was wrapped between carrot slices to bait for *Ceratocystis* spp. (Brito et al., 2019; Moller and DeVay, 1968). Baiting was also performed by inserting diseased tissue into freshly harvested cacao pods and cucumber fruit in an attempt to isolate *Phytophthora*.

Initial identification and cultural characteristics. Initial identification was performed based on morphological characteristics of teleomorphs and anamorphs. Isolates were characterized from two-week-old cultures grown on 2% malt extract agar (MEA). One hundred measurements of each teleomorph and anamorph structure from each representative isolate were made with an Olympus microscope and an OptiLab camera system (Yogyakarta, Indonesia). The average (mean) and standard deviation (stdv) of measurements were computed and presented as mean minus stdv-mean plus stdv. Morphological characteristics were compared with *Ceratocystis* isolates from ~~*Acacia*~~*A. mangium* (Tarigan et al., 2011) and sweet potato (Engelbrecht and Harrington, 2005).

DNA isolation, PCR, and sequence analyses. Two representative isolates (WRC and WBC), isolated from the diseased duku trees were further used for DNA sequence analysis. DNA was isolated from mycelia cultured at 27°C for seven days in malt extract broth (Difco Laboratories, Sparks, MD) in plastic Petri dishes. Total DNA was extracted using bead-beating technology and the silica spin filter method (Mo Bio and Geneaid Kit) according to the manufacturer's instructions. DNA concentration and purity were measured spectrophotometrically. The ITS1/5.8 S rDNA/ITS2 (ITS) region of *Ceratocystis* isolates was amplified by PCR, using ITS1 (forward: 5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (reverse: 5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The β-tubulin gene (TUB) region was amplified by PCR, using βt1a (forward: 5'-TTCCCCCGTCTCCACTTCTTCATG-3') and βt1b (5'-GACGAGATCGTTCATGTTGAACTC-3') (Glass & Donaldson, 1995). PCR reaction mixtures consisted of 1 µl of each primer (10 mM), 15 µl of 1st BASE REDIANT 2X PCR Master Mix (#BIO-5185), 3 µl of DNA template (2-10 ng) and 10 µl nuclease-free water to make up 30 µl total volume reactions. PCR was performed using Thermal Cycler (Agilent, SureCycler 8800) with a 5-minute 95°C denaturation step followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 56°C for *ITS* and 55°C for TUB, and 40 s extension at 72°C, followed by a final extension of 5 min at 72°C. Negative controls (without template DNA) were applied in each assay. The PCR products of ITS and TUB regions were sequenced at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia.

Identification of isolates was accomplished by BLAST searches of the *ITS* and *TUB* sequences on the GenBank database (http://www.ncbi.nlm.nih.gov). BLAST identification suggested that both isolates belonged to the species *Ceratocystis fimbriata*. To determine relatedness of isolates from duku with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014) and phylogenetic analyses were performed. Representative sequences of ITS haplotypes of *C.*

fimbriata as designated by Harrington et al. (2014) and ITS sequences of accession numbers KF878326, KF650948, AM712445, AM292204, MF033455, EU588656, KC261853~~EU588656 and KC261853~~, which most closely matched with isolates from duku, were used in the analyses. *C. variospora* (accessions AF395683) ~~*C. cacaofunesta* isolate C1004~~ was used as the outgroup taxon. There were ~~33~~ 35 ITS sequences in the dataset (Table 1) and the sequences were initially aligned using clustal-W and then manually adjusted by adding gaps in MEGA X (Kumar et al., 2018). The relationships between ITS sequences of isolates from *L. domesticum* and other representative genotypes of the *C. fimbriata* sensu stricto (Harrington et al., 2014; Oliveira et al., 2015) were analysed using genetic distance matrices, unweighted pair group method with arithmetic means (UPGMA), and 1000 bootstrap replications under PAUP 4.0b10 (Swofford, 2003).~~Phylogenetic analysis was performed to reconstruct maximum parsimony trees with (PAUP) version 4.0b10 (Swofford, 2003). Characters were unweighted and unordered with gaps treated as fifth state. A total of 1,000 bootstrap replicates with simple stepwise addition and TBR swapping was implemented.~~

Pathogenicity tests. Two isolates identified using DNA sequence data were used to test for pathogenicity. Pathogenicity tests were conducted on one-year-old duku (*Lansium domesticum* var. *domesticum*) seedlings grown in a partially flooded and in a non-flooded nursery. Seedlings were grown in 20 cm diameter plastic pots containing a mixture of topsoil and compost under a 25% shading net. The pots from the flooded nursery were placed in a tray filled with tap water, which was maintained to a depth of 2-3 cm. Pathogenicity was also tested on three-month-old ~~Acacia~~ acacia (~~*Acacia*~~ *A. mangium*) and six-month-old mango (*Mangifera indica* cv. Arumanis) seedlings.

Preliminary tests showed that stem inoculations with a mycelial plug were ineffective unless the bark was wounded. Therefore, wound inoculation was used throughout the experiments. Wounds were made by puncturing three points on the bark to a 3-mm depth using

a sterile 28g needle, and a 2 × 2 mm agar plug taken from an actively growing colony on 2% MEA was placed in the wound with the mycelium downward. This was covered with a section (10 × 10 mm) of wetted tissue paper and wrapped with clear tape to reduce contamination and desiccation. The inoculum along with the wrapping plastic was removed at three days post-inoculation. Each isolate was injected into ten seedlings for each flooded and non-flooded group of seedlings. For uninoculated controls~~control experiments~~, wounded bark was wrapped with sterile MEA plugs. Whole experiments were repeated twice and data were pooled after verifying the variance homogeneity using the Levene test. ~~with similar results and the results from the second experiment was presented.~~

Disease severity was assessed 20 days post-inoculation based on the length of wood discolouration. Sections were cut from the margins of lesions, surface-sterilized, and plated on MEA or inserted into a carrot dish to re-isolate the inoculated fungus to complete Koch's postulates. Fungal identity was verified by colony, anamorph, and teleomorph morphology.

Results

Field observations and symptom development. Diseased trees were characterized by wilting of some twigs or branches, followed by defoliation and dieback. In most cases, total plant wilt or death was observed within six months from the first appearance of wilt (Fig. 1A, 1B). Bark canker was eventually found on heavily infected trunks or dead trees (Fig. 1D). Scraping the bark down to the wood along the wilted side of the trunk up to the branch revealed extensive areas of discoloured tissue (Fig. 1E, 1F). The discoloured wood typically had a streaked appearance, turning a uniform dark brown with age and could be found beneath the outermost layers of sapwood (Fig. 1E) and in some cases, discolouration extended to the heartwood (Fig. 1F). All diseased trees had been attacked by squirrels (Fig. 1C) and lesions appeared to

originate from surrounding beetle entry/exit holes (Fig. 1E) on the peeled-off bark, indicating the involvement of ~~either an insect-borne or~~a wound pathogen.

The disease was observed along the watershed of the Komering River, including Lubuk Batang (OKU District) and Rasuan (OKU Timur District), all in South Sumatra Province of Sumatra. Affected trees ranged from young (< 5 years) to old (> 50 years) in age. Disease incidence and severity were highest in Lubuk Batang Lama, where the disease first appeared. The disease progress both in term of incidence and severity was fast. All trees (100%) from eight sampled duku orchards in Ogan Komering Ulu (OKU) District of South Sumatra where the disease originated had wilted and died in the November 2017 survey (Table 2). In the 2019 field observation, the disease was found to have sporadically killed duku trees in Ogan Komering Ulu Timur (OKUT) District (within 100 km of the disease origin). Squirrel attacks were not found on the recently infected trees. Disease was not found in other duku orchards of South Sumatra in OKI, PALI and Muara Enim Districts. There was no appearance of squirrel scratches in those disease-free orchards.

Culture characteristics and morphology. Fungi typical of genus *Ceratocystis* were consistently isolated from direct plating of diseased wood on to both MEA and carrot slices. Colonisation of *Phytophthora* on diseased wood was not detected by baiting using cacao pods and cucumber fruit. *Ceratocystis* isolates from *L. domesticum* trees were typical of *Ceratocystis* spp. in the *C. fimbriata* s.l. species complex, having characteristic olive-green colonies and the typical banana-fruit odour. They had globose to sub-globose ascomata with long necks and typical divergent ostiolar hyphae at their tips (Fig. 2). Teleomorph and anamorph structures were produced within two weeks on MEA cultures. Two isolates (WRC and WBC) were described and both had ascopore (4-7×3-5 µm), cylindrical conidia (14-25×4-5 µm), and aleuroconidia sizes (11-16×7-11 µm) within the range of those of *C. fimbriata s.s.* neotype BPI

595863 (Engelbrecht and Harrington, 2005). ~~WRC~~ Both isolates produced a barrel-shaped (doliform) conidia (8-10×6-8 µm) in chain (Fig. 2).

~~ascopore, cylindrical conidia, and aleuroconidia with similar sizes and shapes to *C. acaciivora*, a pathogen of cancer and wilt of *A. mangium* in Indonesia (Tarigan et al., 2011) (Table 3).~~

~~BLAST searches of the ITS region and β-tubulin (TUB) of WBC and WRC isolates resulted in > 93% hits of significant sequence homology (99.64-100% similarity) with *Ceratocystis fimbriata* against the NCBI GenBank database. A significant sequence similarity (99.66-100%) also resulted with *C. acaciivora* and *C. manginecans* (Table 3). WBC and WBC isolates had differences in two bases of ITS sequence (99.6% similarity) and 100% homology in TUB sequence. Manual alignment and phylogenetic analysis of the ITS rDNA sequence of WRC grouped the isolate into ITS5 haplotype of *C. fimbriata* as designated by Harrington et al. (2014). Manual alignment of ITS sequence could not group WBC within any ITS haplotype, but phylogenetic analysis clustered the isolate within ITS5, ITS6, and ITS7 (Fig. 3).~~ **Sequence Analyses.** WBC and WBC isolates had differences in two bases of ITS sequence (99.6% similarity), but had a 100% similarity in the TUB sequence. BLAST searches of the ITS region of WRC (MT229127) and WBC (MT229128) identified both sequences with the GenBank deposits for *Ceratocystis fimbriata* with 100% of similarity and query coverage. A similar BLAST result was obtained with the TUB sequence (MW013766 and MW013767 for WBC and WBC, respectively) and confirmed the assignment to *Ceratocystis fimbriata* with 100% of similarity and query coverage.

Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al., 2014) grouped the isolates into ITS5 and ITS6z haplotype of *C. fimbriata* for WRC and WBC, respectively. The WRC showed 100% similarity with other ITS5 haplotype of *C. fimbriata* isolated from tea tree (KF650948), taro (AM712445), pomegranate

(AM292204) in China; from eucalyptus (KF878326) in Zimbabwe; from acacia (MF033455) in Vietnam; and from acacia (EU588656) in Indonesia. WBC had 100% similarity with member of ITS6z haplotype of *C. fimbriata* isolated from *Hypocryphalus mangifera* (KC261853) in Oman. UPGMA analysis clustered both isolates from *L. domesticum* within a single group consisted of both ITS5 and ITS6 haplotypes (Fig. 3).

Pathogenicity Test. In pathogenicity tests, initial symptoms appeared as water-soaked brown lesions on the wound site within three days after inoculation. The lesions remained small at inoculation sites on bark, but scraping the bark down to the wood revealed extensive areas of discoloured xylem tissue upward and downward from the inoculated site (Fig. 4A). Upward extension of xylem discolouration from the inoculation site was more extensive ($P<0.0001$) than downward extension on duku seedling inoculated with WRC. However, no significant difference ($P\geq0.05$) between upward and downward discolouration extension was exhibited by WRC on acacia and mango and by WBC on all hosts (Table 3). This kind of discoloured xylem was similar to a typical symptom of diseased trees in the field. The WRC isolate was more pathogenic on duku seedling than WBC as it induced significantly ($P<0.05$) longer lesions and caused more ($P<0.05$) plant wilt and death (Fig. 4A). Plant wilt and death was observed within 20 days post-inoculation and later the wilting incidence gradually increased. Regrowth of lateral shoots was observed on wilted plants. The control plants, inoculated with malt extract agar, remained asymptomatic and had only a trace of xylem discolouration (less than 5 mm in length) at the wound site (Table 3). Partial flooding of duku seedling did not significantly ($P=0.163$) affect extension of the xylem discoloration, but plant mortality by WRC was lower ($P<0.05$) than on non-flooded seedling (Table 3). ~~Disease was less developed on plants growing under partial flooding. Inoculated plants growing in partially flooded pots exhibited less xylem discolouration than non-flooded plants. Wilting and dead plants were also observed less among the partially flooded plants than the non-flooded plants (Table 4).~~ Fungus with the same

morphological characteristics was re-isolated from diseased wood of inoculated seedlings, but not from any of the control plants.

Ceratocystis isolates also induced xylem discolouration and wilt symptoms on inoculated *A. mangium* seedlings (Fig. 4B), similar to that observed on duku seedlings. Xylem discolouration on ~~Acacia~~ acacia developed faster than on duku and was equally extensive (P≥0.05) for both upward and downward expansion (Table 3). Plant wilt and death was observed earlier on a~~A~~cacia compared to duku with half the WRC-inoculated ~~Acacia~~ acacia dying within 20 days post-inoculation. Similar to what was observed on duku seedlings, the WRC isolate caused significantly (P<0.05) longer lesion and more death on acacia and therefore, proved to be more pathogenic than WBC (Table 3). *Ceratocystis* isolates were also pathogenic on mango (*M. indica*), but did not induce wilting symptoms (Fig. 4C). Mycelial plug inoculation on stems of mango resulted in wood discolouration similar to the symptoms on duku and ~~Acacia~~ acacia (Fig. 4C), but with less expansive discolouration (Table 3).

Discussion

This study presents the first report of *Ceratocystis fimbriata* associated with massive mortality of *L. domesticum* trees in South Sumatra, Indonesia. This fungus was shown to be pathogenic by producing expansive wood discolouration and causing lethal wilt on inoculated duku seedlings similar to that found in the field. Fungus with the same morphological characteristics was easily re-isolated from diseased wood of inoculated seedlings, suggesting fulfilment of Koch's postulates. Inoculation experiments on ~~Acacia~~ acacia seedlings suggested that the pathogen was also pathogenic there by producing more expansive wood discolouration, bark canker, wilting symptoms, and plant death. *Ceratocystis* isolates from duku proved to be less pathogenic on mango, as less wood discolouration was induced, without wilting and plant death.

Morphological characteristics showed ~~and molecular-based identification suggested~~ that the pathogen belonged to the species ~~was closely related to~~ *C. fimbriata*~~, *C. acaciivora* and *C. manginecans*~~. (Engelbrecht & Harrington, 2005). ~~Isolate WRC was more pathogenic than WBC on both *L. domesticum* and *A. mangium*. WRC produced ascopore, cylindrical conidia, and aleuroconidia with similar sizes and shapes to *C. acaciivora*, a pathogen of cancer and wilt of *A. mangium* in Indonesia (Tarigan et al., 2011).~~ Both *Ceratocystis* isolates from duku (WRC and WBC) had a similar morphology to *C. fimbriata s.s.* neotype BPI 595863 (Engelbrecht and Harrington, 2005), except for doliform conidia that were absent on BPI 595863. ~~Harrington et al. (2014, 2015) and Oliveira et al. (2015) considered *C. acaciivora* and *C. manginecans* to be synonyms or conspecifics of *C. fimbriata* sensu stricto.~~

The ITS rDNA sequence of the most pathogenic isolate, WRC (MT229127), had an identical sequence to the isolates of *C. fimbriata* from tea tree (KF650948), taro (AM712445), and pomegranate (AM292204) in China; from eucalyptus (KF878326) from Zimbabwe; from acacia (MF033455) in Vietnam; and from acacia (EU588656) in Indonesia. All these isolates were confirmed belong to ITS5 haplotype of *C. fimbriata* (Harrington et al., 2014; Li et al., 2016). Some of these isolates were previously identified as *C. acaciivora* (Tarigan et al., 2011) and subsequently reconsidered as *C. manginecans* (Fourie et al., 2015), but Oliveira et al., (2015) considered those cryptic species to be synonyms or conspecifics of *C. fimbriata* sensu stricto. The ITS5 haplotype is an aggressive genotype of *C. fimbriata* causing a lethal wilt disease of economically important plants worldwide. This genotype represented the native *C. fimbriata* populations in Brazilian forest plantations of *Eucalyptus* spp. (Harrington et al., 2014;). This ITS haplotype was also found infecting *Acacia* spp. and its original host, *Eucalyptus* spp. in China, Indonesia, South Africa, Thailand, Uruguay (Harrington et al., 2014), Zimbabwe (Jimu et al. 2015) and Vietnam (Trang et al. 2017). The member of this *Eucalyptus* population of *C. fimbriata* cause the wilt epidemic on kiwifruit in Brazil (Ferreira et al., 2017).

In China, the ITS5 genotype has been considered to be introduced from Brazil through *Eucalyptus* cuttings and reported to cause epidemics on pomegranate, loquat, and taro (Harrington et al., 2015; Li et al., 2016), and tea tree (Xu et al., 2019). The less pathogenic isolate, WBC, showed homology sequence to the type Y of the ITS rDNA of *C. fimbriata* isolate CMW13582 (KC261853) from *Hypocryphalus mangifera* in Oman (Naidoo, 2013). Both isolates were grouped to ITS6z haplotype of *C. fimbriata* (Harrington et al., 2014). In this study, WBC showed also a weak aggressiveness on an Indonesian cultivar of mango. Report on disease epidemic caused by this genotype of *C. fimbriata* was not available, and it is likely that the ITS6z haplotype is a less aggressive pathogen.

~~Manual alignment and phylogenetic analysis of the ITS rDNA sequence of WRC grouped the isolate into ITS5 haplotype of *C. fimbriata* as designated by Harrington et al. (2014). Manual alignment of ITS sequence could not group the less pathogenic isolate (WBC) within any of ITS haplotypes, but phylogenetic analysis clustered the isolate within ITS5, ITS6, and ITS7. ITS5 haplotype of *C. fimbriata* represented isolates from *Eucalyptus* spp. and *Acacia mearnsii*. Members of ITS6 and ITS7 represented isolates from *Eucalyptus* spp. and *Acacia* spp. in Indonesia, and isolates from mango in Oman, Pakistan and Brazil (Harrington et al., 2014).~~

C. fimbriata has been known to infect a wide variety of annual and perennial host plants throughout the world. In Indonesia, diseases caused by *C. fimbriata* are considered to be of minor importance due to non-lethal and sporadic infestation. The fungal infection has long been noted to cause a non-lethal disease known as mouldy rot on the trunk of rubber trees (Tayler and Stephens, 1929). The role of fungal infection as the primary causal agent of the disease has been dismissed since mouldy rot is considered an advanced stage of a physiological disorder induced by excessive tapping and ethylene overstimulation (Putranto et al. 2015) and the disease can be eliminated by treatment with non-fungicidal biostimulants (Suwandi et al.,

2018). In the last decade, disease incited by *C. fimbriata* has been one of the most destructive and economically important diseases on ~~A~~acacia plantations in Indonesia, shortly after an outbreak on the industrial forest plantations throughout the world (Roux and Wingfield, 2009). Outbreaks of *Ceratocystis* disease have forced the replacement of thousands of hectares of *A. mangium* plantations in eastern Sabah, Malaysia (Brawner et al., 2015). In Indonesia, *Ceratocystis* infection has contributed to 2% mortality by the fourth rotation of *A. mangium* in Sumatra, Indonesia (Hardie et al., 2017). Pathogens causing lethal wilt of duku belong to ITS haplotype 5, which represented *C. fimbriata* populations from forest plantations of *Acacia* spp. and *Eucalyptus* spp. Pathogenicity tests also confirmed that *A. mangium* is more susceptible than the original host (duku tree), suggesting the establishment of *C. fimbriata* pathogenicity on ~~Acacia~~ acacia as the main host. Similar disease symptoms caused by *Ceratocystis* infections were found to be endemic on ~~Acacia~~ acacia and eucalyptus plantations located about 30 km away from the site of study. It is likely that population of *C. fimbriata* pathogenic on acacia plantation could extend their host range to native fruit tree such as *Lansium* and cause a serious threat to the neighbouring fruit tree species. The host-range extension by the ITS5 haplotype of *C. fimbriata* to the susceptible neighbouring plants occurred in Brazil, in which the genotype from eucalyptus showed strong aggressiveness on taro (Harrington et al., 2011) and caused epidemic on grapevine (Ferreira et al., 2017). Similar host extension by the ITS5 haplotype also occurred in China, in which the eucalyptus population caused epidemic on pomegranate, loquat, and taro (Harrington et al., 2015; Li et al., 2016), and tea tree (Xu et al., 2019).

All sampled diseased trees had been previously attacked by squirrels and lesions appeared to originate from surrounding beetle entry/exit holes on peeled-off bark from squirrel scratches, suggesting the involvement of the wild vertebrate as the wound creator and beetles for fungal spore ~~transmission and infection~~dispersion. Fungal feeding insects, such as ~~*nitidulid and ambrosia beetles*~~*Hypocryphalus mangiferae*, ~~are~~ have been ~~known~~ suggested to be associated

with the rapid distribution of *C. fimbriata* in Oman and Pakistan ~~as the main vectors of the *Ceratocystis* disease~~ (~~Hinds, 1972;~~ Al Adawi et al., 2013). Squirrel attacks on either diseased or healthy duku trees were found only during the disease outbreaks in 2013-2014~~on disease-affected orchards~~ and these attacks were likely due to the limitation of squirrel feed sources in the field. All affected orchards had grown duku in a monoculture. Pathogenicity tests supported the idea that partial flooding was not likely to predispose duku trees to *Ceratocystis* infection as the disease did not develop well under partial flooding. Recent field observations in areas near the disease origin suggested that the disease spreads sporadically~~, but without evidence of massive~~ with limited mortality. Squirrel attacks were not found on recently infected trees, suggesting the possible involvement of the wild vertebrate wounds on the massive disease spread in duku orchards. Vertebrate-incited wounds, such as those from squirrels and monkeys, are considered to contribute to the spread of *Ceratocystis* wilt on *A. mangium* plantations (Brawner et al. 2015; Hardie et al. 2017; Nasution et al., 2019).

~~Initial field symptoms of most diseased duku trees started from an infected point on twigs or branches and the wood discolouration expanded to both lower and upper parts of trees. Upward or downward lesion expansion was also confirmed in inoculated seedlings of duku, Acacia and mango in this study and has been previously reported on Acacia (Tarigan et al., 2011) and mango (Al Adawi et al. 2013). Proper pruning of diseased branches at the healthy area under the discoloured wood could stop the downward lesion expansion and control the disease. Pruned trees were ready to produce new healthy shoots and continue growing without apparent re-infection for three years as practically applied in an orchard and shown in a pot trial. On Acacia plantations, improper pruning techniques are considered to promote dispersal of *Ceratocystis* wilt (Roux and Wingfield, 2009; Tarigan et al., 2011b), but tip pruning using hand shears has been reported to reduce disease infestation (Chi et al., 2019).~~

Acknowledgements

This research was supported by the Sriwijaya University Priority Applied Research Project 023/SP2H/LT/DPRM/II/2016.

References

Al Adawi, A. O., Al Jabri, R. M., Deadman, M. L., Barnes, I., Wingfield, B. D., Wingfield, M. J. 2013. The mango sudden decline pathogen, *Ceratocystis manginecans*, is vectored by *Hypocryphalus mangiferae* (Coleoptera: Scolytinae) in Oman. *Eur J Plant Pathol* 135: 243-251.

BPS-Statistics Indonesia, 2018. Indonesian Statistic of Annual Fruit and Vegetable Plants. https://www.bps.go.id/publication/2018/10/05/081665ec9eb65fdce8a69473/statistik-tanaman-buah---buahan-dan-sayuran-tahunan-indonesia-2017 [17 March 2020].

Brawner, J., Japarudin, Y., Lapammu, M., Rauf, R., Boden, D. and Wingfield, M. J. 2015. Evaluating the inheritance of *Ceratocystis acaciivora* symptom expression in a diverse *Acacia mangium* breeding population. *South. Forests* 77: 83-90.

Brito, R. A. S., Cavalcante, G. P., Borel, F. C. and Maffia, L. A. 2019. Detection and isolation of *Ceratocystis fimbriata* in mango trees on semi-selective medium. *Eur. J. Plant Pathol.* 155:667-669.

~~Chi, N. M., Thu, P. Q., Hinh, T. X. and Dell, B. 2019. Management of *Ceratocystis manginecans* in plantations of *Acacia* through optimal pruning and site selection. *Australas. Plant Pathol.* 48:343-350.~~Engelbrecht, C. J. B. and Harrington, T. C., 2005. Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. *Mycologia* 97:57-69.

Ferreira, M. A., Harrington, T. C., Alfenas, A. C. and Mitzubuti, E. S. G. 2011. Movement of genotypes of *Ceratocystis fimbriata* within and among *Eucalyptus* plantations in Brazil. *Phytopathology* 101:1005-1012.

Ferreira, M. A., Harrington, T. C., Piveta, G., and Alfenas, A. C. 2017. Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil. *Trop. plant pathol.* 42:86–95.

Glass, N. L. and Donaldson, G. C. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl. Environ. Microbiol.* 61:1323-1330.

Hardie, M., Akhmad, N., Mohammed, C., Mendham, D., Corkrey, R., Gafur, A. and Siregar, S. 2018. Role of site in the mortality and production of *Acacia mangium* plantations in Indonesia. *South. Forests* 80:37-50.

Harrington, T. C., Huang, Q., Ferreira, M. A. and Alfenas, A. C. 2015. Genetic analyses trace the Yunnan, China population of *Ceratocystis fimbriata* on pomegranate and taro to populations on eucalyptus in Brazil. *Plant Dis.* 99:106-111.

Harrington, T. C., Kazmi, M. R., Al-Sadi, A. M. and Ismail, S. I. 2014. Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata* sensu stricto. *Mycologia* 106:224-242.

Harrington, T. C., Thorpe, D. J., and Alfenas, A. C. 2011. Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of *Ceratocystis fimbriata*. *Phytopathology* 101:555-566.

Hinds, T. E. 1972. Insect transmission of *Ceratocystis* species associated with aspen cankers. *Phytopathology* 62:221-225.

Jimu, L., Wingfield, M. J., Mwenje, E. and Jolanda Roux, J. 2015. Diseases on *Eucalyptus* species in Zimbabwean plantations and woodlots. *South. For.* 77: 221-230.

Keith, L. M., Matsumoto, T. K. and McQuate, G. T. 2013. First report of *Dolabra nepheliae* associated with corky bark disease of langsat in Hawaii. *Plant Dis.* 97:990.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* 35:1547-1549.

Li, Q., Harrington, T. C., McNew, D., Li, J., Huang, Q., Somasekhara, Y. M. and Alfenas, A. C. 2016. Genetic Bottlenecks for Two Populations of *Ceratocystis fimbriata* on Sweet Potato and Pomegranate in China. *Plant Dis*. 100: 2266-2274.

Moller, W. J. and DeVay, J. E. 1968. Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology* 58:123-124.

Naidoo, K., Steenkamp, E. T., Coetzee, M. P., Wingfield, M. J. dan Wingfield, B. D. (2013). Concerted evolution in the ribosomal RNA cistron. *PloS one*, *8*(3), e59355.

Nasution, A., Glen, M., Beadle, C. and Mohammed, C. 2019. Ceratocystis wilt and canker - a disease that compromises the growing of commercial acacia-based plantations in the tropics. *Aus. For.* 82: 80-93.

Oliveira, L. S., Harrington, T. C., Ferreira, M.A., Damacena, M. B., Al-Sadi, A. M., Al-Mahmooli, I. H. and Alfenas, A. C. 2015. Species or genotypes? Reassessment of four recently described species of the ceratocystis wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology* 105:1229-1244.

Putranto, R. A., Herlinawati, E., Rio, M., Leclercq, J., Piyatrakul, P., Gohet, E., Sanier, C., Oktavia, F., Pirrello, J., Kuswanhadi, Montoro P. 2015. Involvement of ethylene in the latex metabolism and tapping panel dryness of *Hevea brasiliensis*. *Int. J. Mol. Sci.* 16:17885-17908.

~~Roux, J. and Wingfield, M. J. 2009. *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. *South. For.* 71:115-120.~~

Suwandi, S., Junita, A., Suparman, S., Umayah, A., Hamidson, H., Muslim, A. and Irsan, C. 2018. Curative activity of watery fermented compost extract as a bark treatment against tapping panel dryness. *Open Agr. J.* 12:74-83.

Swofford, D. L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods), v. 4.0b10*. Sunder-land, MA: Sinauer Associates.

Tarigan, M., Roux, J., van Wyk, M., Tjahjono, B. and Wingfield, M. J. 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.

~~Tarigan, M., Wingfield, M. J., van Wyk, M., Tjahjono, B. and Roux, J. 2011b. Pruning quality affects infection of *Acacia mangium* and *A. crassicarpa* by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*. *South. For.* 73:187-191.~~

Tayler, V. A. and Stephens, J., 1929. *Native Rubber in the Dutch East Indies*. Rubber Growers Association, London, UK, 48 pp.

Techavuthiporn, C. 2018. Langsat- *Lansium domesticum*. In: *Exotic fruits*, eds. by S. Rodrigues, E. D. O. Silva and E. S. D. Brito, pp. 279-283. Academic Press, New York, USA.

Trang, T. T., Eyles, A., Davies, N., Glen, M., Ratkowsky, D. and Mohammed, C. Screening for host responses in *Acacia* to a canker and wilt pathogen, *Ceratocystis manginecans*. *For Path*. 48:e12390.

Van Wyk, M., Al Adawi, A. O., Khan, I. A., Deadman, M. L., Wingfield, B. D. and Wingfield, M. J. 2007. *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Divers*. 27:213-230.

Van Wyk, M., Al-Adawi, A. O., Wingfield, B. D., Al-Subhi, A. M., Deadman, M. L. and Wingfield, M. J. 2005. DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman. Australas. *Plant Pathol*. 34:587-590.

White, T. J., Bruns, T. D., Lee, S. B. and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*, eds. by M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, pp. 315-322. Academic Press, New York, USA.

Whitman, W. F. 1980. Growing and fruiting the langsat in Florida. *Proc. Fla. State Hort. Soc.* 93:136-140.

Xu, K. C., Zhang, R. Q., Li, J., Bai, Y. H., Yang, X. D., Sun, Y. X. and Huang, Q. 2019. *Camellia sinensis*, a New Host Plant of *Ceratocystis fimbriata* from China. *Plant Dis.* 103:2670.

Yaacob, O. and Bamroongrugsa, N. 1991. *Lansium domesticum* Correa. In: *Plant Resources of South-East Asia no. 2: 4dible fruits and nuts*, eds. by E. W. M. Verheij and R. E. Coronel, pp. 186-190. Pudoc, Wageningen, The Netherlands.

Table 1. Collection details and GenBank accession number of ITS sequence for isolates of *Ceratocystis fimbriata* included in this study

Isolate	GenBank Accession no.	ITS haplotype	Host	Origin	Reference
C1418	AY157956	1a	*Ipomoea batatas*	USA	Harrington et al., 2014
C1857	HQ157542	1	*Ficus carica*	Brazil	Harrington et al., 2014
CMW4797	FJ236733	1b	*Eucalyptus* sp.	Congo	Harrington et al., 2014
CMW9998	FJ236721	1b	*Eucalyptus* sp.	South Africa	Harrington et al., 2014
C1655	HQ157546	2	*Mangifera indica*	Brazil	Harrington et al., 2014
C1440	HQ157544	3	*Eucalyptus* sp.	Brazil	Harrington et al., 2014

CMW5328	AF395686	3	*Eucalyptus grandis*	Uganda	Harrington et al., 2014
C1442	HQ157545	4	*Eucalyptus* sp.	Brazil	Harrington et al., 2014
CMW38737	KF878326	5	*Eucalyptus grandis*	Zimbabwe	Jimu et al., 2015
C1345	AY157966	5	*Eucalyptus* sp.	Brazil	Harrington et al., 2014
A59662	KF650948	5	*Camellia sinensis*	China	Xu et al., 2019
YM061	AM712445	5	*Colocasia esculenta*	China	Li et al., 2016
P20053	AM292204	5	*Punica granatum*	China	Li et al., 2016
C1	MF033455	5	*Acacia* sp.	Vietnam	Trang et al., 2017
CMW22563	EU588656	5	*Acacia mangium*	Indonesia	Tarigan et al., 2011
WRC	MT229127	5	*Lansium domesticum*	Indonesia	Present study
C2055	HQ157548	6	*Mangifera* sp.	Brazil	Harrington et al., 2014
CMW13582	KC261853	6z	*Hypocryphalus mangifera*	Oman	Naidoo et al., 2013
WBC	MT229128	6z	*Lansium domesticum*	Indonesia	Present study
CMW13851	AY953383	7b	*Mangifera indica*	Oman	Van Wyk et al., 2005
CMW23634	EF433302	7b	*Mangifera indica*	Pakistan	Van Wyk et al., 2007
CMW22579	EU588658	7b	*Acacia mangium*	Indonesia	Tarigan et al 2011
CMW8856	AY233867	8a	*Citrus* sp.	Colombia	Harrington et al., 2014
CMW17808	EF127990	8c	*Eucalyptus* sp.	Colombia	Harrington et al., 2014
CMW22092	FJ151432	8e	*Eucalyptus deglupta*	Ecuador	Harrington et al., 2014
C1558	AY157965	9	*Mangifera indica*	Brazil	Harrington et al., 2014
C1914	HQ157540	9	*Colocasia esculenta*	Brazil	Harrington et al., 2014
C994	AY157964	10	*Mangifera indica*	Brazil	Harrington et al., 2014
Cf 4	EF042605	10a	*Mangifera indica*	Brazil	Harrington et al., 2014
C1865	AY526286	11	*Colocasia esculenta*	Brazil	Harrington et al., 2014
C1926	HQ157541	12	*Colocasia esculenta*	Brazil	Harrington et al., 2014

C1688	AY526291	14	*Mangifera indica*	Brazil	Harrington et al., 2014
C925	AY157967	15	*Gmelina arborea*	Brazil	Harrington et al., 2014
C924	HQ157539	16	*Gmelina arborea*	Brazil	Harrington et al., 2014

1

2

1 **Table 2**. Incidence of *Ceratocystis* wilt in duku orchards of OKU District, South Sumatra

2

Location (trees/location)	Incidence (%)		
	February 2014	August 2014	November 2017
Belatung (n=66)	36	86	100
Lubuk Batang Baru (n=85)	38	55	100
Lubuk Batang Lama (n=69)	63	100	100

3

4

Table 3. Pathogenicity of *Ceratocystis fimbriata* isolates on one-year-old duku (*Lansium domesticum* var. *domesticum*), three-month-old *Acacia mangium*, and six-month-old *Mangifera indica* cv. Arumanis seedlings

Isolate and plant species	Flooding stress	Length (mm) of wood discolouration[1)]			Wilting and death at 20 dpi	Wilting and death at 60 dpi[2)]
		Downward	Upward	Total		
Lansium domesticum						
WRC	Partial flooding	11.3 ± 1.7*	22.8 ± 6.1	34.1 ± 6.4 ab	1/20	7/20 b
	Without flooding	12.6 ± 1.9*	37.3 ± 11.1	49.9 ± 11.4 a	5/20	15/20 a
WBC	Partial flooding	6.2 ± 0.8	9.6 ± 3.3	15.8 ± 3.4 bc	0/20	0/20 c
	Without flooding	5.0 ± 0.5	5.6 ± 0.8	10.6 ± 1.3 c	0/20	2/20 bc
MEA (control)	Partial flooding	1.9 ± 0.1	2.0 ± 0.1	3.9 ± 0.2 d	0/20	0/20 c
	Without flooding	1.9 ± 0.2	1.9 ± 0.1	3.8 ± 0.3 d	0/20	0/20 c
Acacia mangium						
WRC	Without flooding	42.1 ± 3.5	34.9 ± 7.3	76.9 ± 14.8 a	6/20	17/20 a
WBC	Without flooding	17.8 ± 4.1	18.0 ± 8.4	35.8 ± 6.3 b	1/20	5/20 b
MEA (control)	Without flooding	2.1 ± 0.2	2.1 ± 0.2	4.1 ± 0.4 c	0/20	0/20 c
Mangifera indica cv. Arumanis						
WRC	Without flooding	5.1 ± 1.0	5.6 ± 0.9	9.7 ± 1.7 a	0/20	0/20
WBC	Without flooding	7.1 ± 1.3	7.3 ± 1.1	14.4 ± 1.7 a	0/20	0/20

MEA (control)	Without flooding	1.3 ± 0.1	1.3 ± 0.1	2.6 ± 0.1 b	0/20	0/20

1) Wood discolouration was measured 20 d post inoculation (dpi). Means of downward lesion length labelled with * are significantly different from upward lesion according to the Welch two sample t-test. Means of total lesion length by different plant species followed by common letter are not significantly different according to the HSD test.
2) Number of death plants by different plant species labelled by same letter are not significantly different according to the Fisher's exact test of independence with applying the Bonferroni corrected alpha level

Fig. 1. Symptoms of *Ceratocystis* wilt on duku trees (*Lansium domesticum* var. *domesticum*). (A) Partial wilting and fast dieback of upper twigs and branches. (B) Total plant wilt and dieback after six months of partial wilting. (C) Peeled-off bark of branches due to squirrel attacks on diseased tree. (D) Bark canker on heavily infected trunk. (E) The discoloured wood beneath the outermost layers of sapwood and a beetle entry/exit hole on affected wood. (F) The discoloured wood extended to the heartwood of the basal stem.

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolate WRC from bark canker of *Lansium domesticum*. (A) Globose ascomata with long neck. (B) Ascospores. (C) Cylindrical conidia. (D) Primary phialidic conidiophore with emerging cylindrical conidia. (E) Chlamydosphore. (F) Barrel-shaped conidia in chain. Scale bars A = 100 µm; B = 10 µm; C-F = 50 µm.

Fig. 3. Dendrogram generated by UPGMA showing the genetic relatedness of representative the ITS rDNA genotypes (sequences) ~~One of the three most parsimonious trees based on the ITS rDNA sequences of the representative isolate~~ of the *Ceratocystis fimbriata* sensu stricto or its synonym. The GenBank accession numbers, s~~S~~train numbers, ITS haplotypes, host genera and countries of origin are given for the representatives of each haplotype. Isolates from *Lansium domesticum* in Indonesia were ~~coloured blue~~marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). *C. ~~cacaofunesta~~ variospora* was used as the outgroup taxon. ~~The consistency index, retention index, and the composite index were 0.614, 0.866, and 0.674, respectively.~~ Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on

appropriate nodes. Scale bar indicates genetic distance~~Bar indicates the number of nucleotide substitutions~~.

Fig. 4. Symptoms reproduced from mycelial plug inoculation with *Ceratocystis fimbriata* isolates (WRC and WBC) from *Lansium domesticum* 20 days after inoculation. (A) Symptoms on one-year-old duku seedlings (*Lansium domesticum*) inoculated with malt extract agar plug (control) (I), restricted wood discolouration and non-wilted plant inoculated with WBC (II), partial and total wilting of plant inoculated with WRC (III, IV), upward extensive wood discolouration from inoculated site (red arrow) (V). (B) Symptoms on three-month-old seedlings of *Acacia mangium* showing extensive wood discolouration by WRC and limited lesions by WBC. New lateral shoot growth on diseased *Acacia* (yellow arrow). (C) Symptoms on six-month-old seedlings of *Mangifera indica* cv. Arumanis showing wood discolouration at site of inoculation (red arrow).

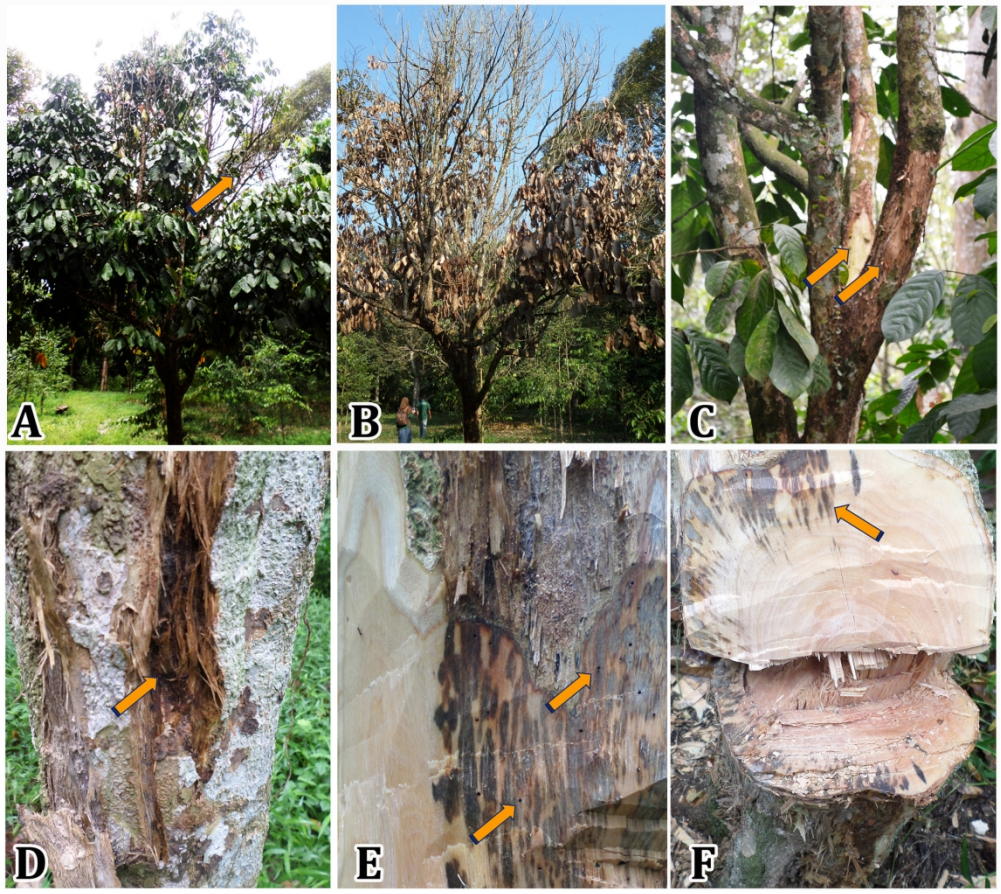

Fig. 1. Symptoms of *Ceratocystis* wilt on duku trees (*Lansium domesticum* var. *domesticum*). (A) Partial wilting and fast dieback of upper twigs and branches. (B) Total plant wilt and dieback after six months of partial wilting. (C) Peeled-off bark of branches due to squirrel attacks on diseased tree. (D) Bark canker on heavily infected trunk. (E) The discoloured wood beneath the outermost layers of sapwood and a beetle entry/exit hole on affected wood. (F) The discoloured wood extended to the heartwood of the basal stem

182x162mm (300 x 300 DPI)

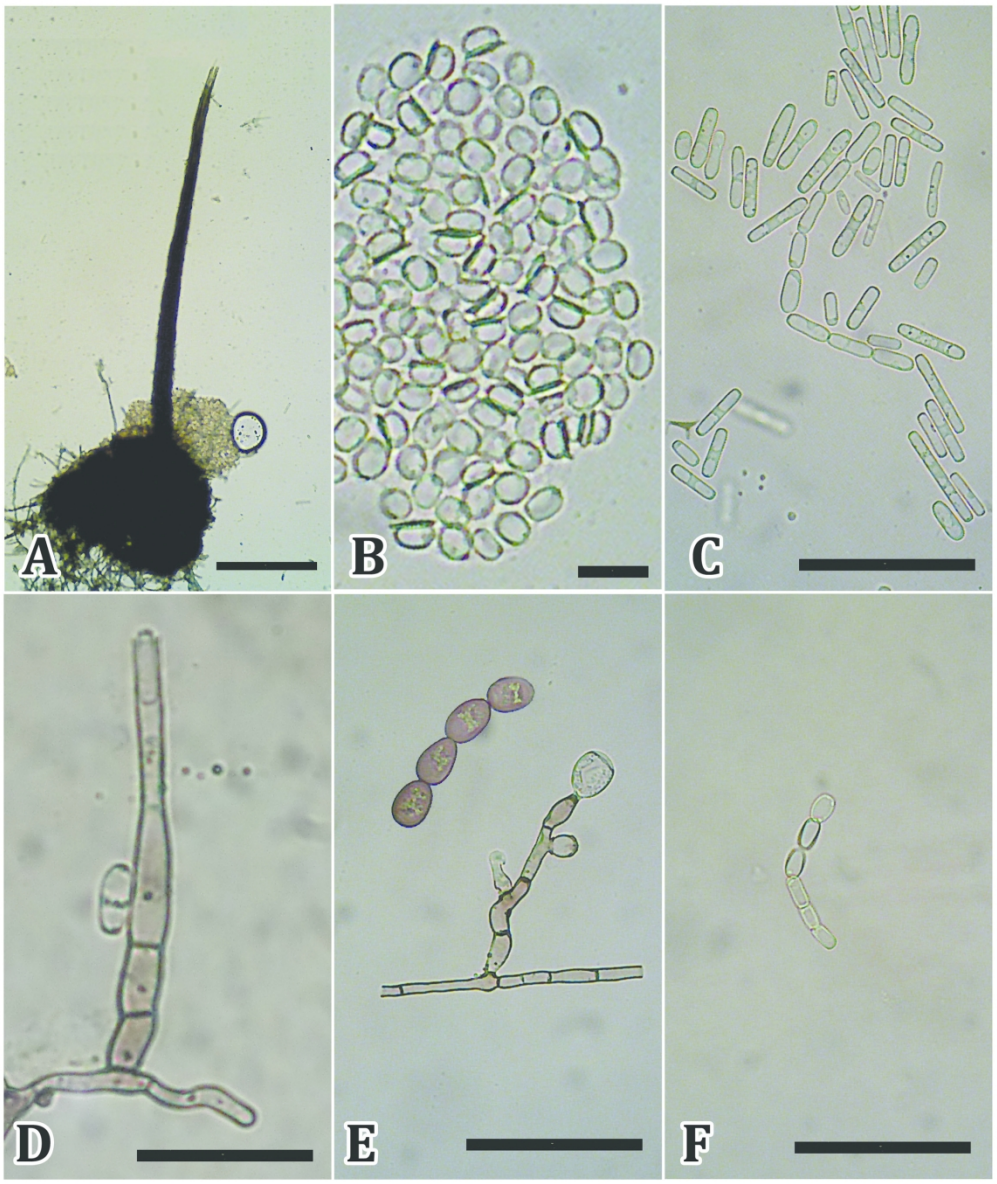

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolate WRC from bark canker of *Lansium domesticum*. (A) Globose ascomata with long neck. (B) Ascospores. (C) Cylindrical conidia. (D) Primary phialidic conidiophore with emerging cylindrical conidia. (E) Chlamydospore. (F) Barrel-shaped conidia in chain. Scale bars A = 100 μm; B = 10 μm; C-F = 50 μm

182x216mm (300 x 300 DPI)

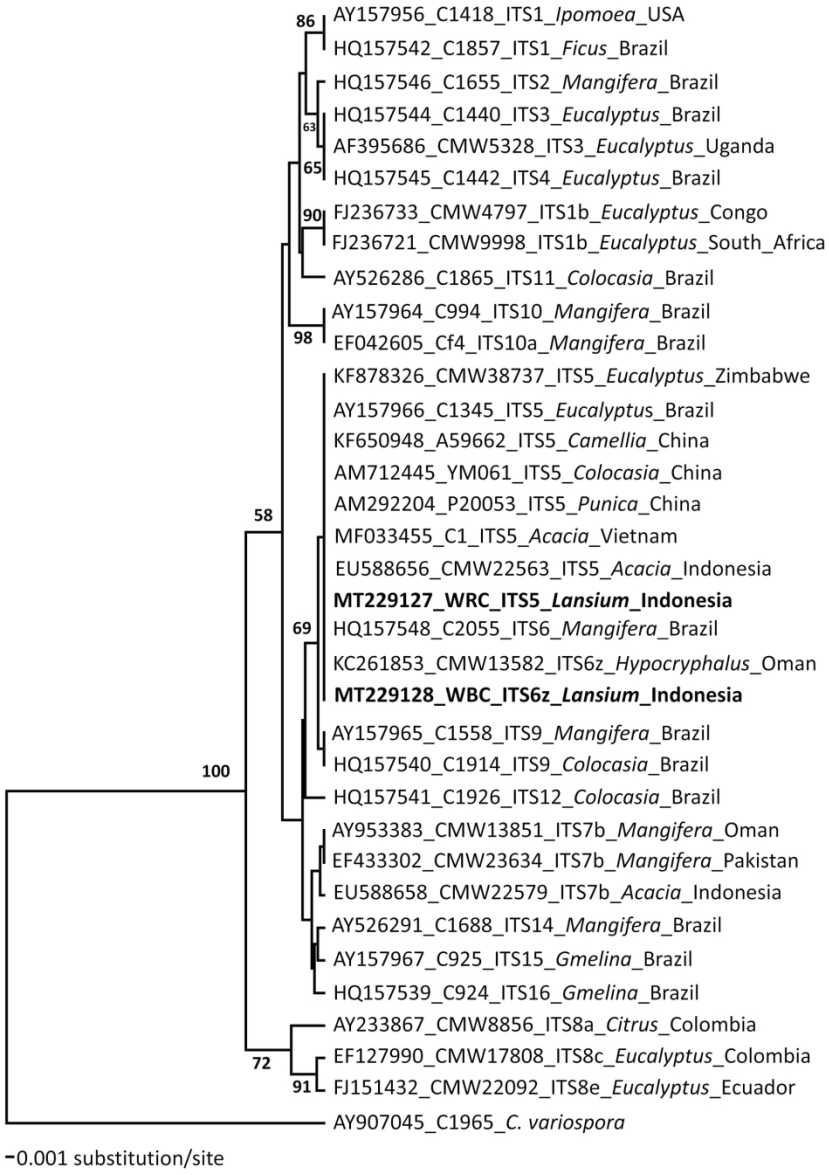

Fig. 3. Dendrogram generated by UPGMA showing the genetic relatedness of representative the ITS rDNA genotypes (sequences) of the *Ceratocystis fimbriata* sensu stricto. The GenBank accession numbers, strain numbers, ITS haplotypes, host genera and countries of origin are given for the representatives of each haplotype. Isolates from *Lansium domesticum* in Indonesia were marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). *C. variospora* was used as the outgroup taxon. Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on appropriate nodes. Scale bar indicates genetic distance.

163x224mm (300 x 300 DPI)

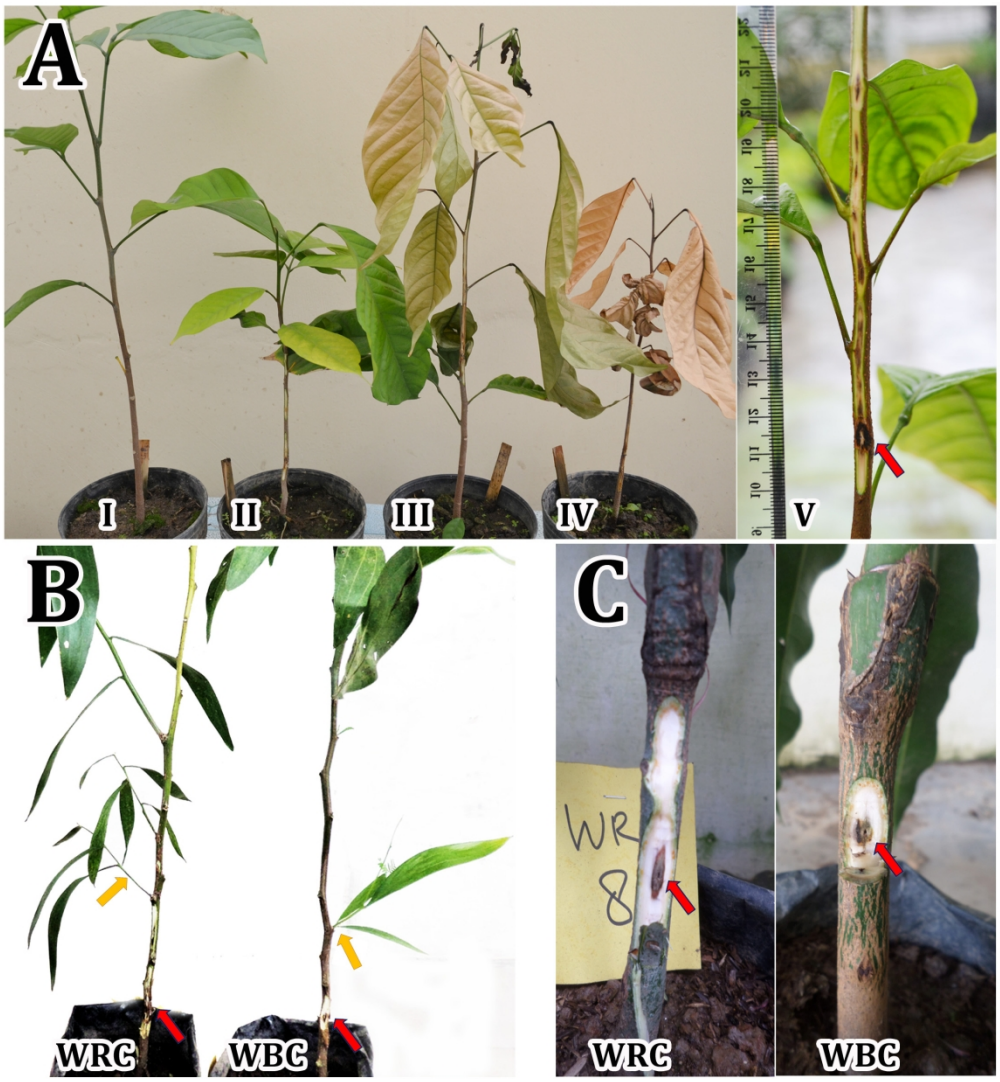

Fig. 4. Symptoms reproduced from mycelial plug inoculation with *Ceratocystis fimbriata* isolates (WRC and WBC) from *Lansium domesticum* 20 days after inoculation. (A) Symptoms on one-year-old duku seedlings (*Lansium domesticum*) inoculated with malt extract agar plug (control) (I), restricted wood discolouration and non-wilted plant inoculated with WBC (II), partial and total wilting of plant inoculated with WRC (III, IV), upward extensive wood discolouration from inoculated site (red arrow) (V). (B) Symptoms on three-month-old seedlings of *Acacia mangium* showing extensive wood discolouration by WRC and limited lesions by WBC. New lateral shoot growth on diseased Acacia (yellow arrow). (C) Symptoms on six-month-old seedlings of *Mangifera indica* cv Arumanis showing wood discolouration at site of inoculation (red arrow).

183x198mm (300 x 300 DPI)

The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-08-2020-0147.R1

1 message

The Plant Pathology Journal <onbehalfof@manuscriptcentral.com> Sun, Dec 20, 2020 at 6:34 PM
Reply-To: paper@kspp.org
To: suwandi@fp.unsri.ac.id, suwandi.saleh@gmail.com
Cc: kiwoo@knu.ac.kr

20-Dec-2020

Dear Dr. Suwandi Suwandi:

Manuscript ID PPJ-OA-08-2020-0147.R1 entitled "Identification and characterization of Ceratocystis fimbriata causing a lethal wilt on Lansium tree in Indonesia" which you submitted to The Plant Pathology Journal, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into https://mc.manuscriptcentral.com/ppj and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using colored text to indicate the altered portion.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to The Plant Pathology Journal, your revised manuscript should be uploaded as soon as possible. If it is not possible for you to submit your revision in a reasonable amount of time, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to The Plant Pathology Journal and I look forward to receiving your revision.

Sincerely,
Prof. Ki Woo Kim
Editor
The Plant Pathology Journal
kiwoo@knu.ac.kr

Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author
The manuscript is greatly improved with a more careful analysis and comparisons of ITS sequences. But the discussion of the ITS haplotypes could be clearer. The two ITS sequences (ITS5 and ITS6z) deposited in GenBank and discussed would fit into the group of ITS5, ITS6, and ITS7b sequences reported in Asia populations. Many isolates in Asia and Oman have mixed ITS sequences due to crosses between these ITS haplotypes, and direct sequencing is often difficult and ambiguous because of intragenomic mixtures. I assume the authors did not discover this sort of ambiguity among

their reads, but if they did it could be added to the manuscript. It would seem that the population of C. fimbriata that they discovered in Sumatra is a combination of ITS5, ITS6, and ITS7b, with ITS6z a result of crossing of these haplotypes. I noted in the alignment that they show C2759 as ITS6z, but this was just one of many ITS haplotypes found for this isolate. and not the most common. C2759 was from Dalbergia in Pakistan. I think it would be best to conclude that you found two haplotypes in Indonesia, one consistent with that found in Oman and Pakistan on the mango bark beetle and Dalbergia (and other hosts) and a second sequence found in China, Indonesia, Vietnam and Brazil on various hosts, including Acacia.

Reviewer: 2

Comments to the Author
This paper reports on one of ophiostomatoid group pathogens, Ceratocystis fimbriata which could detrimental to Lansium trees in Indonesia. The study was performed to identify the causal fungus based on morphology and molecular analysis of ITS and tublin gene sequences. The work was scientifically sound. Two types of isolates were resolved within the C. fimbriata species. Pathogenicity test proved the isolates are the causal agent. Overall, the report contains useful information on Lansium tree disease.
There were several typo errors need to be polished.
Page 7 line 25. ascopore to ascospore
Page 15 line 9. dan to and
Page 16 line 18. Australas to Australas (italic)
Page 24 line 5. Chlamydosphore to Chlamydospore

Some questions remains regarding the experiment.
1. Did the authors observe by microscope the inside part of bark of the tree with discoloured region and insect attacked holes in Fig. 1E? Is there any insect gallery or fungal structures such as perithecia or synnemata (coremia)?
2. Ceratocytis fungi are known to be sensitive to cycloheximide. Have you tried to grow Ceratocystis fimbriata isolates on cycloheximide supplemented PDA or MEA?
3. In the Fig. 4-V, discoloured region was extended to upper part. Can you reisolate the pathogen from the upper discoloured region? How much did the inoculated pathogen move to the discoloured part?
4. What is the mechanism of wood discoloration in Fig. 1 or 4? Fig. 4
5. Regarding sequence analysis, UPGMA tree was constructed. But it is suggested that one of neighbour joining tree, parsimony tree, and maxium likelihood tree would be better. And include a tree based on the tubulin gene sequence.

January 25, 2021

Dear Prof. Ki Woo Kim,
Editor of The Plant Pathology Journal

Enclosed you will find a second revised version with tracked or highlighted changes of the manuscript ID PPJ-OA-08-2020-0147 entitled " Identification and characterisation of Ceratocystis fimbriata causing lethal wilt on the Lansium tree in Indonesia" by S. Suwandi, C. Irsan, H. Hamidson, A. Umayah, and K.D. Asriyani which we would like to re-submit for publication in The Plant Pathology Journal.

Reviewers recommended some revisions that we have made corrections accordingly. We would like to thank for all reviewers' suggestions and corrections. We have noted the contribution from the two anonymous reviewers in our acknowledgement.

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

Reviewer's #1 comment [1]: The manuscript is greatly improved with a more careful analysis and comparisons of ITS sequences. But the discussion of the ITS haplotypes could be clearer.
Our response: We are very appreciating and agreeing for valuable reviewer comments and suggestions. The revisions have been carefully considered and implemented to improve our manuscript

Reviewer's #2 comment [1]: There were several typo errors need to be polished.
Page 7 line 25. ascopore to ascospore
Page 15 line 9. dan to and
Page 16 line 18. Australas to Australas (italic)
Page 24 line 5. Chlamydosphore to Chlamydospore.
Our response: Thank you for reviewer correction, the change has been made accordingly.

Reviewer's #2 comment [2]: Did the authors observe by microscope the inside part of bark of the tree with discoloured region and insect attacked holes in Fig. 1E? Is there any insect gallery or fungal structures such as perithecia or synnemata (coremia)?
Our response: No, we did not make detailed microscope observations on insect digging holes, but we found mycelial masses and perithecia structures on some advanced discoloured wood sections or after incubation in humid condition.

Reviewer's #2 comment [3]: Ceratocytis fungi are known to be sensitive to cycloheximide. Have you tried to grow Ceratocystis fimbriata isolates on cycloheximide supplemented PDA or MEA?
Our response: We did not use cycloheximide-contained PDA or MEA to confirm the species identity. Cycloheximide was not available in our laboratory and the chemical needs more than 3 months for ordering. In this study, we prefer to identify the isolate based on the data of DNA sequences as the sequence analyses are considered appropriate for species identification.

***Reviewer's #2 comment [4]*:** In the Fig. 4-V, discoloured region was extended to upper part. Can you reisolate the pathogen from the upper discoloured region? How much did the inoculated pathogen move to the discoloured part?

Our response: Yes, the inoculated fungus could be reisolated from most of the upper parts of discoloured wood indicating fungal colonization and upward movement along the xylem. We could reisolate the inoculated fungus for 10-30 cm upward (from the inoculation point) on stem of duku seedlings. Therefore, this kind of tissue colonization is a typical systemic infection of vascular wilt pathogen.

***Reviewer's #2 comment [5]*:** What is the mechanism of wood discoloration in Fig. 1 or 4?
Our response: Wood or xylem discolouration could be mainly caused by the accumulation of fungal mycelium and aleurioconidia on the xylem tissues. Fungal colonization and proliferation within the xylem may trigger tyloses, gums and polysaccharides accumulation and cause a water blockage and expose the plant to water stresses. Radial dark striations of wood may also be induced by water stress following the water blockage through xylem vessels. Phytotoxic compounds, to a lesser extent, have also been reported to be associated with necrosis and discoloration of wood and bark tissues.

***Reviewer's #2 comment [6]*:** Regarding sequence analysis, UPGMA tree was constructed. But it is suggested that one of neighbor joining tree, parsimony tree, and maximum likelihood tree would be better.
Our response: We agree with the reviewer comment that neighbor joining tree, parsimony tree, and maximum likelihood tree would be better than UPGMA for construction of phylogenetic tree. MP analysis with our ITS sequences resulted a tree with higher homoplasy index (0.331). NJ tree that is known to comparatively rapid and generally gives better results than UPGMA method, but resulted in a relatively poor bootstrap support compared to the UPGMA with our ITS sequences. The UPGMA method was also have been implemented in the reference studies regarding phylogenetic analysis of ITS haplotype in *C. fimbriata* (Oliveira et al., 2015; Li et al., 2016).

***Reviewer's #2 comment [7]*:** And include a tree based on the tubulin gene sequence.
Our response: We are very appreciating for this comment and agree to include a phylogenetic tree based on the tubulin sequences. A robust MP tree was produced and our Ceratocystis isolates could be clearly identified as C. fimbriata. The MP trees of β-tubulin sequence also supported the conclusion that the population of C. fimbriata causing disease on duku and acacia in Sumatra is a combination of ITS5, ITS6, and ITS7b, with the ITS6z a result of crossing of these haplotypes.

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in the The Plant Pathology Journal. Please feel free to contact me if you need any additional information or clarification.

Sincerely,
Suwandi Suwandi
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: suwandi@fp.unsri.ac.id; suwandi.saleh@gmail.com

The Plant Pathology Journal

Identification and characterization of *Ceratocystis fimbriata* causing a lethal wilt on *Lansium* tree in Indonesia

Journal:	*The Plant Pathology Journal*
Manuscript ID	PPJ-OA-08-2020-0147.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	25-Jan-2021
Complete List of Authors:	Suwandi, Suwandi; Sriwijaya University Faculty of Agriculture, Plant Protection Irsan, Chandra; Sriwijaya University Faculty of Agriculture, Plant Protection Hamidson, Harman; Sriwijaya University Faculty of Agriculture, Plant Protection Umayah, Abu; Sriwijaya University Faculty of Agriculture, Plant Protection Asriyani, Khoirotun ; Sriwijaya University Faculty of Agriculture, Plant Protection
Keyword:	A. Plant Pathogens

Identification and characterisation of *Ceratocystis fimbriata* causing lethal wilt on the *Lansium* tree in Indonesia

Running text: *Ceratocystis fimbriata*, wilt pathogen of *Lansium* tree

Suwandi Suwandi*, Chandra Irsan, Harman Hamidson, Abu Umayah, Khoirotun Dwi Asriyani

Department of Plant Protection, Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32 Indralaya, Palembang, Indonesia

Corresponding author: S. Suwandi, E-mail: suwandi@fp.unsri.ac.id, Tel./fax. +62-711-580059, ORCID ID: 0000-0003-3096-5797

Abstract

Bark canker, wood discolouration, and wilting of the duku tree (*Lansium domesticum* Corr.) along the watershed of Komering River, South Sumatra Province, Indonesia first appeared in 2013. The incidence of tree mortality was 100% within three years in badly infected orchards. A *Ceratocystis* species was consistently isolated from the diseased tissue and identified by morphological and sequence analyses of the ITS and TUB regions. Pathogenicity tests were conducted and Koch's postulates were confirmed. The fungus was also pathogenic on *Acacia mangium*, but was less pathogenic on mango. Partial flooding was unfavourable for disease development. Two described isolates (WRC and WBC) had minor variation in morphology and DNA sequences, but the former exhibited a more pathogenic on both duku and acacia. The

ITS phylogenies grouped the most pathogenic isolate (WRC) causing wilting of the duku tree within the aggressive and widely distributed ITS5 haplotype of *C. fimbriata*.

Keywords: *Ceratocystis* canker and wilt, *Ceratocystis fimbriata*, *Lansium* tree, *Acacia mangium*

Introduction

The duku (*Lansium domesticum Corr.*), also known as the langsat and the kokosan is a tropical lowland fruit tree native to western Southeast Asia, from Borneo in the east (Indonesia) to peninsular Thailand in the west. It occurs wild and cultivated in its native countries and is one of the most widely cultivated fruits (Techavuthiporn, 2018; Yaacob and Bamroongrugsa, 1991). Duku is among the most popular local fruits in Indonesia. In 2017, the total number of harvested duku trees in Indonesia was 2.4 million trees, with a total yield of 138.4 metric tons (BPS-Statistics Indonesia, 2018). The most famous cultivars are grown in South Sumatra (duku Palembang and duku Komering) due to their sweet flavour combined with a subacid taste and having few seeds, or even being seedless. In South Sumatra, duku is mainly grown as a backyard or garden tree in combination with other native fruit trees along the watershed of the Musi, Komering, Ogan, Lematang and Rawas Rivers.

Lethal disease has rarely been evident on duku trees growing in the wild or cultivated orchard areas. Anthracnose caused by *Colletotrichum gloeosporioides*, appearing as brownish spots on the fruit bunch and often resulting in premature fruit drop and post-harvest losses, is commonly evidenced throughout the tropics (Yaacob and Bamroongrugsa, 1991). Corky bark disease, which makes the bark become rough and corky and flake off, often resulting in little to no fruit production has been reported on dukus in tropical USA (Keith et al., 2013; Whitman, 1980). In Hawaii, a corky bark canker is associated with an Ascomycete fungus, *Dolabra*

nepheliae, and insect larvae of *Araecerus* sp. (Coleoptera: Anthribidae) and *Corticeus* sp. (Coleoptera: Tenebrionidae) feeding under the loosened bark (Keith et al., 2013).

During early January 2014, massive mortality of duku trees along the watershed of the Komering River in OKU District was reported by most local and some national newspapers. In total, more than 2,000 trees of the most popular cultivar, duku Komering, died. The symptoms first appeared during the early rainy season of October 2013. Most of the trees that died were predisposed due to partial flooding to a depth of about 20 cm for about one month from the end of December 2013 to January 2014. However, some affected trees were found growing on non-flooded sites, indicating an infectious disease. In this study, we describe a new bark canker and wilting associated with massive mortality of duku trees in Indonesia, illustrate morphological and molecular-based identification of the pathogen, and describe the pathogenicity of the causal fungus on duku trees and other hosts. Disease progress and spread for five years is also discussed.

Materials and Methods

Disease incidence and isolation of the causal agent. Incidence of diseased trees was assessed in 2014 and 2017 at eight duku orchards in Ogan Komering Ulu (OKU) District of South Sumatra. In each orchard, five 10 × 10 m plots starting from the centre of the diseased trees were selected. The trees were recorded as infected if any part of the shoot or stem showed disease symptoms. Twenty diseased duku trees were randomly selected from the affected orchards. Sections of the discoloured wood from the stem were cut, wrapped in a paper towel and transported to the laboratory for examination. Isolation of the fungal pathogen was performed from discoloured wood that had been surface-sterilized with 70% ethanol for 30 s and 1% NaOCl for 2 min. Small sections (5 × 5 mm) from the margin of discolouration were placed on a malt extract agar (MEA) amended with 50 µg/ml streptomycin in Petri dishes.

Another subset of surface-sterilized wood sections was wrapped between carrot slices to bait for *Ceratocystis* spp. (Brito et al., 2019; Moller and DeVay, 1968). Baiting was also performed by inserting diseased tissue into freshly harvested cacao pods and cucumber fruit in an attempt to isolate *Phytophthora*.

Initial identification and cultural characteristics. Initial identification was performed based on morphological characteristics of teleomorphs and anamorphs. Isolates were characterized from two-week-old cultures grown on 2% malt extract agar (MEA). One hundred measurements of each teleomorph and anamorph structure from each representative isolate were made with an Olympus microscope and an OptiLab camera system (Yogyakarta, Indonesia). The average (mean) and standard deviation (stdv) of measurements were computed and presented as mean minus stdv-mean plus stdv. Morphological characteristics were compared with *Ceratocystis* isolates from *A. mangium* (Tarigan et al., 2011) and sweet potato (Engelbrecht and Harrington, 2005).

DNA isolation, PCR, and sequence analyses. Two representative isolates (WRC and WBC), isolated from the diseased duku trees were further used for DNA sequence analysis. DNA was isolated from mycelia cultured at 27°C for seven days in malt extract broth (Difco Laboratories, Sparks, MD) in plastic Petri dishes. Total DNA was extracted using bead-beating technology and the silica spin filter method (Mo Bio and Geneaid Kit) according to the manufacturer's instructions. DNA concentration and purity were measured spectrophotometrically. The ITS1/5.8 S rDNA/ITS2 (ITS) region of *Ceratocystis* isolates was amplified by PCR, using ITS1 (forward: 5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (reverse: 5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The β-tubulin gene (TUB) region was amplified by PCR, using βt1a (forward: 5'-TTCCCCCGTCTCCACTTCTTCATG-3') and βt1b (5'-GACGAGATCGTTCATGTTGAACTC-3') (Glass & Donaldson, 1995). PCR

reaction mixtures consisted of 1 µl of each primer (10 mM), 15 µl of 1st BASE REDIANT 2X PCR Master Mix (#BIO-5185), 3 µl of DNA template (2-10 ng) and 10 µl nuclease-free water to make up 30 µl total volume reactions. PCR was performed using Thermal Cycler (Agilent, SureCycler 8800) with a 5-minute 95°C denaturation step followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 56°C for *ITS* and 55°C for TUB, and 40 s extension at 72°C, followed by a final extension of 5 min at 72°C. Negative controls (without template DNA) were applied in each assay. The PCR products of ITS and TUB regions were sequenced at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia.

Identification of isolates was accomplished by BLAST searches of the *ITS* and *TUB* sequences on the GenBank database (http://www.ncbi.nlm.nih.gov). BLAST identification suggested that both isolates belonged to the species *Ceratocystis fimbriata*. Phylogenetic analyses were performed to identify the species of *Ceratocystis* most closely related to the *Lansium* isolate from Indonesia. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade of the *Ceratocystis fimbriata* species complex (Barnes et al., 2018; Fourie et al., 2015; Oliveira et al., 2015). The β-tubulin sequences (Table 1) were aligned using the online software MAFFTv.7 (Katoh et al., 2019) with the best alignment strategy was automatically selected by the software. Sequence alignments were manually edited in MEGA X (Kumar et al., 2018). There were 34 aligned datasets and the sequences were used for phylogenetic tree construction using a maximum parsimony (MP) analysis under PAUP 4.0b10 (Swofford, 2003). To determine relatedness of isolates from duku with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014) and phylogenetic analyses were performed. Representative sequences of ITS haplotypes of *C. fimbriata* as designated by Harrington et al. (2014) and ITS sequences of accession numbers KF878326, KF650948, AM712445, AM292204, MF033455, EU588656, KC261853, which

most closely matched with isolates from duku, were used in the analyses. *C. variospora* (accessions AF395683) was used as the outgroup taxon. There were 35 ITS sequences in the dataset (Table 1) and the sequences were initially aligned using MAFFTv.7 (Katoh et al., 2019) and then manually adjusted and trimmed in MEGA X (Kumar et al., 2018). The relationships between ITS sequences of isolates from *L. domesticum* and other representative genotypes of the *C. fimbriata* sensu stricto (Harrington et al., 2014; Oliveira et al., 2015) were analysed using genetic distance matrices, unweighted pair group method with arithmetic means (UPGMA), and 1000 bootstrap replications under PAUP 4.0b10 (Swofford, 2003).

Pathogenicity tests. Two isolates identified using DNA sequence data were used to test for pathogenicity. Pathogenicity tests were conducted on one-year-old duku (*Lansium domesticum* var. *domesticum*) seedlings grown in a partially flooded and in a non-flooded nursery. Seedlings were grown in 20 cm diameter plastic pots containing a mixture of topsoil and compost under a 25% shading net. The pots from the flooded nursery were placed in a tray filled with tap water, which was maintained to a depth of 2-3 cm. Pathogenicity was also tested on three-month-old acacia (*A. mangium*) and six-month-old mango (*Mangifera indica* cv. Arumanis) seedlings.

Preliminary tests showed that stem inoculations with a mycelial plug were ineffective unless the bark was wounded. Therefore, wound inoculation was used throughout the experiments. Wounds were made by puncturing three points on the bark to a 3-mm depth using a sterile 28g needle, and a 2 × 2 mm agar plug taken from an actively growing colony on 2% MEA was placed in the wound with the mycelium downward. This was covered with a section (10 × 10 mm) of wetted tissue paper and wrapped with clear tape to reduce contamination and desiccation. The inoculum along with the wrapping plastic was removed at three days post-inoculation. Each isolate was injected into ten seedlings for each flooded and non-flooded group of seedlings. For uninoculated controls, wounded bark was wrapped with sterile MEA

plugs. Whole experiments were repeated twice and data were pooled after verifying the variance homogeneity using the Levene test.

Disease severity was assessed 20 days post-inoculation based on the length of wood discolouration. Sections were cut from the margins of lesions, surface-sterilized, and plated on MEA or inserted into a carrot dish to re-isolate the inoculated fungus to complete Koch's postulates. Fungal identity was verified by colony, anamorph, and teleomorph morphology.

Results

Field observations and symptom development. Diseased trees were characterized by wilting of some twigs or branches, followed by defoliation and dieback. In most cases, total plant wilt or death was observed within six months from the first appearance of wilt (Fig. 1A, 1B). Bark canker was eventually found on heavily infected trunks or dead trees (Fig. 1D). Scraping the bark down to the wood along the wilted side of the trunk up to the branch revealed extensive areas of discoloured tissue (Fig. 1E, 1F). The discoloured wood typically had a streaked appearance, turning a uniform dark brown with age and could be found beneath the outermost layers of sapwood (Fig. 1E) and in some cases, discolouration extended to the heartwood (Fig. 1F). All diseased trees had been attacked by squirrels (Fig. 1C) and lesions appeared to originate from surrounding beetle entry/exit holes (Fig. 1E) on the peeled-off bark, indicating the involvement of a wound pathogen.

The disease was observed along the watershed of the Komering River, including Lubuk Batang (OKU District) and Rasuan (OKU Timur District), all in South Sumatra Province of Sumatra. Affected trees ranged from young (< 5 years) to old (> 50 years) in age. Disease incidence and severity were highest in Lubuk Batang Lama, where the disease first appeared. The disease progress both in term of incidence and severity was fast. All trees (100%) from eight sampled duku orchards in Ogan Komering Ulu (OKU) District of South Sumatra where

the disease originated had wilted and died in the November 2017 survey (Table 2). In the 2019 field observation, the disease was found to have sporadically killed duku trees in Ogan Komering Ulu Timur (OKUT) District (within 100 km of the disease origin). Squirrel attacks were not found on the recently infected trees. Disease was not found in other duku orchards of South Sumatra in OKI, PALI and Muara Enim Districts. There was no appearance of squirrel scratches in those disease-free orchards.

Culture characteristics and morphology. Fungi typical of genus *Ceratocystis* were consistently isolated from direct plating of diseased wood on to both MEA and carrot slices. Colonisation of *Phytophthora* on diseased wood was not detected by baiting using cacao pods and cucumber fruit. *Ceratocystis* isolates from *L. domesticum* trees were typical of *Ceratocystis* spp. in the *C. fimbriata* sensu lato species complex, having characteristic olive-green colonies and the typical banana-fruit odour. They had globose to sub-globose ascomata with long necks and typical divergent ostiolar hyphae at their tips (Fig. 2). Teleomorph and anamorph structures were produced within two weeks on MEA cultures. Two isolates (WRC and WBC) were described and both had ascospore (4-7×3-5 μm), cylindrical conidia (14-25×4-5 μm), and aleuroconidia sizes (11-16×7-11 μm) within the range of those of *C. fimbriata* sensu stricto neotype BPI 595863 (Engelbrecht and Harrington, 2005). Both isolates produced a barrel-shaped (doliform) conidia (8-10×6-8 μm) in chain (Fig. 2).

Sequence Analyses. WBC and WBC isolates had differences in two bases of ITS sequence (99.6% similarity), but had a 100% similarity in the TUB sequence. BLAST searches of the ITS region of WRC (MT229127) and WBC (MT229128) identified both sequences with the GenBank deposits for *Ceratocystis fimbriata* with 100% of similarity and query coverage. A similar BLAST result was obtained with the TUB sequence (MW013766 and MW013767 for

WBC and WBC, respectively) and confirmed the assignment to *Ceratocystis fimbriata* with 100% of similarity and query coverage.

MP analyses for the β-tubulin resulted in single most parsimonious tree of 84 steps (Fig. 3), with a homoplasy index = 0.036, consistency index = 0.964, rescaled consistency index =0.979, and retention index= 0.944. *Ceratocystis* isolates from *Lansium* in Indonesia reside in the LAC of *C. fimbriata* sensu lato and they are phylogenetically clustered closely with ex-type and ex-paratype of *C. manginecans* and *C. fimbriata*. *C. manginecans* is considered synonym or conspecific of *C. fimbriata* sensu stricto (Harrington et al., 2014; Oliveira et al., 2015).

Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al., 2014) grouped the isolates into ITS5 and ITS6z haplotype of *C. fimbriata* for WRC and WBC, respectively. The WRC showed 100% similarity with other ITS5 haplotype of *C. fimbriata* isolated from tea tree (KF650948), taro (AM712445), pomegranate (AM292204) in China; from eucalyptus (KF878326) in Zimbabwe; from acacia (MF033455) in Vietnam; and from acacia (EU588656) in Indonesia. WBC had 100% similarity with member of ITS6z haplotype of *C. fimbriata* isolated from *Hypocryphalus mangiferae* (KC261853) in Oman. UPGMA analysis clustered both isolates from *L. domesticum* within a single group consisted of both ITS5 and ITS6 haplotypes (Fig. ~~3~~4).

Pathogenicity Test. In pathogenicity tests, initial symptoms appeared as water-soaked brown lesions on the wound site within three days after inoculation. The lesions remained small at inoculation sites on bark, but scraping the bark down to the wood revealed extensive areas of discoloured xylem tissue upward and downward from the inoculated site (Fig. ~~4A~~5A). Upward extension of xylem discolouration from the inoculation site was more extensive ($P<0.0001$) than downward extension on duku seedling inoculated with WRC. However, no significant difference ($P\geq0.05$) between upward and downward discolouration extension was exhibited by WRC on acacia and mango and by WBC on all hosts (Table 3). This kind of discoloured xylem

was similar to a typical symptom of diseased trees in the field. The WRC isolate was more pathogenic on duku seedling than WBC as it induced significantly ($P<0.05$) longer lesions and caused more ($P<0.05$) plant wilt and death (Fig. ~~4A~~5A). Plant wilt and death was observed within 20 days post-inoculation and later the wilting incidence gradually increased. Regrowth of lateral shoots was observed on wilted plants. The control plants, inoculated with malt extract agar, remained asymptomatic and had only a trace of xylem discolouration (less than 5 mm in length) at the wound site (Table 3). Partial flooding of duku seedling did not significantly ($P=0.163$) affect extension of the xylem discoloration, but plant mortality by WRC was lower ($P<0.05$) than on non-flooded seedling (Table 3). Fungus with the same morphological characteristics was re-isolated from diseased wood of inoculated seedlings, but not from any of the control plants.

Ceratocystis isolates also induced xylem discolouration and wilt symptoms on inoculated *A. mangium* seedlings (Fig. ~~4B~~5B), similar to that observed on duku seedlings. Xylem discolouration on acacia developed faster than on duku and was equally extensive ($P\geq0.05$) for both upward and downward expansion (Table 3). Plant wilt and death was observed earlier on acacia compared to duku with half the WRC-inoculated acacia dying within 20 days post-inoculation. Similar to what was observed on duku seedlings, the WRC isolate caused significantly ($P<0.05$) longer lesion and more death on acacia and therefore, proved to be more pathogenic than WBC (Table 3). *Ceratocystis* isolates were also pathogenic on mango (*M. indica*), but did not induce wilting symptoms (Fig. ~~4C~~5C). Mycelial plug inoculation on stems of mango resulted in wood discolouration similar to the symptoms on duku and acacia (Fig. ~~4C~~5C), but with less expansive discolouration (Table 3).

Discussion

This study presents the first report of *Ceratocystis fimbriata* associated with massive mortality of *L. domesticum* trees in South Sumatra, Indonesia. This fungus was shown to be

pathogenic by producing expansive wood discolouration and causing lethal wilt on inoculated duku seedlings similar to that found in the field. Fungus with the same morphological characteristics was easily re-isolated from diseased wood of inoculated seedlings, suggesting fulfilment of Koch's postulates. Inoculation experiments on acacia seedlings suggested that the pathogen was also pathogenic there by producing more expansive wood discolouration, bark canker, wilting symptoms, and plant death. *Ceratocystis* isolates from duku proved to be less pathogenic on mango, as less wood discolouration was induced, without wilting and plant death.

~~Morphological characteristics showed that the pathogen belonged to the species *C. fimbriata* (Engelbrecht & Harrington, 2005). Both *Ceratocystis* isolates from duku (WRC and WBC) had a similar morphology to *C. fimbriata s.s.* neotype BPI 595863 (Engelbrecht and Harrington, 2005), except for doliform conidia that were absent on BPI 595863.~~

The ITS rDNA sequence of the most pathogenic isolate, WRC (MT229127), had an identical sequence to the isolates of *C. fimbriata* from tea tree (KF650948), taro (AM712445), and pomegranate (AM292204) in China; from eucalyptus (KF878326) from Zimbabwe; from acacia (MF033455) in Vietnam; and from acacia (EU588656) in Indonesia. All these isolates were confirmed belong to ITS5 haplotype of *C. fimbriata* (Harrington et al., 2014; Li et al., 2016). Some of these isolates were previously identified as *C. acaciivora* (Tarigan et al., 2011) and subsequently reconsidered as *C. manginecans* (Fourie et al., 2015), but Oliveira et al., (2015) considered those cryptic species to be synonyms or conspecifics of *C. fimbriata* sensu stricto. The ITS5 haplotype is an aggressive genotype of *C. fimbriata* causing a lethal wilt disease of economically important plants worldwide. This genotype represented the native *C. fimbriata* populations in Brazilian forest plantations of *Eucalyptus* spp. (Harrington et al., 2014; Harrington et al., 2015; Li et al., 2016). This ITS haplotype was also found infecting *Acacia* spp. and its original host, *Eucalyptus* spp. in China, Indonesia, South Africa, Thailand, Uruguay (Harrington et al., 2014), Zimbabwe (Jimu et al. 2015) and Vietnam (Trang et al. 2017). The

member of this *Eucalyptus* population of *C. fimbriata* cause the wilt epidemic on kiwifruit in Brazil (Ferreira et al., 2017). In China, the ITS5 genotype has been considered to be introduced from Brazil through *Eucalyptus* cuttings and reported to cause epidemics on pomegranate, loquat, and taro (Harrington et al., 2015; Li et al., 2016), and tea tree (Xu et al., 2019).

The less pathogenic isolate, WBC, is grouped as ITS6z, a minor haplotype derived from a single haploid strain of C2759 (CBS 135868). The C2759 was originated from *Dalbergia sissoo* in Pakistan and its single-ascospore culture yielded many different haplotypes with the ITS7b as the major genotype (Harrington et al., 2014). WBC had 100% similarity with other member of ITS6z haplotype (type Y = KC261853) of *C. fimbriata* isolate CMW13582 originated from the bark beetle, *H. mangiferae* in Oman (Naidoo et al., 2013). The ITS7b is a common ITS genotype of *C. fimbriata* from Oman, Pakistan, and Indonesia that previously described as *C. manginecans* (Harrington et al., 2014; Oliveira et al., 2015). Many isolates in Asia and Oman have mixed ITS sequences due to crosses between the ITS5, ITS6, and ITS7b genotypes (Oliveira et al., 2015). In this study, *Ceratocystis* isolates from Indonesia (ITS5 and ITS6z) and members of ITS7b haplotype (CMW13851 and CMW23634 from Oman and Pakistan, respectively) are grouped into a single phylogenetic cluster of *C. fimbriata* sensu stricto based on partial β-tubulin sequence. It is likely that the population of *C. fimbriata* causing disease on duku and acacia in Sumatra is a combination of ITS5, ITS6, and ITS7b, with the ITS6z a result of crossing of these haplotypes.~~The less pathogenic isolate, WBC, showed homology sequence to the type Y of the ITS rDNA of *C. fimbriata* isolate CMW13582 (KC261853) from *Hypocryphalus mangifera* in Oman (Naidoo, 2013). Both isolates were grouped to ITS6z haplotype of *C. fimbriata* (Harrington et al., 2014). In this study, WBC showed also a weak aggressiveness on an Indonesian cultivar of mango. Report on disease epidemic caused by this genotype of *C. fimbriata* was not available, and it is likely that the ITS6z haplotype is a less aggressive pathogen.~~

Morphological characteristics showed that the pathogen belonged to the species *C. fimbriata* (Engelbrecht & Harrington, 2005). Both *Ceratocystis* isolates from duku (WRC and WBC) had a similar morphology to *C. fimbriata* sensu stricto neotype BPI 595863 (Engelbrecht and Harrington, 2005), except for doliform conidia that were absent on BPI 595863. Phylogenetic analyses based on the ITS and β-tubulin regions showed conclusively that *Ceratocystis* isolates causing bark canker and lethal wilt on duku tree in Indonesia is identified as *C. fimbriata* sensu stricto. There were two ITS genotypes of *C. fimbriata* associated with disease on *Lansium* tree in Indonesia, one consistent with that found in Oman and Pakistan on the mango bark beetle and *Dalbergia* (and other hosts) and a second sequence found in China, Indonesia, Vietnam and Brazil on various hosts, including acacia.

C. fimbriata has been known to infect a wide variety of annual and perennial host plants throughout the world. In Indonesia, diseases caused by *C. fimbriata* are considered to be of minor importance due to non-lethal and sporadic infestation. The fungal infection has long been noted to cause a non-lethal disease known as mouldy rot on the trunk of rubber trees (Tayler and Stephens, 1929). The role of fungal infection as the primary causal agent of the disease has been dismissed since mouldy rot is considered an advanced stage of a physiological disorder induced by excessive tapping and ethylene overstimulation (Putranto et al. 2015) and the disease can be eliminated by treatment with non-fungicidal biostimulants (Suwandi et al., 2018). In the last decade, disease incited by *C. fimbriata* has been one of the most destructive and economically important diseases on acacia plantations in Indonesia, shortly after an outbreak on the industrial forest plantations throughout the world (Roux and Wingfield, 2009). Outbreaks of *Ceratocystis* disease have forced the replacement of thousands of hectares of *A. mangium* plantations in eastern Sabah, Malaysia (Brawner et al., 2015). In Indonesia, *Ceratocystis* infection has contributed to 2% mortality by the fourth rotation of *A. mangium* in Sumatra, Indonesia (Hardie et al., 2017). Pathogens causing lethal wilt of duku belong to ITS

haplotype 5, which represented *C. fimbriata* populations from forest plantations of *Acacia* spp. and *Eucalyptus* spp. Pathogenicity tests also confirmed that *A. mangium* is more susceptible than the original host (duku tree), suggesting the establishment of *C. fimbriata* pathogenicity on acacia as the main host. Similar disease symptoms caused by *Ceratocystis* infections were found to be endemic on acacia and eucalyptus plantations located about 30 km away from the site of study. It is likely that population of *C. fimbriata* pathogenic on acacia plantation could extend their host range to native fruit tree such as *Lansium* and cause a serious threat to the neighbouring fruit tree species. The host-range extension by the ITS5 haplotype of *C. fimbriata* to the susceptible neighbouring plants occurred in Brazil, in which the genotype from eucalyptus showed strong aggressiveness on taro (Harrington et al., 2011) and caused epidemic on grapevine (Ferreira et al., 2017). Similar host extension by the ITS5 haplotype also occurred in China, in which the eucalyptus population caused epidemic on pomegranate, loquat, and taro (Harrington et al., 2015; Li et al., 2016), and tea tree (Xu et al., 2019).

All sampled diseased trees had been previously attacked by squirrels and lesions appeared to originate from surrounding beetle entry/exit holes on peeled-off bark from squirrel scratches, suggesting the involvement of the wild vertebrate as the wound creator and beetles for fungal spore dispersion. Fungal feeding insects, such as *H. mangiferae*, have been suggested to be associated with the rapid distribution of *C. fimbriata* in Oman and Pakistan (Al Adawi et al., 2013). Squirrel attacks on either diseased or healthy duku trees were found only during the disease outbreaks in 2013-2014 and these attacks were likely due to the limitation of squirrel feed sources in the field. All affected orchards had grown duku in a monoculture. Pathogenicity tests supported the idea that partial flooding was not likely to predispose duku trees to *Ceratocystis* infection as the disease did not develop well under partial flooding. Recent field observations in areas near the disease origin suggested that the disease spreads sporadically with limited mortality. Squirrel attacks were not found on recently infected trees, suggesting

the possible involvement of the wild vertebrate wounds on the massive disease spread in duku orchards. Vertebrate-incited wounds, such as those from squirrels and monkeys, are considered to contribute to the spread of *Ceratocystis* wilt on *A. mangium* plantations (Brawner et al. 2015; Hardie et al. 2017; Nasution et al., 2019).

Acknowledgements

We thank the editors and reviewers for critical review of the manuscript. This research was supported by the Sriwijaya University Priority Applied Research Project 023/SP2H/LT/DPRM/II/2016.

References

Al Adawi, A. O., Al Jabri, R. M., Deadman, M. L., Barnes, I., Wingfield, B. D. and Wingfield, M. J. 2013. The mango sudden decline pathogen, *Ceratocystis manginecans*, is vectored by *Hypocryphalus mangiferae* (Coleoptera: Scolytinae) in Oman. *Eur. J. Plant Pathol.* 135:243-251.

Barnes, I., Fourie, A., Wingfield, M. J., Harrington, T. C., McNew, D. L., Sugiyama, L. S., Luiz, B. C., Heller, W. P. and Keith, L. M. 2018. New Ceratocystis species associated with rapid death of *Metrosideros polymorpha* in Hawai`i. *Persoonia* 40:154-181.

BPS-Statistics Indonesia, 2018. Indonesian Statistic of Annual Fruit and Vegetable Plants. https://www.bps.go.id/publication/2018/10/05/081665ec9eb65fdce8a69473/statistik-tanaman-buah---buahan-dan-sayuran-tahunan-indonesia-2017 [17 March 2020].

Brawner, J., Japarudin, Y., Lapammu, M., Rauf, R., Boden, D. and Wingfield, M. J. 2015. Evaluating the inheritance of *Ceratocystis acaciivora* symptom expression in a diverse *Acacia mangium* breeding population. *South. Forests* 77:83-90.

Brito, R. A. S., Cavalcante, G. P., Borel, F. C. and Maffia, L. A. 2019. Detection and isolation of *Ceratocystis fimbriata* in mango trees on semi-selective medium. *Eur. J. Plant Pathol.* 155:667-669.

Chen, S., Van Wyk, M., Roux, J., Wingfield, M. J., Xie, Y. and Zhou X. 2013. Taxonomy and pathogenicity of Ceratocystis species on Eucalyptus trees in South China, including *C. chinaeucensis* sp. nov. *Fungal Divers.* 58:267-279.

Engelbrecht, C. J. B. and Harrington, T. C., 2005. Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. *Mycologia* 97:57-69.

Ferreira, M. A., Harrington, T. C., Alfenas, A. C. and Mitzubuti, E. S. G. 2011. Movement of genotypes of *Ceratocystis fimbriata* within and among *Eucalyptus* plantations in Brazil. *Phytopathology* 101:1005-1012.

Ferreira, M. A., Harrington, T. C., Piveta, G. and Alfenas, A. C. 2017. Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil. *Trop. Plant Pathol.* 42:86-95.

Fourie, A., Wingfield, M. J., Wingfield, B. D. and Barnes, I. 2015. Molecular markers delimit cryptic species in *Ceratocystis sensu stricto*. *Mycol. Progress* 14: 1020.

Glass, N. L. and Donaldson, G. C. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl. Environ. Microbiol.* 61:1323-1330.

Hardie, M., Akhmad, N., Mohammed, C., Mendham, D., Corkrey, R., Gafur, A. and Siregar, S. 2018. Role of site in the mortality and production of *Acacia mangium* plantations in Indonesia. *South. Forests* 80:37-50.

Harrington, T. C., Huang, Q., Ferreira, M. A. and Alfenas, A. C. 2015. Genetic analyses trace the Yunnan, China population of *Ceratocystis fimbriata* on pomegranate and taro to populations on eucalyptus in Brazil. *Plant Dis.* 99:106-111.

Harrington, T. C., Kazmi, M. R., Al-Sadi, A. M. and Ismail, S. I. 2014. Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata* sensu stricto. *Mycologia* 106:224-242.

Harrington, T. C., Thorpe, D. J. and Alfenas, A. C. 2011. Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of *Ceratocystis fimbriata*. *Phytopathology* 101:555-566.

Hinds, T. E. 1972. Insect transmission of *Ceratocystis* species associated with aspen cankers. *Phytopathology* 62:221-225.

Jimu, L., Wingfield, M. J., Mwenje, E. and Jolanda Roux, J. 2015. Diseases on *Eucalyptus* species in Zimbabwean plantations and woodlots. *South. For.* 77: 221-230.

Katoh, K., Rozewicki, J. and Yamada, K. D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform.* 20: 1160-1166.

Keith, L. M., Matsumoto, T. K. and McQuate, G. T. 2013. First report of *Dolabra nepheliae* associated with corky bark disease of langsat in Hawaii. *Plant Dis.* 97:990.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* 35:1547-1549.

Li, Q., Harrington, T. C., McNew, D., Li, J., Huang, Q., Somasekhara, Y. M. and Alfenas, A. C. 2016. Genetic Bottlenecks for Two Populations of *Ceratocystis fimbriata* on Sweet Potato and Pomegranate in China. *Plant Dis*. 100:2266-2274.

Luchi, N., Ghelardini, L., Belbahri, L., Quartier, M. and Santini, A. 2013. Rapid detection of *Ceratocystis platani* inoculum by quantitative real-time PCR assay. *Appl. Environ. Microbiol.* 79:5394-5404.

Moller, W. J. and DeVay, J. E. 1968. Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology* 58:123-124.

Naidoo, K., Steenkamp, E. T., Coetzee, M. P., Wingfield, M. J. and Wingfield, B. D. 2013. Concerted evolution in the ribosomal RNA cistron. *PloS one*, *8*(3), e59355.

Nasution, A., Glen, M., Beadle, C. and Mohammed, C. 2019. Ceratocystis wilt and canker - a disease that compromises the growing of commercial acacia-based plantations in the tropics. *Aus. For.* 82:80-93.

Oliveira, L. S., Harrington, T. C., Ferreira, M.A., Damacena, M. B., Al-Sadi, A. M., Al-Mahmooli, I. H. and Alfenas, A. C. 2015. Species or genotypes? Reassessment of four recently described species of the ceratocystis wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology* 105:1229-1244.

Putranto, R. A., Herlinawati, E., Rio, M., Leclercq, J., Piyatrakul, P., Gohet, E., Sanier, C., Oktavia, F., Pirrello, J., Kuswanhadi and Montoro P. 2015. Involvement of ethylene in the latex metabolism and tapping panel dryness of *Hevea brasiliensis*. *Int. J. Mol. Sci.* 16:17885-17908.

Suwandi, S., Junita, A., Suparman, S., Umayah, A., Hamidson, H., Muslim, A. and Irsan, C. 2018. Curative activity of watery fermented compost extract as a bark treatment against tapping panel dryness. *Open Agr. J.* 12:74-83.

Swofford, D. L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods), v. 4.0b10*. Sunder-land, MA: Sinauer Associates.

Tarigan, M., Roux, J., van Wyk, M., Tjahjono, B. and Wingfield, M. J. 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.

Tayler, V. A. and Stephens, J., 1929. *Native Rubber in the Dutch East Indies*. Rubber Growers Association, London, UK, 48 pp.

Techavuthiporn, C. 2018. Langsat- *Lansium domesticum*. In: *Exotic fruits*, eds. by S. Rodrigues, E. D. O. Silva and E. S. D. Brito, pp. 279-283. Academic Press, New York, USA.

Trang, T. T., Eyles, A., Davies, N., Glen, M., Ratkowsky, D. and Mohammed, C. Screening for host responses in *Acacia* to a canker and wilt pathogen, *Ceratocystis manginecans*. *For Path*. 48:e12390.

Van Wyk, M., Al Adawi, A. O., Khan, I. A., Deadman, M. L., Wingfield, B. D. and Wingfield, M. J. 2007. *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Divers*. 27:213-230.

Van Wyk, M., Al-Adawi, A. O., Wingfield, B. D., Al-Subhi, A. M., Deadman, M. L. and Wingfield, M. J. 2005. DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman. *Australas*. *Plant Pathol*. 34:587-590.

Van Wyk, M., Wingfield, B. D., Marin, M. and Wingfield, M. J. 2010. New *Ceratocystis* species infecting coffee, cacao, citrus and native trees in Colombia. *Fungal Divers.* 40:103-117.

White, T. J., Bruns, T. D., Lee, S. B. and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*, eds. by M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, pp. 315-322. Academic Press, New York, USA.

Whitman, W. F. 1980. Growing and fruiting the langsat in Florida. *Proc. Fla. State Hort. Soc.* 93:136-140.

Xu, K. C., Zhang, R. Q., Li, J., Bai, Y. H., Yang, X. D., Sun, Y. X. and Huang, Q. 2019. *Camellia sinensis*, a New Host Plant of *Ceratocystis fimbriata* from China. *Plant Dis*. 103:2670.

Yaacob, O. and Bamroongrugsa, N. 1991. *Lansium domesticum* Correa. In: *Plant Resources of South-East Asia no. 2: 4dible fruits and nuts*, eds. by E. W. M. Verheij and R. E. Coronel, pp. 186-190. Pudoc, Wageningen, The Netherlands.

Table 1. Collection details and GenBank accession number of ITS and β-tubulin sequence for isolates of *Ceratocystis fimbriata* included in this study

Isolate	GenBank Accession no.		Species and ITS haplotype	Host	Origin	Reference
	ITS	β-tubulin				
C1418	AY157956	-	*C. fimbriata* ITS1a	*Ipomoea batatas*	USA	Harrington et al., 2014
C1857	HQ157542	-	*C. fimbriata* ITS1	*Ficus carica*	Brazil	Harrington et al., 2014
CMW4797	FJ236733	-	*C. fimbriata* ITS1b	*Eucalyptus* sp.	Congo	Harrington et al., 2014
CMW9998	FJ236721	-	*C. fimbriata* ITSb	*Eucalyptus* sp.	South Africa	Harrington et al., 2014
C1655	HQ157546	-	*C. fimbriata* ITS2	*Mangifera indica*	Brazil	Harrington et al., 2014
C1440	HQ157544	-	*C. fimbriata* ITS3	*Eucalyptus* sp.	Brazil	Harrington et al., 2014
CMW5328	AF395686	-	*C. fimbriata* ITS3	*E. grandis*	Uganda	Harrington et al., 2014
C1442	HQ157545	-	*C. fimbriata* ITS4	*Eucalyptus* sp.	Brazil	Harrington et al., 2014
CMW38737	KF878326	KF878335	*C. fimbriata* ITS5	*E. grandis*	Zimbabwe	Jimu et al., 2015
C1345	AY157966	-	*C. fimbriata* ITS5	*Eucalyptus* sp.	Brazil	Harrington et al., 2014
A59662	KF650948	-	*C. fimbriata* ITS5	*Camellia sinensis*	China	Xu et al., 2019
YM061	AM712445	-	*C. fimbriata* ITS5	*Colocasia esculenta*	China	Li et al., 2016
P20053	AM292204	-	*C. fimbriata* ITS5	*Punica granatum*	China	Li et al., 2016
C1	MF033455	MF040712	*C. fimbriata* ITS5	*Acacia* sp.	Vietnam	Trang et al., 2017
CMW22563	EU588656	EU588636	*C. fimbriata* ITS5	*A. mangium*	Indonesia	Tarigan et al., 2011
WRC	**MT229127**	**MW013766**	***C. fimbriata* ITS5**	***Lansium domesticum***	**Indonesia**	**Present study**
C2055	HQ157548	-	*C. fimbriata* ITS6	*Mangifera* sp.	Brazil	Harrington et al., 2014
CMW13582	KC261853	-	*C. fimbriata* ITS6z	*Hypocryphalus mangifera*	Oman	Naidoo et al., 2013
WBC	**MT229128**	**MW013767**	***C. fimbriata* ITS6z**	***L. domesticum***	**Indonesia**	**Present study**
CMW13851	AY953383	EF433308	*C. fimbriata* ITS7b	*M. indica*	Oman	Van Wyk et al., 2005
CMW23634	EF433302	EF433311	*C. fimbriata* ITS7b	*M. indica*	Pakistan	Van Wyk et al., 2007
CMW22579	EU588658	-	*C. fimbriata* ITS7b	*A. mangium*	Indonesia	Tarigan et al 2011
CMW8856	AY233867	-	*C. fimbriata* ITS8a	*Citrus* sp.	Colombia	Harrington et al., 2014
CMW17808	EF127990	-	*C. fimbriata* ITS8c	*Eucalyptus* sp.	Colombia	Harrington et al., 2014
CMW22092	FJ151432	-	*C. fimbriata* ITS8e	*E. deglupta*	Ecuador	Harrington et al., 2014

C1558	AY157965	-	*C. fimbriata* ITS9	*M. indica*	Brazil	Harrington et al., 2014
C1914	HQ157540	-	*C. fimbriata* ITS9	*C. esculenta*	Brazil	Harrington et al., 2014
C994	AY157964	-	*C. fimbriata* ITS10	*M. indica*	Brazil	Harrington et al., 2014
Cf 4	EF042605	-	*C. fimbriata* ITS10a	*M. indica*	Brazil	Harrington et al., 2014
C1865	AY526286	-	*C. fimbriata* ITS11	*C. esculenta*	Brazil	Harrington et al., 2014
C1926	HQ157541	-	*C. fimbriata* ITS12	*C. esculenta*	Brazil	Harrington et al., 2014
C1688	AY526291	-	*C. fimbriata* ITS14	*M. indica*	Brazil	Harrington et al., 2014
C925	AY157967	-	*C. fimbriata* ITS15	*Gmelina arborea*	Brazil	Harrington et al., 2014
C924	HQ157539	-	*C. fimbriata* ITS16	*G. arborea*	Brazil	Harrington et al., 2014
CMW6569	-	DQ371652	*C. pirilliformis*	*E. nitens*	Australia	Barnes et al., 2018
CMW6579	-	DQ371653	*C. pirilliformis*	*E. nitens*	Australia	Barnes et al., 2018
CMW17808	-	EU881898	*C. neglecta*	*E. grandis*	Colombia	Fourie et al., 2015
CMW18194	-	EU881899	*C. neglecta*	*E. grandis*	Colombia	Fourie et al., 2015
CMW5751	-	AY177225	*C. colombiana*	*Coffea arabica*	Colombia	Fourie et al., 2015
CMW5761	-	AY177224	*C. colombiana*	*C. arabica*	Colombia	Fourie et al., 2015
CMW14803	-	KJ631108	*C. cacaofunesta*	*Theobroma cacao*	Ecuador	Fourie et al., 2015
CMW15051	-	KJ601510	*C. cacaofunesta*	*T. cacao*	Costa Rica	Fourie et al., 2015
CMW8850	-	AY233875	*C. papillata*	*Citrus* × *Tangelo* hybrid	Colombia	Van Wyk et al., 2010
CMW8856	-	AY233874	*C. papillata*	*Citrus limon*	Colombia	Van Wyk et al., 2010
CMW14797	-	EF433307	*C. fimbriata*	*M. indica*	Brazil	Barnes et al., 2018
CMW28907	-	FJ200270	*C. fimbriata*	*M. indica*	Brazil	Barnes et al., 2018
CMW1547	-	EF070443	*C. fimbriata*	*I. batatas*	Papua New Guinea	Barnes et al., 2018
C1421	-	KF302689	*C. fimbriata*	*I. batatas*	USA	Barnes et al., 2018
CMW24174	-	EF190951	*C. fimbriatomima*	*Eucalyptus* hybrid	Venezuela	Fourie et al., 2015
CMW24176	-	EF190952	*C. fimbriatomima*	*Eucalyptus* hybrid	Venezuela	Fourie et al., 2015
CMW21127	-	EU588643	*C. fimbriata*	*A. crassicarpa*	Indonesia	Oliveira et al., 2015
CMW24664	-	JQ862720	*C. fimbriata*	*Eucalyptus* hybrid	China	Chen et al., 2013
CBS115173	-	KF302700	*C. fimbriata*	*Gmelina arborea*	Brazil	Luchi et al., 2013
CBS14653	-	KF302702	*C. fimbriata*	*C. arabica*	Suriname	Luchi et al., 2013

CMW14802	-	EF070425	*C. platani*	*Platanus occidentalis*	USA	Barnes et al., 2018
CMW23450	-	KJ601513	*C. platani*	*P. occidentalis*	Greece	Barnes et al., 2018
CMW11424	-	AY528966	*C. polychroma*	*Syzygium aromaticum*	Indonesia	Barnes et al., 2018
CMW11436	-	AY528967	*C. polychroma*	*S. aromaticum*	Indonesia	Barnes et al., 2018
CMW19383	-	EF070430	*C. atrox*	*E. grandis*	Australia	Barnes et al., 2018
CMW19385	-	EF070431	*C. atrox*	*E. grandis*	Australia	Barnes et al., 2018

1

2

Table 2. Incidence of *Ceratocystis* wilt in duku orchards of OKU District, South Sumatra

Location (trees/location)	Incidence (%)		
	February 2014	August 2014	November 2017
Belatung (n=66)	36	86	100
Lubuk Batang Baru (n=85)	38	55	100
Lubuk Batang Lama (n=69)	63	100	100

Table 3. Pathogenicity of *Ceratocystis fimbriata* isolates on one-year-old duku (*Lansium domesticum* var. *domesticum*), three-month-old *Acacia mangium*, and six-month-old *Mangifera indica* cv. Arumanis seedlings

Isolate and plant species	Flooding stress	Length (mm) of wood discolouration[1)]			Wilting and death at 20 dpi	Wilting and death at 60 dpi[2)]
		Downward	Upward	Total		
Lansium domesticum						
WRC	Partial flooding	11.3 ± 1.7*	22.8 ± 6.1	34.1 ± 6.4 ab	1/20	7/20 b
	Without flooding	12.6 ± 1.9*	37.3 ± 11.1	49.9 ± 11.4 a	5/20	15/20 a
WBC	Partial flooding	6.2 ± 0.8	9.6 ± 3.3	15.8 ± 3.4 bc	0/20	0/20 c
	Without flooding	5.0 ± 0.5	5.6 ± 0.8	10.6 ± 1.3 c	0/20	2/20 bc
MEA (control)	Partial flooding	1.9 ± 0.1	2.0 ± 0.1	3.9 ± 0.2 d	0/20	0/20 c
	Without flooding	1.9 ± 0.2	1.9 ± 0.1	3.8 ± 0.3 d	0/20	0/20 c
Acacia mangium						
WRC	Without flooding	42.1 ± 3.5	34.9 ± 7.3	76.9 ± 14.8 a	6/20	17/20 a
WBC	Without flooding	17.8 ± 4.1	18.0 ± 8.4	35.8 ± 6.3 b	1/20	5/20 b
MEA (control)	Without flooding	2.1 ± 0.2	2.1 ± 0.2	4.1 ± 0.4 c	0/20	0/20 c
Mangifera indica cv. Arumanis						
WRC	Without flooding	5.1 ± 1.0	5.6 ± 0.9	9.7 ± 1.7 a	0/20	0/20
WBC	Without flooding	7.1 ± 1.3	7.3 ± 1.1	14.4 ± 1.7 a	0/20	0/20

MEA (control)	Without flooding	1.3 ± 0.1	1.3 ± 0.1	2.6 ± 0.1 b	0/20	0/20

1) Wood discolouration was measured 20 d post inoculation (dpi). Means of downward lesion length labelled with * are significantly different from upward lesion according to the Welch two sample t-test. Means of total lesion length by different plant species followed by common letter are not significantly different according to the HSD test.
2) Number of death plants by different plant species labelled by same letter are not significantly different according to the Fisher's exact test of independence with applying the Bonferroni corrected alpha level

Fig. 1. Symptoms of *Ceratocystis* wilt on duku trees (*Lansium domesticum* var. *domesticum*). (A) Partial wilting and fast dieback of upper twigs and branches. (B) Total plant wilt and dieback after six months of partial wilting. (C) Peeled-off bark of branches due to squirrel attacks on diseased tree. (D) Bark canker on heavily infected trunk. (E) The discoloured wood beneath the outermost layers of sapwood and a beetle entry/exit hole on affected wood. (F) The discoloured wood extended to the heartwood of the basal stem.

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolate WRC from bark canker of *Lansium domesticum*. (A) Globose ascomata with long neck. (B) Ascospores. (C) Cylindrical conidia. (D) Primary phialidic conidiophore with emerging cylindrical conidia. (E) ~~Chlamydosphore~~Chlamydospore. (F) Barrel-shaped conidia in chain. Scale bars A = 100 µm; B = 10 µm; C-F = 50 µm.

Fig. 3. Phylogenetic tree generated from maximum parsimony analysis of the β-tubulin sequences showing the relationship between *C. fimbriata* from *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *Ceratocystis fimbriata* species complex. The strain numbers, host genera, countries of origin, and species are given for the representatives of each isolate. Species names considered to be synonyms of *C. fimbriata* sensu stricto are in parentheses (Harrington et al., 2014; Oliveira et al., 2015). *C. variospora* was used as the outgroup taxon. Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on appropriate nodes.

Fig. ~~3~~4. Dendrogram generated by UPGMA showing the genetic relatedness of representative the ITS rDNA genotypes (sequences) of the *Ceratocystis fimbriata* sensu stricto. The GenBank accession numbers, strain numbers, ITS haplotypes, host genera and countries of origin are given for the representatives of each haplotype. Isolates from *Lansium domesticum* in Indonesia were marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). *C. variospora* was used as the outgroup taxon. Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on appropriate nodes. Scale bar indicates genetic distance.

Fig. 45. Symptoms reproduced from mycelial plug inoculation with *Ceratocystis fimbriata* isolates (WRC and WBC) from *Lansium domesticum* 20 days after inoculation. (A) Symptoms on one-year-old duku seedlings (*Lansium domesticum*) inoculated with malt extract agar plug (control) (I), restricted wood discolouration and non-wilted plant inoculated with WBC (II), partial and total wilting of plant inoculated with WRC (III, IV), upward extensive wood discolouration from inoculated site (red arrow) (V). (B) Symptoms on three-month-old seedlings of *Acacia mangium* showing extensive wood discolouration by WRC and limited lesions by WBC. New lateral shoot growth on diseased *Acacia* (yellow arrow). (C) Symptoms on six-month-old seedlings of *Mangifera indica* cv. Arumanis showing wood discolouration at site of inoculation (red arrow).

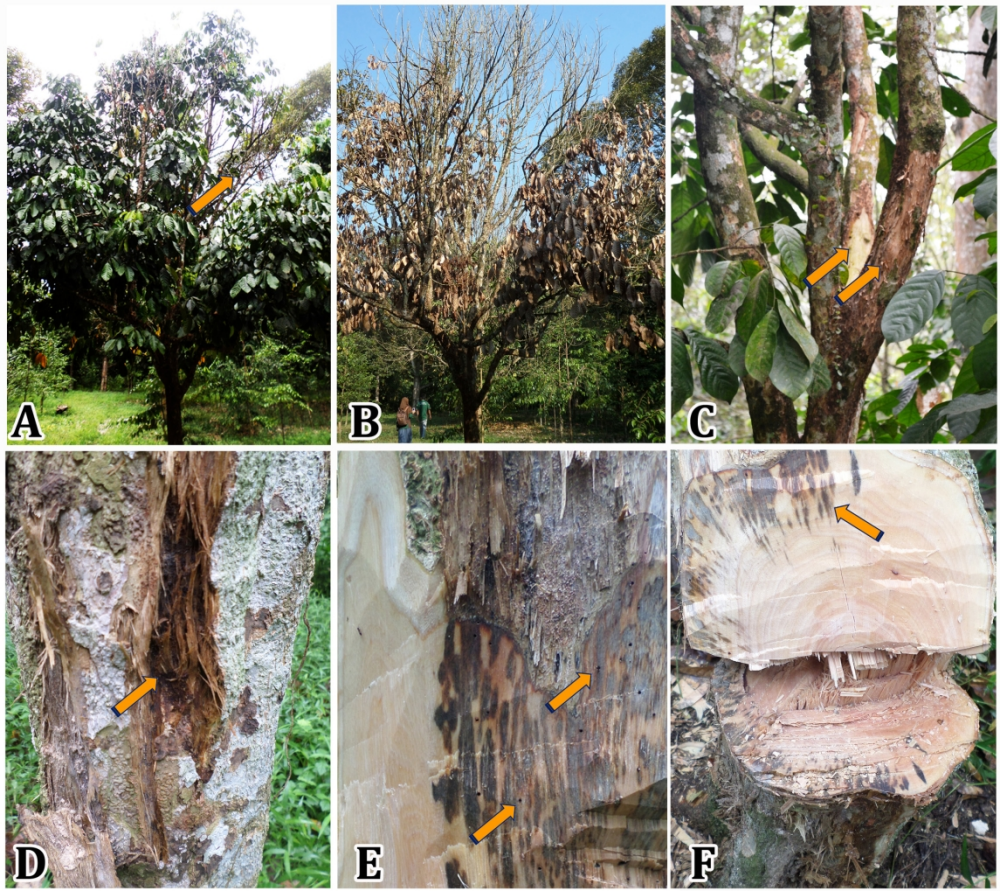

Fig. 1. Symptoms of *Ceratocystis* wilt on duku trees (*Lansium domesticum* var. *domesticum*). (A) Partial wilting and fast dieback of upper twigs and branches. (B) Total plant wilt and dieback after six months of partial wilting. (C) Peeled-off bark of branches due to squirrel attacks on diseased tree. (D) Bark canker on heavily infected trunk. (E) The discoloured wood beneath the outermost layers of sapwood and a beetle entry/exit hole on affected wood. (F) The discoloured wood extended to the heartwood of the basal stem

182x162mm (300 x 300 DPI)

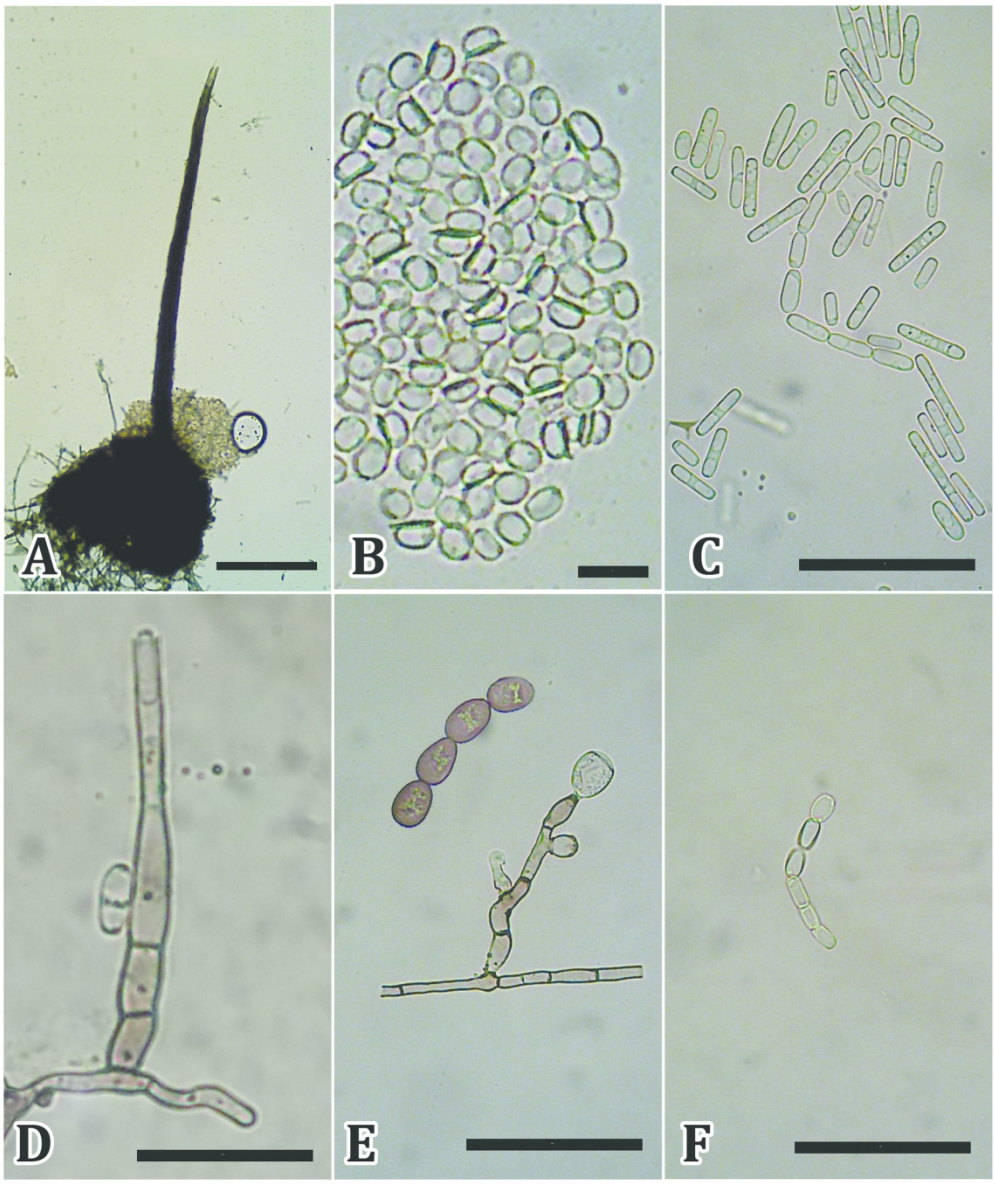

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolate WRC from bark canker of *Lansium domesticum*. (A) Globose ascomata with long neck. (B) Ascospores. (C) Cylindrical conidia. (D) Primary phialidic conidiophore with emerging cylindrical conidia. (E) Chlamydospore. (F) Barrel-shaped conidia in chain. Scale bars A = 100 μm; B = 10 μm; C-F = 50 μm

182x216mm (300 x 300 DPI)

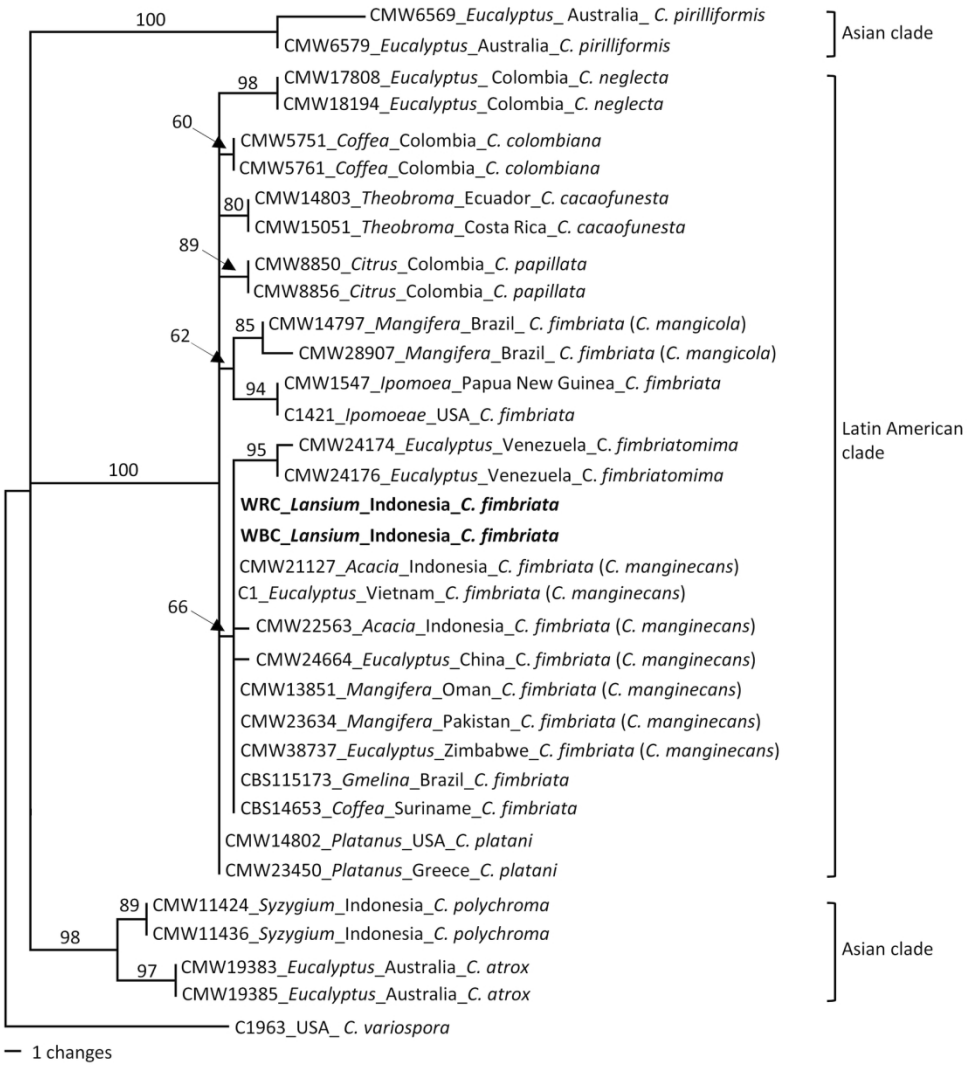

Fig. 3. Phylogenetic tree generated from maximum parsimony analysis of the β-tubulin sequences showing the relationship between *C. fimbriata* from *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *Ceratocystis fimbriata* species complex. The strain numbers, host genera, countries of origin, and species are given for the representatives of each isolate. Species names considered to be synonyms of *C. fimbriata* sensu stricto are in parentheses (Harrington et al., 2014; Oliveira et al., 2015). *C. variospora* was used as the outgroup taxon. Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on appropriate nodes.

158x176mm (300 x 300 DPI)

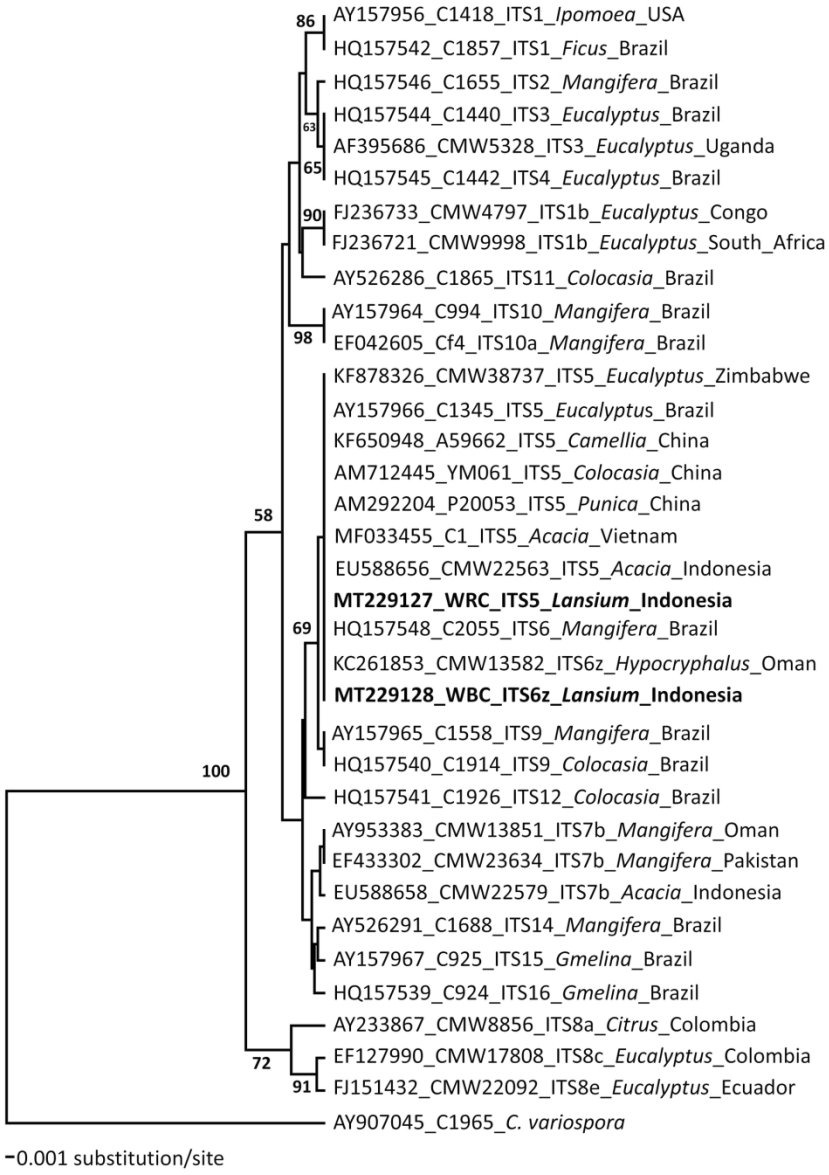

Fig. 4. Dendrogram generated by UPGMA showing the genetic relatedness of representative the ITS rDNA genotypes (sequences) of the *Ceratocystis fimbriata* sensu stricto. The GenBank accession numbers, strain numbers, ITS haplotypes, host genera and countries of origin are given for the representatives of each haplotype. Isolates from *Lansium domesticum* in Indonesia were marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). *C. variospora* was used as the outgroup taxon. Bootstrap values greater than 50% obtained after a bootstrap test with 1,000 replications are indicated on appropriate nodes. Scale bar indicates genetic distance.

163x224mm (300 x 300 DPI)

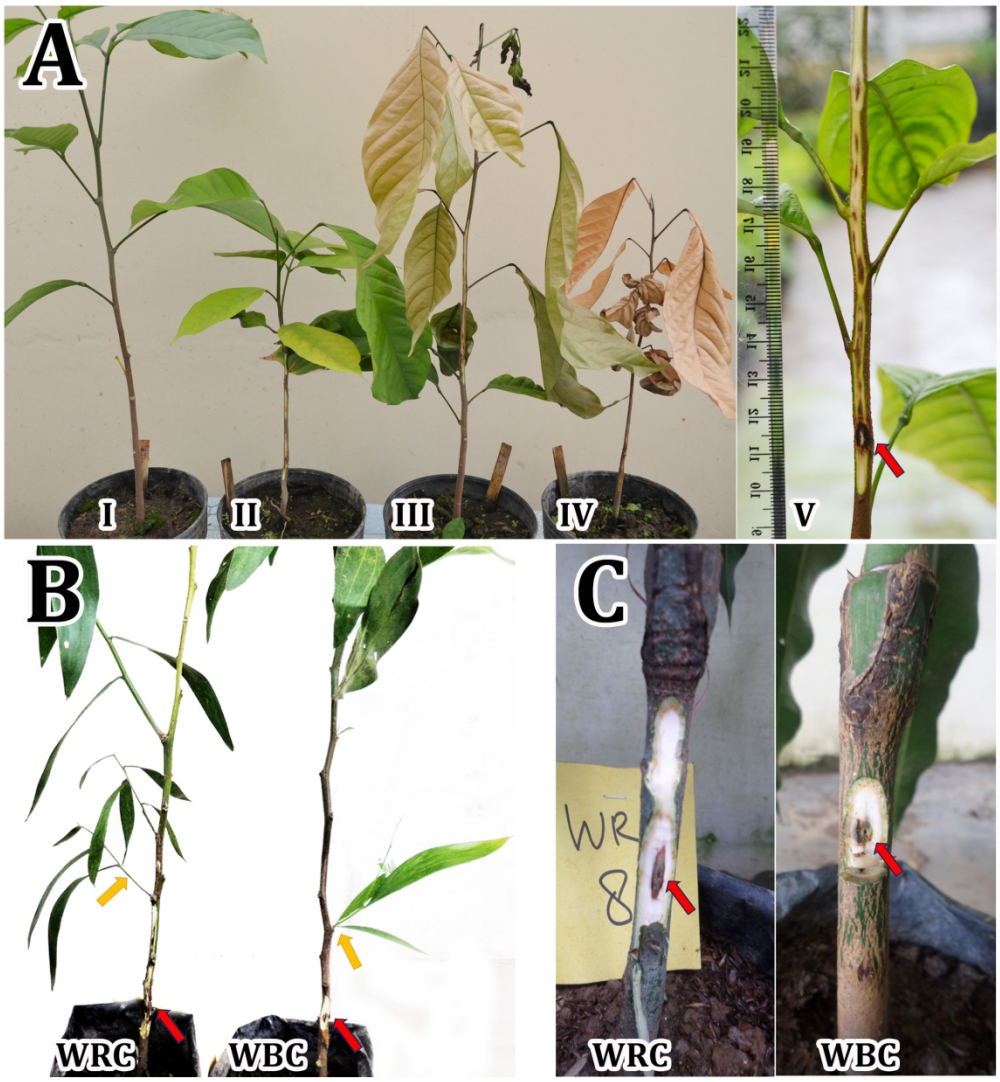

Fig. 5. Symptoms reproduced from mycelial plug inoculation with *Ceratocystis fimbriata* isolates (WRC and WBC) from *Lansium domesticum* 20 days after inoculation. (A) Symptoms on one-year-old duku seedlings (*Lansium domesticum*) inoculated with malt extract agar plug (control) (I), restricted wood discolouration and non-wilted plant inoculated with WBC (II), partial and total wilting of plant inoculated with WRC (III, IV), upward extensive wood discolouration from inoculated site (red arrow) (V). (B) Symptoms on three-month-old seedlings of *Acacia mangium* showing extensive wood discolouration by WRC and limited lesions by WBC. New lateral shoot growth on diseased Acacia (yellow arrow). (C) Symptoms on six-month-old seedlings of *Mangifera indica* cv. Arumanis showing wood discolouration at site of inoculation (red arrow).

183x198mm (300 x 300 DPI)

Unsri Google Apps

Suwandi S <suwandi@fp.unsri.ac.id>

PPJ 2020-0147: Final Proof Corrections & Invoice

1 message

한국식물병리학회 편집위원회 <paper@kspp.org> Tue, Feb 23, 2021 at 9:24 AM
Reply-To: "\"한국식물병리학회 편집위원회\"" <paper@kspp.org>
To: suwandi@fp.unsri.ac.id, suwandi.saleh@gmail.com

Dear Prof. Suwandi Suwandi:

Your manuscript is to be published in The Plant Pathology Journal, 37(2), April issue, 2021.
Please return the checked galley proofs via e-mail within 72 hours (by Feb 26, 2021).

Furthermore, kindly find attached the invoice of publication fee.
Please check your invoice and finish the payment by April 7, 2020.

If you have any questions or concerns, please don't hesitate to contact us.
Thank you.

Best regards,
Yoonjin Kim
PPJ Administrative Editor

사단법인 한국식물병리학회 Korean Society of Plant Pathology

TEL. +82-02-557-9360 | **FAX.** +82-02-557-9361 | **HOMAPAGE.** www.kspp.org
ADDRESS. (06130) 서울시 강남구 테헤란로 7길 22 한국과학기술회관 신관 904호
#904 (New Bldg.), Korean Science&Technology Center, 22 Teheran-ro 7-Gil, Gangnamgu, Seoul, Korea

3 attachments

PPJ-2021-018-Invoice_Suwandi Suwandi(2020-0147).pdf
153K

PPJ-2020-0147.pdf
4330K

PPJ-2020-0147-Suppl.pdf
966K