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Penulis : Ahmad Muslim, Rahmat Pratama, Suwandi Suwandi, Harman Hamidson

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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, Indonesia

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Keyword:	Ceratocystis wilt, canker, die-back disease

Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

Running title: Ceratocystis Wilt on *Lansium domesticum*

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Abstract

Ceratocystis wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on ITS and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately

pathogenic to *Acacia carsicarpa, Eucalyptus urophylla,* and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata, Hevea brasiliensis,* and *Alstonia scholaris*. Therefore, this pathogen becomes serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.

Keywords: agroforestry plants, canker, *Certocystis fimbriata,* die-back disease.

Introduction

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8, 14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum Corr.* has also been reported to have benefits as larvicides (Ni'mah et al., 2015: Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria spp.* (Nurhayati et al., 2016),

organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Tilaar et al., 2008; Subandrate et al., 2016).

Previous studies conducted in 2014 to 2017 (Suwandi et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in 3 locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis* and *Chukrasia tabularis* (Chi et al., 2019a; Chi et al., 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Alstonia scholaris, Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Material and Methods

Diseases incidence, Sample collection, and Fungal isolation

Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Figure 1). In each

plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Suwandi et al., 2021; Pratama et al., 2021a). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *Lansium domesticum* which showed symptoms of branch wilting, discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of Ceratocystis was carried out based on Carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 minutes, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on Malt Extract Agar (MEA) media at room temperature (25 °C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% malt extract agar (MEA, 20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization

The morphological characteristics of the observed fungi were represented by isolates originating from 8 regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores.

Growth in culture

To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of 8 isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30 °C with an interval of 5 °C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses

The pure cultures used for the DNA extraction were fourteen isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193,

CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, USA).

Amplification and PCR sequencing were obtained from two gene regions, namely Beta tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Germany) (25 mM MgCl2, 0.06 U/µl Taq-DNA-Polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch™ thermal cycler (Bio-Rad, USA). The parameters were initial denaturation for 3 minutes at 94°C, 30 cycles for 30 seconds at 94°C for 30 seconds, for 30 seconds at 52°C, and 1 minute at 72 °C for. Amplification was completed at 72°C for 10 minutes and the PCR product was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al. 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For maximum parsimony analysis, the metrics calculated included tree length (TL), retention index (RI), and consistency index (CI). Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials

These studies were conducted using ten isolates of *C. fimbriata* from two disease severely affected areas, namely Ogan Komering Ulu and Musi Banyuasin (Table 1). Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2–3 cm and a height of 50–60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *Acacia mangium, Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Hevea brasiliensis, Alstonia scholaris,* and *Melaleuca cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2–3 cm and a height of 70–80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were

arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package. Furthermore, the Analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey'sHSD) test was used to determine the significant differences in the mean comparisons of the different treatments.

Results and discussion

Diseases incidence, Sample collection, and Fungal isolation

Ceratocystis wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu district, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Figure 1). It has spread widely in other plantations in the Ogan Komering Ulu district covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and

this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya.

From 2020 to 2021, there were similar disease incidence on the duku plantations in Ogan Komering Ilir (OKI), within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestation were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively. Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Figure 2 a and b). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Figures 2c and d). There is a wound on the diseased tree caused by a squirrel scratch (Figure 2e). In general, holes will appear on the infected duku stem caused by *Hypocryphalus mangiferae* (Figure 2 f) which is a vector insect for *Ceratocystis* (Figure 2g).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East

Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80 %, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include (CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192) from Ogan Komering Ulu, (CAL32159, CAL32156, CAL32157, and CAL32158) from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Morphological characterization and Growth in culture

The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Figure 3a) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Figure 3b). This fungus also produced chained barrel-shaped conidia (Figure 3c), and chlamydospores (Figure 3d), it also had hat-shaped ascospores (Figure 3e). Cylindrical conidia (Figure 3g) were generated from the primary phialidic conidiophore (Figure 3f).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *Mangifera indica* (van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and

the diseased *Acacia mangium* (Tarigan et al. 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. Fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Figure 4).

DNA extraction, amplification, sequencing, and phylogenetic analyses

For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Table 4). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences. Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*.

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the maximum parsimony (MP) method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis, Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figures 5 and 6). The phylogeny was assessed and analyzed using bootstrap analysis with 1000 replications, as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials

L. domesticum seedlings inoculated in the first experiment showed discoloration in the bundle vessels, whereby 90% and 100% of it dies 45, as well as 70 days after pathogen inoculation respectively (Fig. 6a; b). Analysis of variance for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 5). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings, and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (figure 6c;d). There was no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 6). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa, E. urophylla,* and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata, H. brasiliensis,* and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The reisolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted in 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006; Al Adawi et al., 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007; Tarigan, 2011). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to the stem, which is spread by squirrels and insects. This disease also occur from the root and continue up to the base of the stem. the infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities to *C. fimbriata* which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were 2 variations of the ITS rDNA sequence from 2 isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype.

ITS5 haplotype was the most common genotype since of it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin district. ITS6z was not isolated from this study. It might due to the haplotype have a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Tarigan et al., 2011; Pratama et al., 2021a; Pratama et al., 2021b). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *Lansium domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010; Tarigan et al., 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *Colocasia esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the

perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa, E. urophylla,* and *M. cajuputi,* as well as weakly pathogenic to *D. costulata, A. scholaris,* and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, with the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *Lansium domesticum* caused by *Ceratocystis Fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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522 **Table 1.** Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (n = 89)	53.9	64	85.4
Saleman (n = 74)	41.9	58.1	95.9
Singapura (n = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (n = 91)	59.3	72.5	84.6
Tebat Agung (n = 67)	10.5	16.4	31.3
Padang Bindu (n = 71)	5.6	15.5	19.7
Kepayang (n = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5

Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

529 **Table 2.** Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood
530 onto the MEA from samples collected from dying *Lansium domesticum* trees in Ogan
531 Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*
Ogan Komering Ulu	Kartamulya	2019	2/5 (40 %)
	Saleman	2019	5/5 (100 %)
	Singapura	2019	2/5 (40 %)
	Pengaringan	2020	5/5 (100 %)
	Reksa Jiwa	2020	2/5 (40 %)
	Tebat Agung	2020	3/5 (60 %)
	Padang Bindu	2020	2/5 (40 %)
	Kepayang	2020	5/5 (100 %)
		Total	26/40 (65%)
East Ogan Komering Ulu	Bantan Pelita	2021	4/5 (80%)
		Total	4/5 (80%)
South Ogan Komering Ulu	Simpang	2021	4/5 (80%)
	Tanjung Sari	2021	2/5 (40%)
	Tanjung	2021	4/5 (80%)
	Beringin	2021	2/5 (40%)
	Kisau	2021	2/5 (40%)
		Total	14/25 (56%)
Ogan Komering Ilir	Penyandingan	2020	3/5 (60%)
	Ulak Kemang	2020	3/5 (60%)
	Tanjung Lubuk	2020	2/5 (40%)
		Total	8/15 (53.3%)

Musi Banyuasin	Kasmaran	2021		1/5 (20 %)
	Babat Toman	2021		2/5 (40 %)
	Beruge	2021		1/5 (20 %)
	Sereka	2021		2/5 (40 %)
	Sanga Desa	2021		5/5 (100 %)
	Tanjung Raya	2021		5/5 (100 %)
			Total	16/30 (53.3 %)
Musi Rawas	Tuah Negri	2021		4/5 (80%)
	Mambang	2021		5/5 (100%)
	Lubuk Tuo	2021		3/5 (60%)
			Total	12/15 (80%)
North Musi Rawas	Beringin Jaya	2021		3/5 (60%)
	Lawang Agung	2021		5/5 (100%)
	Karang Waru	2021		3/5 (60%)
	Rantau Kadam	2021		3/5 (60%)
	Lesung Batu	2021		2/5 (40%)
			Total	16/25 (64%)
Muara Enim	Ujan mas	2020		3/5 (60%)
			Total	3/5 (60%)

535 **Table 3.** Morphology of selected *Ceratocystis Fimbriata* isolates from a different district in South Sumatra

536

Isolates/Morphological characters[a]	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3 to 312.4	122.9 to 291.4	135.7 to 325.2	141.3 to 317.1	137.9 to 321.1	132.1 to 334.9	137.9 to 346.1	122.1 to 316.9
Ascomatal base (l)	153.1 to 404.4	131 to 315.4	148.1 to 398.4	151.1 to 411.4	143.1 to 398.4	152.4 to 394.1	139.1 to 421.8	157.1 to 412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4 to 768.4	354.9 to 677.7	413.7 to 798.8	439.9 to 736.4	475.8 to 813.6	484.6 to 790.9	463.8 to 723.6	484.6 to 780.9
Neck (w) top	11.5 to 26.8	7.06 to 18.4	11.3 to 21.9	11.1 to 25.4	10.1 to 17.9	11.3 to 21.7	11.1 to 22.9	11.3 to 21.7

Neck (w) bottom	24.8 to 47.9	20.3 to 39.7	23.6 to 42.6	22.6 to 51.2	23.7 to 43.8	22.67 to 42.9	23.7 to 43.6	22.67 to 44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2 to 43.5	30.4 to 40.1	32.7 to 44.7	32.7 to 42.2	33.5 to 43.9	33.7 to 44.8	33.5 to 42.9	31.7 to 44.8
Ascospores								
Hat-shaped ascospores (l)	3.4 to 5.7	3.3 to 5.2	3.2 to 5.4	3.4 to 4.9	3.2 to 4.4	3.1 to 5.1	3.1 to 4.3	3.3 to 4.9
Ascospores (w) without sheath	3.4 to 5.1	3.1 to 4.1	3.3 to 4.7	3.4 to 4.4	3.3 to 4.1	3.4 to 4.5	3.3 to 4.1	3.5 to 4.4
Ascospores (w) with sheath	5 to 6.8	4.1 to 6.1	5.1 to 6.7	5.3 to 6.4	5.2 to 6.5	5.5 to 6.7	5.2 to 6.3	5.4 to 6.6
Primary conidia (l)	12.1 to 27.5	10.6 to 18.9	13.8 to 23.8	12.2 to 29.3	13.2 to 25.7	14.9 to 24.8	12.5 to 21.6	13.7 to 24.6
Primary conidia (w)	3.5 to 7.4	3.2 to 4.3	3.1 to 5.1	3.4 to 4.1	3.2 to 5.1	3.4 to 4.4	3.4 to 4.1	3.5 to 4.7
Secondary Conidia (l)	6.3 to 11.6	5.7 to 10.1	6.6 to 11.8	7.9 to 11.8	6.7 to 11.9	6.8 to 11.5	6.5 to 11.5	6.2 to 11.3

Secondary Conidia (w)	4.5 to 7.6	4.1 to 7.4	4.7 to 7.5	5.6 to 7.9	4.3 to 7.8	4.3 to 7.8	4.3 to 7.1	4.1 to 7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7 to 15.1	8.7 to 15.1	11.3 to 15.6	9.7 to 17.8	10.7 to 15.4	10.1 to 16.5	10.3 to 14.6	10.4 to 14.5
Chlamydospores (w)	7.9 to 13.9	8.3 to 11.1	6.9 to 14.2	6.8 to 13.6	7.6 to 11.8	7.7 to 12.5	7.6 to 11.8	7.6 to 12.9
Culture growth rate at	0	0	0	0	0	0	0	0
10 °C								
15 °C	3.3 to 3.5	2.2 to 2.5	3.2 to 3.5	2.2 to 2.7	3.2 to 3.4	2.2 to 2.8	2.3 to 2.9	2.4 to 2.8
20 °C	3.2 to 3.7	3.1 to 2.9	3.2 to 3.9	3.3 to 3.9	4.2 to 4.4	3.2 to 3.5	4.2 to 4.4	3.2 to 3.5
25 °C	5.1 to 5.3	4.1 to 4.5	4.7 to 5.1	4.4 to 4.7	4.4 to 4.9	4.1 to 4.5	4.4 to 4.9	4.1 to 4.5
30 °C	3.3 to 3.6	3.1 to 3.9	3.5 to 4.6	3.5 to 4.2	3.8 to 4.2	3.1 to 3.4	3.8 to 4.2	3.1 to 3.4

[a] All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm

[b] Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after fourteen days

Table 4. *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. Grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	CAL32194	*Lansium domesticum*	Indonesia	MT373418	MW752140
	ITS5	CAL32191	*L. domesticum*	Indonesia	MT373420	MW752141

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS5	CAL32193	*L. domesticum*	Indonesia	MT373417	MW752142
	ITS5	CAL32196	*L. domesticum*	Indonesia	MT373419	MW752144
	ITS5	CAL32195	*L. domesticum*	Indonesia	MT373416	MW752145
	ITS5	CAL32192	*L. domesticum*	Indonesia	MT373415	MW752146
	ITS5	CAL31663	*L. domesticum*	Indonesia	MT373422	-
	ITS5	CAL32367	*L. domesticum*	Indonesia	MT373421	-
	ITS5	CAL32164	*L. domesticum*	Indonesia	-	-
	ITS5	CAL30673	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31351	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31654	*L. domesticum*	Indonesia	-	-
	ITS5	CMW38737	*E. Grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus Mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS7b	CAL32156	*L. domesticum*	Indonesia	-	MW752143
	ITS7b	CAL32157	*L. domesticum*	Indonesia	-	MW752147
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina Arborea*	Brazil	AY157967	-
	ITS16	C924	*G. Arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin America n clade (LAC)	CMW17808	*E. Grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillate	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	LAC	CBS115173	*Gmelina Arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

554 **Table 5.** Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery
555 condition.
556

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35f	7/10	10/10
CAL32157	10	15.49ef	7/10	8/10
CAL32158	10	12.29cd	5/10	5/10
CAL32159	10	11.02c	2/10	5/10
CAL32191	10	11.73cd	2/10	3/10
CAL32192	10	13.83def	7/10	8/10
CAL32193	10	19.81g	9/10	10/10
CAL32194	10	6.86b	2/10	2/10
CAL32195	10	12.89cde	5/10	6/10
CAL32196	10	11.19cde	5/10	7/10
Control (MEA)	10	0.01a	0/10	0/10
P		<0.001		

557
558 Values followed by the same letters in a column are not different among isolates at P=0.05
559 according to Tukey's HSD multiple range test.
560
561
562

563 **Table 6.** Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition.

564

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*		
		Lesion length (cm)	Wilting and death at 45 dpi*	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25ef	10/10	10/10	9.86de	0/10	1/10	11.32b	0/10	1/10
CAL32157	10	16.32de	10/10	10/10	10.16de	0/10	2/10	11.81b	0/10	1/10
CAL32158	10	14.49cde	8/10	10/10	9.39cd	0/10	1/10	9.33b	0/10	0/10
CAL32159	10	13.59bcd	8/10	10/10	8.26bcd	0/10	1/10	9.86b	0/10	0/10
CAL32191	10	11.73bc	7/10	10/10	7.96bcd	0/10	0/10	9.82b	0/10	0/10
CAL32192	10	15.54cde	10/10	10/10	6.57bc	0/10	0/10	10.59b	0/10	0/10
CAL32193	10	20.93f	10/10	10/10	12.59e	0/10	5/10	11.92b	0/10	3/10
CAL32194	10	9.943b	5/10	10/10	5.97b	0/10	0/10	8.80b	0/10	0/10

CAL32195	10	15.39cde	9/10	10/10	7.82bcd	0/10	2/10	11.20b	0/10	2/10
CAL32196	10	14.64cde	8/10	10/10	8.64bcd	0/10	1/10	11.15b	0/10	1/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001		

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test. * dpi=days post inoculation.

577 **Table 6.** (Continued)

Isolates	Host test	*Dyera costulata*			*Hevea brasiliensis*			*Alstonia scholaris*			*Melaleuca leucadendra*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	4.25b	0/10	0/10	5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10
CAL32157	10	3.91b	0/10	0/10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10
CAL32158	10	3.63b	0/10	0/10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10
CAL32159	10	3.83b	0/10	0/10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10
CAL32191	10	3.57b	0/10	0/10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10
CAL32192	10	5.15b	0/10	0/10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10
CAL32193	10	5.39b	0/10	0/10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10
CAL32194	10	3.05b	0/10	0/10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10

CAL32195	10	4.02b	0/10	0/10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10
CAL32196	10	3.60b	0/10	0/10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001			<0.001		

578

579 Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test.

580

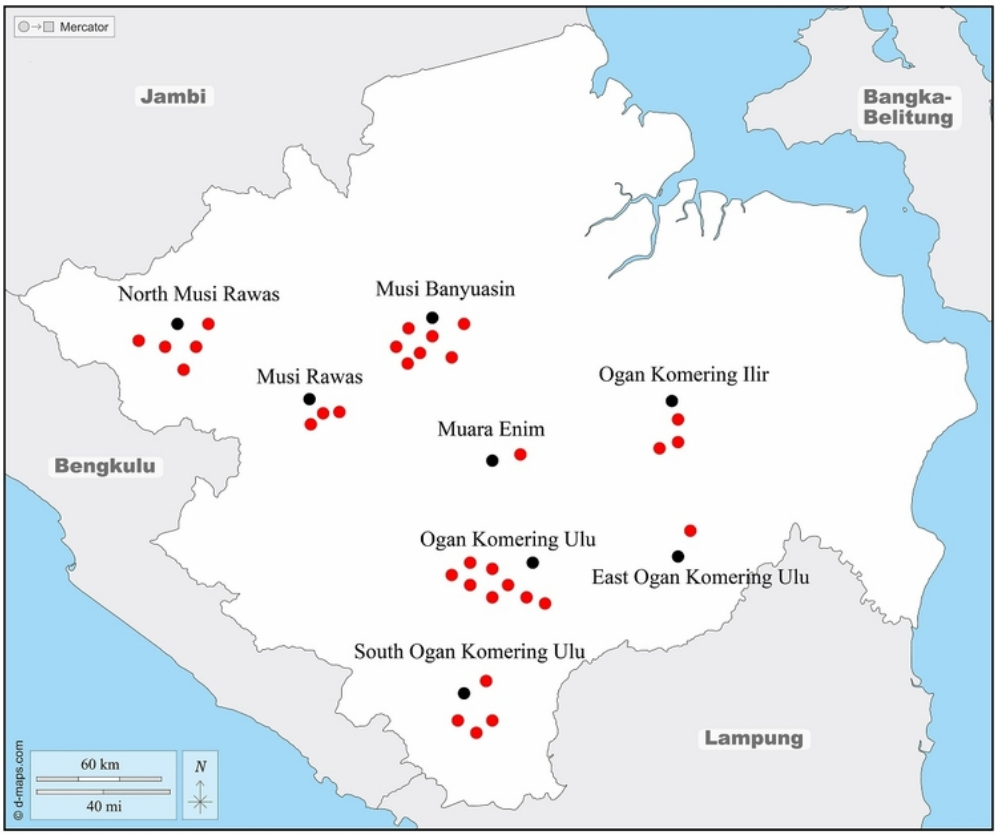

Fig. 1. Map of South Sumatera, red circle showing the collection sites for Ceratocystis fimbriata.

31x26mm (600 x 600 DPI)

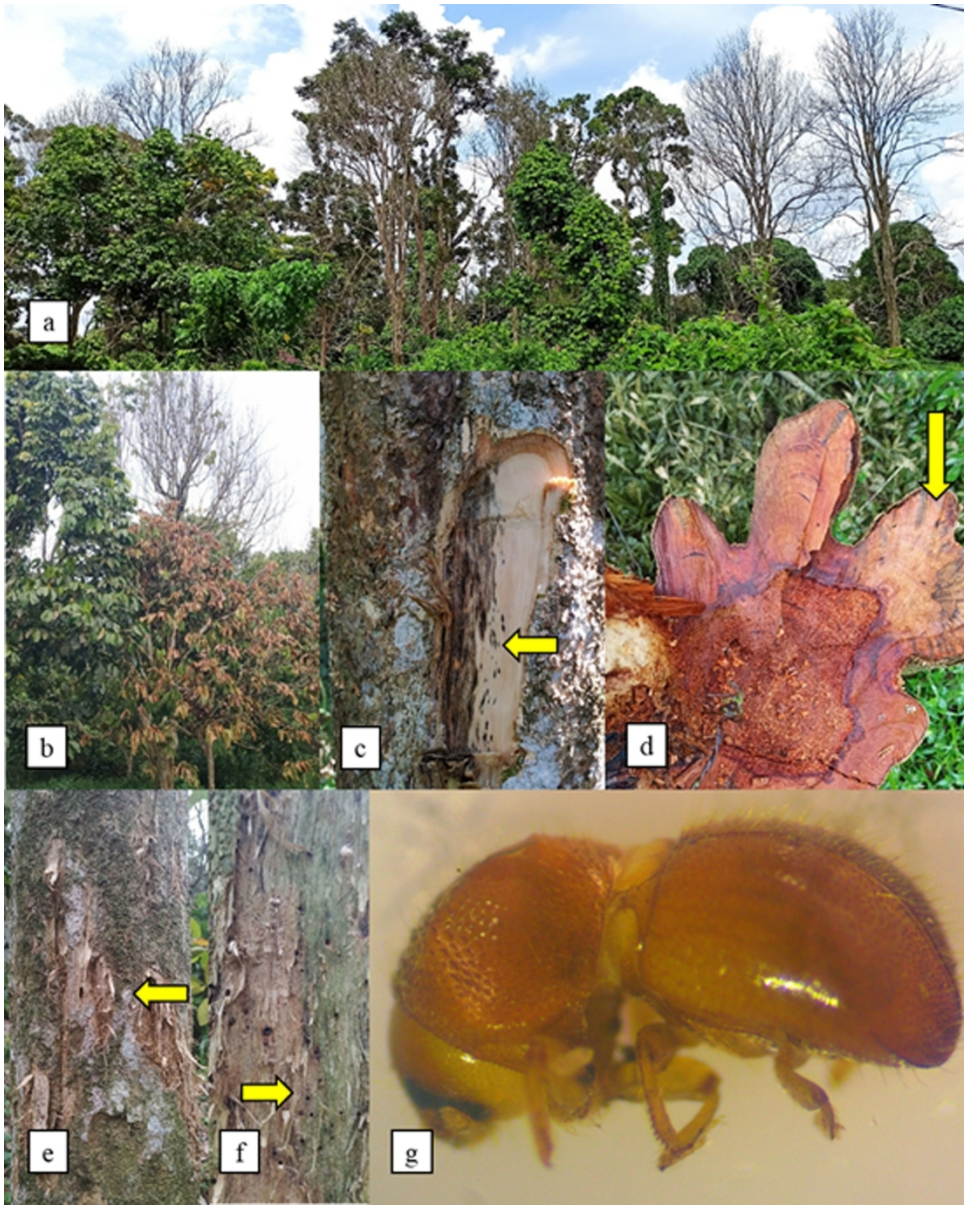

Fig. 2. Symptoms of wilt and die-back on Lansium domesticum. a, b. Trees affected by Ceratocystis fimbriata experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. c, d. Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks. e. Squirrel attacks caused peeled-off bark on diseased tree. f. a beetle hole on affected diseased wood. g. Hypocryphalus mangiferae as a vector for the spread of Ceratocystis.

69x87mm (600 x 600 DPI)

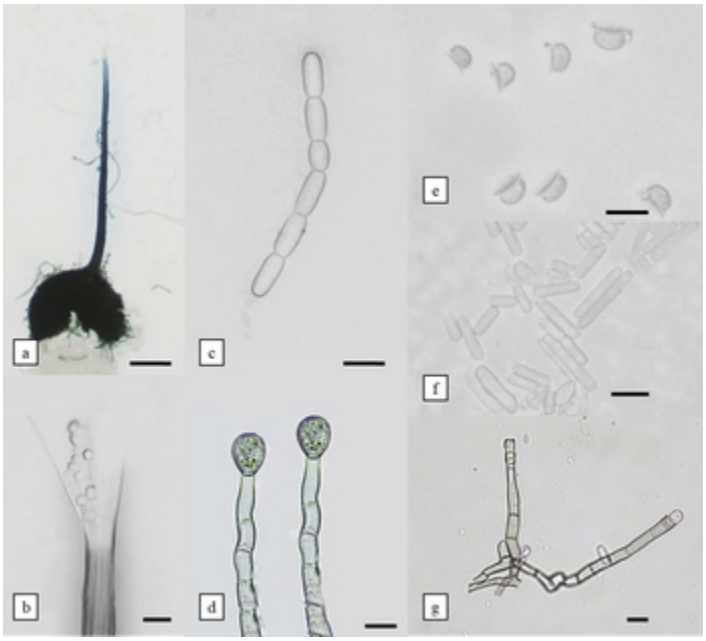

Fig. 3. Morphological characteristics of Ceratocystis fimbriata isolated from Lansium domesticum stem lesion: a. globose ascomata with a long neck, b. divergent ostiolar hyphae, c. barrel-shaped conidia, d. chlamydospores, e. hat-shaped ascospores, f. cylindrical conidia g. conidiophore/phialide, —Scale bars: a = 100 μm; b,c,d,e = 10 μm; f = 5 μm.

14x13mm (600 x 600 DPI)

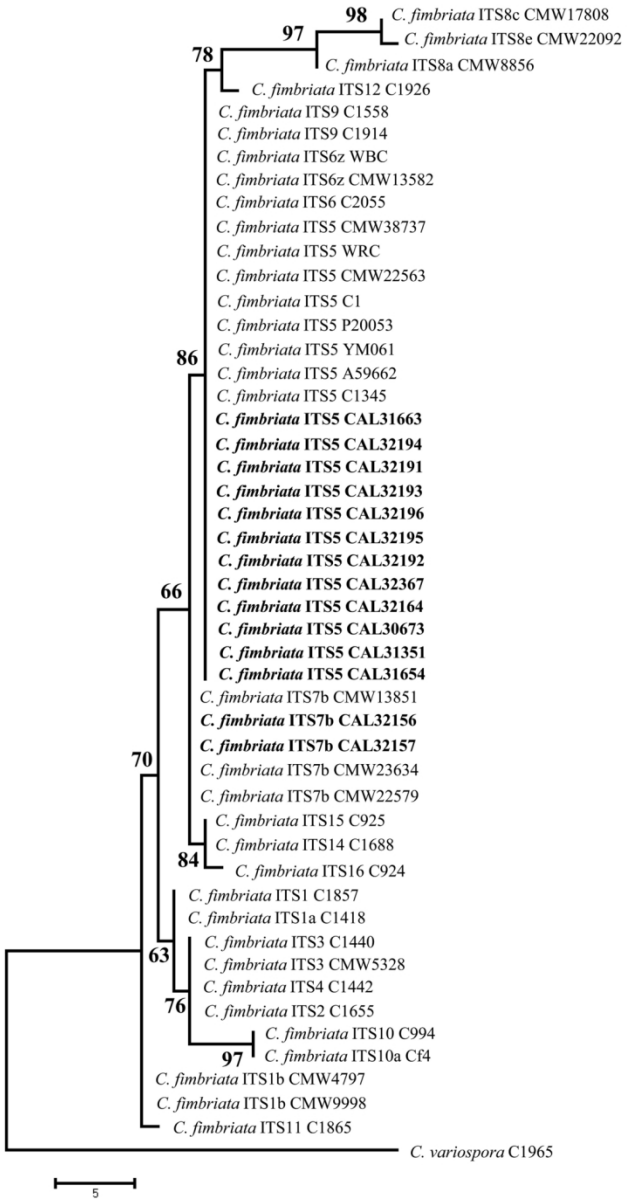

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between Ceratocystis fimbriata from the Lansium tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the C. fimbriata species complex. C. variospora is used as an outgroup.

62x90mm (600 x 600 DPI)

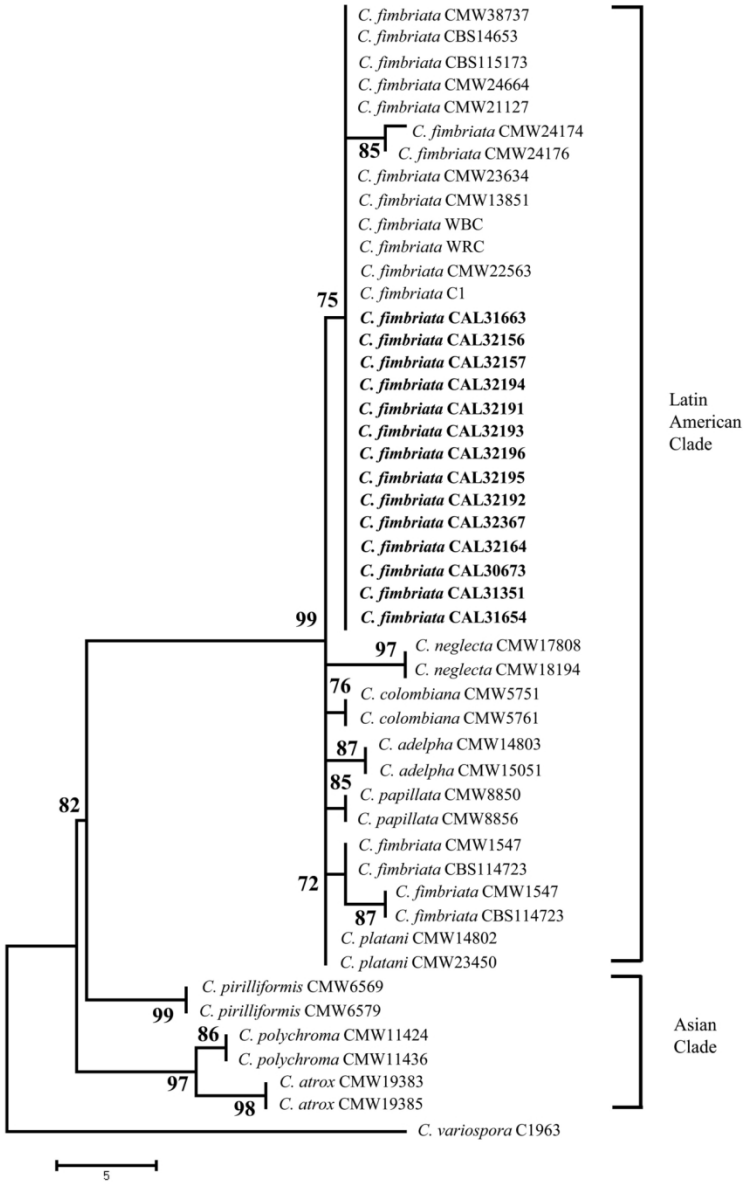

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in Ceratocystis fimbriata sensu stricto. Isolates from Lansium domesticum in Indonesia are marked in bold. The ITS haplotypes of C. fimbriata are numbered following the numerical designation of Harrington et al. (2014). C. variospora is used as an outgroup taxon.

62x90mm (600 x 600 DPI)

Fig. 6. Symptoms of mycelial plug inoculation with Ceratocystis fimbriata isolates (CAL32194 and CAL32159) from Lansium domesticum 45 days after inoculation. a. Symptoms on 2-year-old duku seedlings (L. domesticum) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). b. The formation of an upward lesion from the inoculation site (red arrow) on duku plants after being inoculated by CAL32194 (II) and CAL32159 (III). c. d. 4-month-old Acacia plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (II) and CAL32159 (III). e. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old Eucalyptus, at 45 days of observation did not show any signs of wilting. f. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old Acacia crassicarpa, at 45 days of observation did not show any signs of wilting.

10x12mm (600 x 600 DPI)

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We really hope our manuscript can be published in the Plant Pathology Journal.

Thank you very much for your kindness and consideration of our manuscript.

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Faculty of Agriculture

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Date Sent: 30-Dec-2021

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4.Bukti konfirmasi review dan hasil review pertama (28 Januari 2022)

The Plant Pathology Journal

Preview (PPJ-OA-12-2021-0182)

From: hyuck1857@dau.ac.kr

To: a_muslim@unsri.ac.id

CC:

Subject: The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182

Body: 28-Jan-2022

Dear Dr. Ahmad Muslim:

Manuscript ID PPJ-OA-12-2021-0182 entitled "Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, 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 suggested minor revisions to your manuscript. 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. Jungkwan Lee
Editor In Chief
The Plant Pathology Journal
jungle@dau.ac.kr

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

Comments to the Author
This paper called ‘Diseases severity, genetic variation, and pathogenicity of Ceratocystis wilt on Lansium domesticum in South Sumatra, Indonesia’ seems to be an interesting new achievement. Overall, the contents are well written and covers Ceratocystis wilt could pose a serious threat to Indonesia's biodiversity, and thus will undoubtedly be informative to the readers of PPJ. Nevertheless, I wrote two comments in the manuscript to improve and clarify the work.

Reviewer: 2

Comments to the Author

- Some minor grammar mistakes still are found in the manuscript.

- The number of isolates use for morphological characterization, phylogenetic analysis, pathogenicity test are not in the same number. So, authors should be make a reason why the number of isolates are not in the same number.

- References for morphological characterization in the method should be mentioned.

Date Sent: 28-Jan-2022

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This paper called ‘Diseases severity, genetic variation, and pathogenicity of Ceratocystis wilt on *Lansium domesticum* in South Sumatra, Indonesia’ seems to be an interesting new achievement. Overall, the contents are well written and covers *Ceratocystis* wilt could pose a serious threat to Indonesia's biodiversity, and thus will undoubtedly be informative to the readers of PPJ. Nevertheless, I wrote two comments in the manuscript to improve and clarify the work.

Comments:

1. Table 3 : *Ceratocystis Fimbriata* → *Ceratocystis fimbriata*
2. Table 3 :

Morphological characters	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351

5. Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (31 Januari 2022)

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

The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182

a. muslim unsri <a_muslim@unsri.ac.id> Sun, Jan 30, 2022 at 3:23 PM
To: hyuck1857@dau.ac.kr

January 31, 2022

Prof. Jungkwan Lee
Editor in Chief
The Plant Pathology Journal

Dear Prof. Jungkwan Lee,

Thank you very much for your email regarding reviewer's comments and your suggestion of our manuscript. We would like to thank and appreciate for all reviewers' suggestions and corrections.

We have made corrections and some modifications according to Reviewer's revisions. Here, we enclose our revised manuscript with tracked changes of the manuscript, ID PPJ-OA-12-2021-0182 entitled "Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, Indonesia" by Ahmad Muslim, Rahmat Pratama, Suwandi Suwandi, Harman Hamidson.

In this opportunity, we would like to re-submit our revised manuscript for publication in The Plant Pathology Journal.

Below is a summary of our answers made in response to the reviewer's comments.

1. First reviewer's comment: This paper called 'Diseases severity, genetic variation, and pathogenicity of Ceratocystis wilt on Lansium domesticum in South Sumatra, Indonesia' seems to be an interesting new achievement. Overall, the contents are well written and covers Ceratocystis wilt could pose a serious threat to Indonesia's biodiversity, and thus will undoubtedly be informative to the readers of PPJ. Nevertheless, I wrote two comments in the manuscript to improve and clarify the work.

1. Table 3 : *Ceratocystis Fimbriata* → *Ceratocystis fimbriata*
2. Table 3 :

Morphological characters	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351

Our response:

1. We agree and the sentence has been changed to be "*Ceratocystis fimbriata*".

2. We agree and are grateful for this suggestion, the table 3 has been changed to be reviewer's suggestion. The revised format of the table had inserted in the manuscript.

2. Second Reviewer's comment: Some minor grammar mistakes still are found in the manuscript.

Our response: We have read our manuscript carefully and have revised the minor grammar mistake. The revised minor grammar had inserted in the manuscript.

3. Second Reviewer's comment: The number of isolates use for morphological characterization, phylogenetic analysis, pathogenicity test are not in the same number. So, authors should be make a reason why the number of isolates are not in the same number.

Our response: The isolates were selected from the most severely affected area, namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. This sentence has been added in the materials and methods in section of inoculation trials (line 166-168).

4. Second Reviewer's comment : References for morphological characterization in the method should be mentioned.

Our response: We are appreciating for this comment, we refered to Al Adawi *et al.*, 2013 for morphological characterization in the method and the reference had inserted in the method section (line 118-119).

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-

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in The Plant Pathology Journal.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
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The Plant Pathology Journal

Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, Indonesia

Journal:	*The Plant Pathology Journal*
Manuscript ID	PPJ-OA-12-2021-0182.R1
Manuscript Type:	Original Article
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Complete List of Authors:	Muslim, Ahmad; Sriwijaya University Faculty of Agriculture, Plant Protection Pratama, Rahmat; Sriwijaya University Faculty of Agriculture, Plant Protection Suwandi, Suwandi; Sriwijaya University Faculty of Agriculture, Plant Protection Hamidson, Harman; Sriwijaya University Faculty of Agriculture, Plant Protection
Keyword:	Ceratocystis wilt, canker, die-back disease

Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

Running title: Ceratocystis Wilt on *Lansium domesticum*

Ahmad Muslim*, Rahmat Pratama, Suwandi Suwandi, Harman Hamidson

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

*Corresponding author : Ahmad Muslim (Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra, 30662, Indonesia, +62 811-7826-119, a_muslim@unsri.ac.id, https://orcid.org/0000-0002-3973-7443)

Abstract

Ceratocystis wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on ITS and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately

pathogenic to *Acacia carsicarpa, Eucalyptus urophylla,* and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata, Hevea brasiliensis,* and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.

Keywords: agroforestry plants, canker, *Certocystis fimbriata,* die-back disease.

Introduction

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8, 14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum Corr.* has also been reported to have benefits as larvicides (Ni'mah et al., 2015: Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria spp.* (Nurhayati et al., 2016),

organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Tilaar et al., 2008; Subandrate et al., 2016).

Previous studies conducted in 2014 to 2017 (Suwandi et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in 3 locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis* and *Chukrasia tabularis* (Chi et al., 2019a; Chi et al., 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Alstonia scholaris, Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Material and Methods

Diseases incidence, Sample collection, and Fungal isolation

Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Figure 1). In each

plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Suwandi et al., 2021; Pratama et al., 2021a). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *Lansium domesticum* which showed symptoms of branch wilting, discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of Ceratocystis was carried out based on ~~Carrot~~ carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 minutes, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on Malt Extract Agar (MEA) media at room temperature (25 °C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% malt extract agar (MEA, 20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization

The morphological characteristics of the observed fungi were represented by isolates originating from 8 regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture

To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of 8 isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30 °C with an interval of 5 °C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses

The pure cultures used for the DNA extraction were fourteen isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193,

CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, USA).

Amplification and PCR sequencing were obtained from two gene regions, namely Beta tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Germany) (25 mM MgCl2, 0.06 U/µl Taq-DNA-Polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch™ thermal cycler (Bio-Rad, USA). The parameters were initial denaturation for 3 minutes at 94°C, 30 cycles for 30 seconds at 94°C for 30 seconds, for 30 seconds at 52°C, and 1 minute at 72 °C for. Amplification was completed at 72°C for 10 minutes and the PCR product was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al. 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For maximum parsimony analysis, the metrics calculated included tree length (TL), retention index (RI), and consistency index (CI). Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials

These studies were conducted using ten isolates of *C. fimbriata*. The isolates were selected from the most severely affected area ~~from two disease severely affected areas,~~ namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2–3 cm and a height of 50–60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *Acacia mangium, Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Hevea brasiliensis, Alstonia scholaris,* and *Melaleuca cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2–3 cm and a height of 70–80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.)

into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package. Furthermore, the Analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey'sHSD) test was used to determine the significant differences in the mean comparisons of the different treatments.

Results and discussion

Diseases incidence, Sample collection, and Fungal isolation

Ceratocystis wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu district, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Figure 1). It has spread widely in other plantations in the Ogan Komering Ulu district covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table

1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya.

From 2020 to 2021, there were similar disease incidences on the duku plantations in Ogan Komering Ilir (OKI), within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively. Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Figure 2 a and b). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Figures 2c and d). There is a wound on the diseased tree caused by a squirrel scratch (Figure 2e). In general, holes will appear on the infected duku stem caused by *Hypocryphalus mangiferae* (Figure 2 f) which is a vector insect for *Ceratocystis* (Figure 2g).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80 %, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include (CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192) from Ogan Komering Ulu, (CAL32159, CAL32156, CAL32157, and CAL32158) from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Morphological characterization and Growth in culture

The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Figure 3a) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Figure 3b). This fungus also produced chained barrel-shaped conidia (Figure 3c), and chlamydospores (Figure 3d), it also had hat-shaped ascospores (Figure 3e). Cylindrical conidia (Figure 3g) were generated from the primary phialidic conidiophore (Figure 3f).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *Mangifera indica* (van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *Acacia mangium* (Tarigan et al. 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. Fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Figure 4).

DNA extraction, amplification, sequencing, and phylogenetic analyses

For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Table 4). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences. Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*.

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the maximum parsimony (MP) method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis, Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figures 5 and 6). The phylogeny was assessed and analyzed using bootstrap analysis with 1000 replications, as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic

characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials

L. domesticum seedlings inoculated in the first experiment showed discoloration in the bundle vessels, whereby 90% and 100% of it dies 45, as well as 70 days after pathogen inoculation respectively (Fig. 6a; b). Analysis of variance for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 5). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings, and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (figure 6c;d). There was no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 6). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa, E. urophylla,* and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata, H. brasiliensis,* and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51

cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The reisolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted ~~in~~ from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006; Al Adawi et al., 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by

wind (Harrington, 2007; Tarigan, 2011). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to the stem, which is spread by squirrels and insects. This disease also occur from the root and continues up to the base of the stem. ~~the~~ The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities to *C. fimbriata* which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were 2 variations of the ITS rDNA sequence from 2 isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since ~~of~~ it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin district. ITS6z was not isolated from this study. It might be due to the haplotype ~~have~~ having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Tarigan et al., 2011; Pratama et al., 2021a; Pratama et al., 2021b). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *Lansium domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010; Tarigan et al., 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by

373 *Colocasia esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum*
374 (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A.*
375 *heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the
376 perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting
377 other trees not previously mentioned.

378 Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees
379 like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test
380 on duku showed that a very high attack intensity of 100% causes wilting and death of plants.
381 Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata*
382 derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021),
383 moderately pathogenic to *A. carsicarpa, E. urophylla,* and *M. cajuputi,* as well as weakly
384 pathogenic to *D. costulata, A. scholaris,* and *H. brasiliensis*. This was shown by the formation
385 of lesions on the stems which leads to the death of the inoculated seedlings.

386 The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of
387 seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants
388 showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue
389 until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis*
390 is a very serious economical disease that has attacked *L. domesticum* in all duku production
391 centers in South Sumatra hence it damages the income sources of farmers in this province.
392 Also, ~~with~~ the verification of *M. cajuputi* as an endogenous wetland plant that is infected and
393 causes death, becomes a threat to the indigenous ones. Given the very wide host of
394 *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

395 Sudden wilt disease on *Lansium domesticum* caused by *Ceratocystis Fimbriata* has
396 spread widely to duku production centers in various districts of South Sumatra. Furthermore,
397 the population consisted of individuals with uniform morphology dominated by ITS5 and

ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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524 **Table 1.** Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (*n* = 89)	53.9	64	85.4
Saleman (*n* = 74)	41.9	58.1	95.9
Singapura (*n* = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (*n* = 91)	59.3	72.5	84.6
Tebat Agung (*n* = 67)	10.5	16.4	31.3
Padang Bindu (*n* = 71)	5.6	15.5	19.7
Kepayang (*n* = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2

Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

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531 **Table 2.** Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood
532 onto the MEA from samples collected from dying *Lansium domesticum* trees in Ogan
533 Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*
Ogan Komering Ulu	Kartamulya	2019	2/5 (40 %)
	Saleman	2019	5/5 (100 %)
	Singapura	2019	2/5 (40 %)
	Pengaringan	2020	5/5 (100 %)
	Reksa Jiwa	2020	2/5 (40 %)
	Tebat Agung	2020	3/5 (60 %)
	Padang Bindu	2020	2/5 (40 %)
	Kepayang	2020	5/5 (100 %)
		Total	26/40 (65%)
East Ogan Komering Ulu	Bantan Pelita	2021	4/5 (80%)
		Total	4/5 (80%)
South Ogan Komering Ulu	Simpang	2021	4/5 (80%)
	Tanjung Sari	2021	2/5 (40%)
	Tanjung	2021	4/5 (80%)
	Beringin	2021	2/5 (40%)
	Kisau	2021	2/5 (40%)
		Total	14/25 (56%)
Ogan Komering Ilir	Penyandingan	2020	3/5 (60%)
	Ulak Kemang	2020	3/5 (60%)
	Tanjung Lubuk	2020	2/5 (40%)

			Total	8/15 (53.3%)
Musi Banyuasin	Kasmaran	2021		1/5 (20 %)
	Babat Toman	2021		2/5 (40 %)
	Beruge	2021		1/5 (20 %)
	Sereka	2021		2/5 (40 %)
	Sanga Desa	2021		5/5 (100 %)
	Tanjung Raya	2021		5/5 (100 %)
			Total	16/30 (53.3 %)
Musi Rawas	Tuah Negri	2021		4/5 (80%)
	Mambang	2021		5/5 (100%)
	Lubuk Tuo	2021		3/5 (60%)
			Total	12/15 (80%)
North Musi Rawas	Beringin Jaya	2021		3/5 (60%)
	Lawang Agung	2021		5/5 (100%)
	Karang Waru	2021		3/5 (60%)
	Rantau Kadam	2021		3/5 (60%)
	Lesung Batu	2021		2/5 (40%)
			Total	16/25 (64%)
Muara Enim	Ujan mas	2020		3/5 (60%)
			Total	3/5 (60%)

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537 **Table 3.** Morphology of selected *Ceratocystis Fimbriata* isolates from a different district in South Sumatra

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~~Isolates/~~Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3 to 312.4	122.9 to 291.4	135.7 to 325.2	141.3 to 317.1	137.9 to 321.1	132.1 to 334.9	137.9 to 346.1	122.1 to 316.9
Ascomatal base (l)	153.1 to 404.4	131 to 315.4	148.1 to 398.4	151.1 to 411.4	143.1 to 398.4	152.4 to 394.1	139.1 to 421.8	157.1 to 412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4 to 768.4	354.9 to 677.7	413.7 to 798.8	439.9 to 736.4	475.8 to 813.6	484.6 to 790.9	463.8 to 723.6	484.6 to 780.9
Neck (w) top	11.5 to 26.8	7.06 to 18.4	11.3 to 21.9	11.1 to 25.4	10.1 to 17.9	11.3 to 21.7	11.1 to 22.9	11.3 to 21.7

Neck (w) bottom	24.8 to 47.9	20.3 to 39.7	23.6 to 42.6	22.6 to 51.2	23.7 to 43.8	22.67 to 42.9	23.7 to 43.6	22.67 to 44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2 to 43.5	30.4 to 40.1	32.7 to 44.7	32.7 to 42.2	33.5 to 43.9	33.7 to 44.8	33.5 to 42.9	31.7 to 44.8
Ascospores								
Hat-shaped ascospores (l)	3.4 to 5.7	3.3 to 5.2	3.2 to 5.4	3.4 to 4.9	3.2 to 4.4	3.1 to 5.1	3.1 to 4.3	3.3 to 4.9
Ascospores (w) without sheath	3.4 to 5.1	3.1 to 4.1	3.3 to 4.7	3.4 to 4.4	3.3 to 4.1	3.4 to 4.5	3.3 to 4.1	3.5 to 4.4
Ascospores (w) with sheath	5 to 6.8	4.1 to 6.1	5.1 to 6.7	5.3 to 6.4	5.2 to 6.5	5.5 to 6.7	5.2 to 6.3	5.4 to 6.6
Primary conidia (l)	12.1 to 27.5	10.6 to 18.9	13.8 to 23.8	12.2 to 29.3	13.2 to 25.7	14.9 to 24.8	12.5 to 21.6	13.7 to 24.6
Primary conidia (w)	3.5 to 7.4	3.2 to 4.3	3.1 to 5.1	3.4 to 4.1	3.2 to 5.1	3.4 to 4.4	3.4 to 4.1	3.5 to 4.7
Secondary Conidia (l)	6.3 to 11.6	5.7 to 10.1	6.6 to 11.8	7.9 to 11.8	6.7 to 11.9	6.8 to 11.5	6.5 to 11.5	6.2 to 11.3

Secondary Conidia (w)	4.5 to 7.6	4.1 to 7.4	4.7 to 7.5	5.6 to 7.9	4.3 to 7.8	4.3 to 7.8	4.3 to 7.1	4.1 to 7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7 to 15.1	8.7 to 15.1	11.3 to 15.6	9.7 to 17.8	10.7 to 15.4	10.1 to 16.5	10.3 to 14.6	10.4 to 14.5
Chlamydospores (w)	7.9 to 13.9	8.3 to 11.1	6.9 to 14.2	6.8 to 13.6	7.6 to 11.8	7.7 to 12.5	7.6 to 11.8	7.6 to 12.9
Culture growth rate at	0	0	0	0	0	0	0	0
10 °C								
15 °C	3.3 to 3.5	2.2 to 2.5	3.2 to 3.5	2.2 to 2.7	3.2 to 3.4	2.2 to 2.8	2.3 to 2.9	2.4 to 2.8
20 °C	3.2 to 3.7	3.1 to 2.9	3.2 to 3.9	3.3 to 3.9	4.2 to 4.4	3.2 to 3.5	4.2 to 4.4	3.2 to 3.5
25 °C	5.1 to 5.3	4.1 to 4.5	4.7 to 5.1	4.4 to 4.7	4.4 to 4.9	4.1 to 4.5	4.4 to 4.9	4.1 to 4.5
30 °C	3.3 to 3.6	3.1 to 3.9	3.5 to 4.6	3.5 to 4.2	3.8 to 4.2	3.1 to 3.4	3.8 to 4.2	3.1 to 3.4

[a] All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm

[b] Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after fourteen days

542 **Table 4.** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. Grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	CAL32194	*Lansium domesticum*	Indonesia	MT373418	MW752140
	ITS5	CAL32191	*L. domesticum*	Indonesia	MT373420	MW752141

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS5	CAL32193	*L. domesticum*	Indonesia	MT373417	MW752142
	ITS5	CAL32196	*L. domesticum*	Indonesia	MT373419	MW752144
	ITS5	CAL32195	*L. domesticum*	Indonesia	MT373416	MW752145
	ITS5	CAL32192	*L. domesticum*	Indonesia	MT373415	MW752146
	ITS5	CAL31663	*L. domesticum*	Indonesia	MT373422	-
	ITS5	CAL32367	*L. domesticum*	Indonesia	MT373421	-
	ITS5	CAL32164	*L. domesticum*	Indonesia	-	-
	ITS5	CAL30673	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31351	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31654	*L. domesticum*	Indonesia	-	-
	ITS5	CMW38737	*E. Grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus Mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS7b	CAL32156	*L. domesticum*	Indonesia	-	MW752143
	ITS7b	CAL32157	*L. domesticum*	Indonesia	-	MW752147
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina Arborea*	Brazil	AY157967	-
	ITS16	C924	*G. Arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin America n clade (LAC)	CMW17808	*E. Grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillate	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank no.	accession
					ITS	β-tubulin
	LAC	CBS115173	*Gmelina Arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

556 **Table 5.** Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery
557 condition.
558

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35f	7/10	10/10
CAL32157	10	15.49ef	7/10	8/10
CAL32158	10	12.29cd	5/10	5/10
CAL32159	10	11.02c	2/10	5/10
CAL32191	10	11.73cd	2/10	3/10
CAL32192	10	13.83def	7/10	8/10
CAL32193	10	19.81g	9/10	10/10
CAL32194	10	6.86b	2/10	2/10
CAL32195	10	12.89cde	5/10	6/10
CAL32196	10	11.19cde	5/10	7/10
Control (MEA)	10	0.01a	0/10	0/10
P		<0.001		

559

560 Values followed by the same letters in a column are not different among isolates at P=0.05
561 according to Tukey's HSD multiple range test.
562
563
564

565 **Table 6.** Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition.

566

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*		
		Lesion length (cm)	Wilting and death at 45 dpi*	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25ef	10/10	10/10	9.86de	0/10	1/10	11.32b	0/10	1/10
CAL32157	10	16.32de	10/10	10/10	10.16de	0/10	2/10	11.81b	0/10	1/10
CAL32158	10	14.49cde	8/10	10/10	9.39cd	0/10	1/10	9.33b	0/10	0/10
CAL32159	10	13.59bcd	8/10	10/10	8.26bcd	0/10	1/10	9.86b	0/10	0/10
CAL32191	10	11.73bc	7/10	10/10	7.96bcd	0/10	0/10	9.82b	0/10	0/10
CAL32192	10	15.54cde	10/10	10/10	6.57bc	0/10	0/10	10.59b	0/10	0/10
CAL32193	10	20.93f	10/10	10/10	12.59e	0/10	5/10	11.92b	0/10	3/10
CAL32194	10	9.943b	5/10	10/10	5.97b	0/10	0/10	8.80b	0/10	0/10

CAL32195	10	15.39cde	9/10	10/10	7.82bcd	0/10	2/10	11.20b	0/10	2/10
CAL32196	10	14.64cde	8/10	10/10	8.64bcd	0/10	1/10	11.15b	0/10	1/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001		

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test. * dpi=days post inoculation.

579 **Table 6.** (Continued)

Isolates	Host test	*Dyera costulata*			*Hevea brasiliensis*			*Alstonia scholaris*			*Melaleuca leucadendra*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	4.25b	0/10	0/10	5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10
CAL32157	10	3.91b	0/10	0/10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10
CAL32158	10	3.63b	0/10	0/10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10
CAL32159	10	3.83b	0/10	0/10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10
CAL32191	10	3.57b	0/10	0/10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10
CAL32192	10	5.15b	0/10	0/10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10
CAL32193	10	5.39b	0/10	0/10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10
CAL32194	10	3.05b	0/10	0/10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10

CAL32195	10	4.02b	0/10	0/10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10
CAL32196	10	3.60b	0/10	0/10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001			<0.001		

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test.

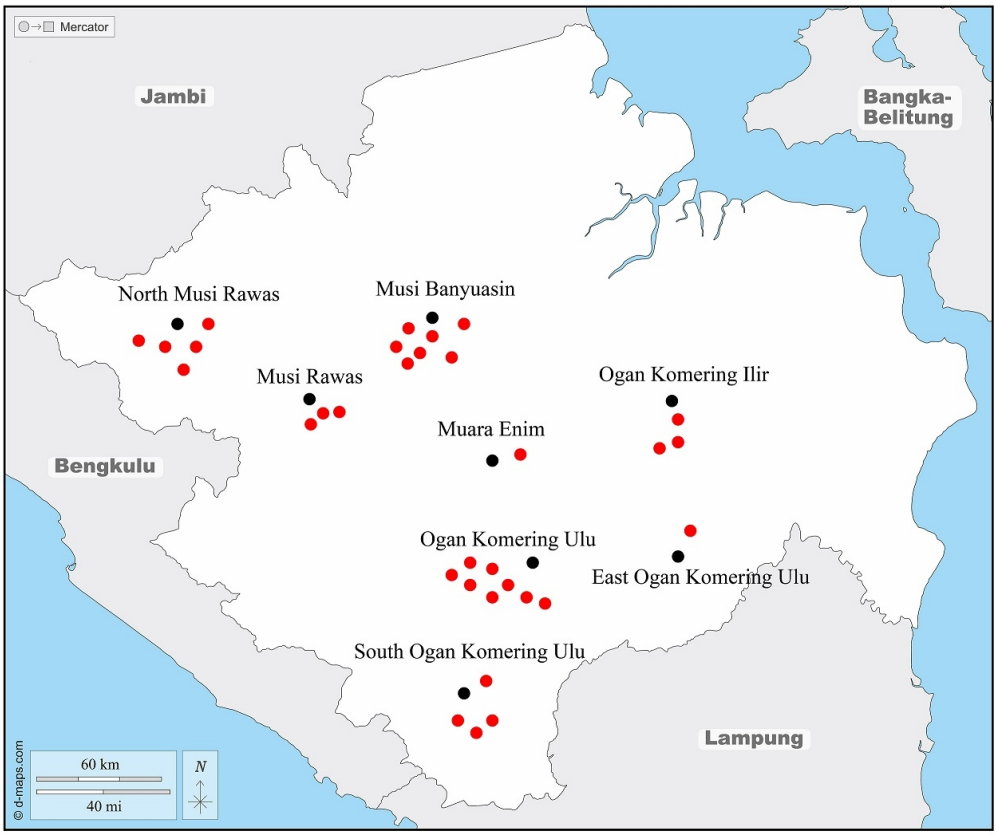

Fig. 1. Map of South Sumatera, red circle showing the collection sites for Ceratocystis fimbriata.

31x26mm (999 x 999 DPI)

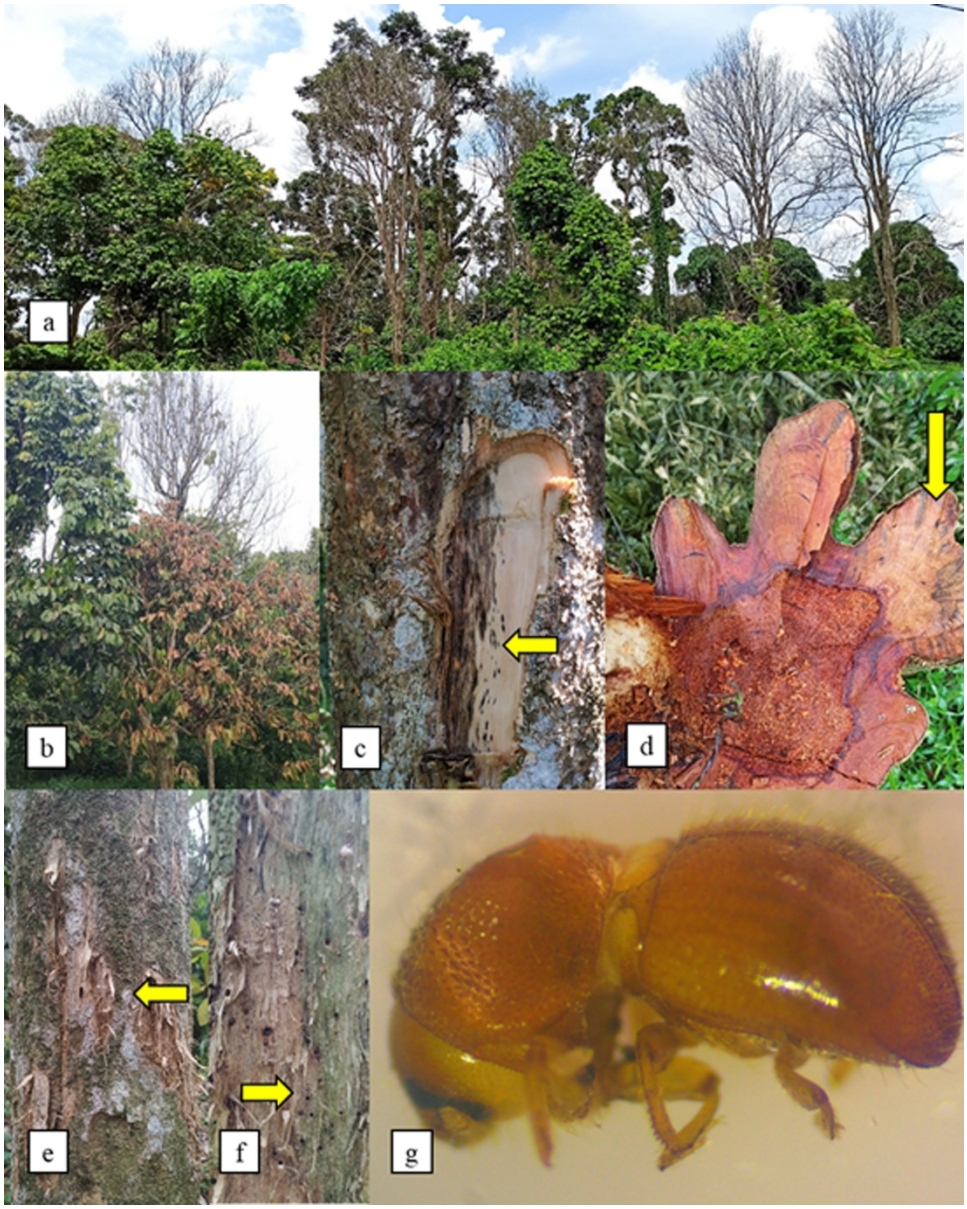

Fig. 2. Symptoms of wilt and die-back on Lansium domesticum. a, b. Trees affected by Ceratocystis fimbriata experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. c, d. Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks. e. Squirrel attacks caused peeled-off bark on diseased tree. f. a beetle hole on affected diseased wood. g. Hypocryphalus mangiferae as a vector for the spread of Ceratocystis.

69x87mm (999 x 999 DPI)

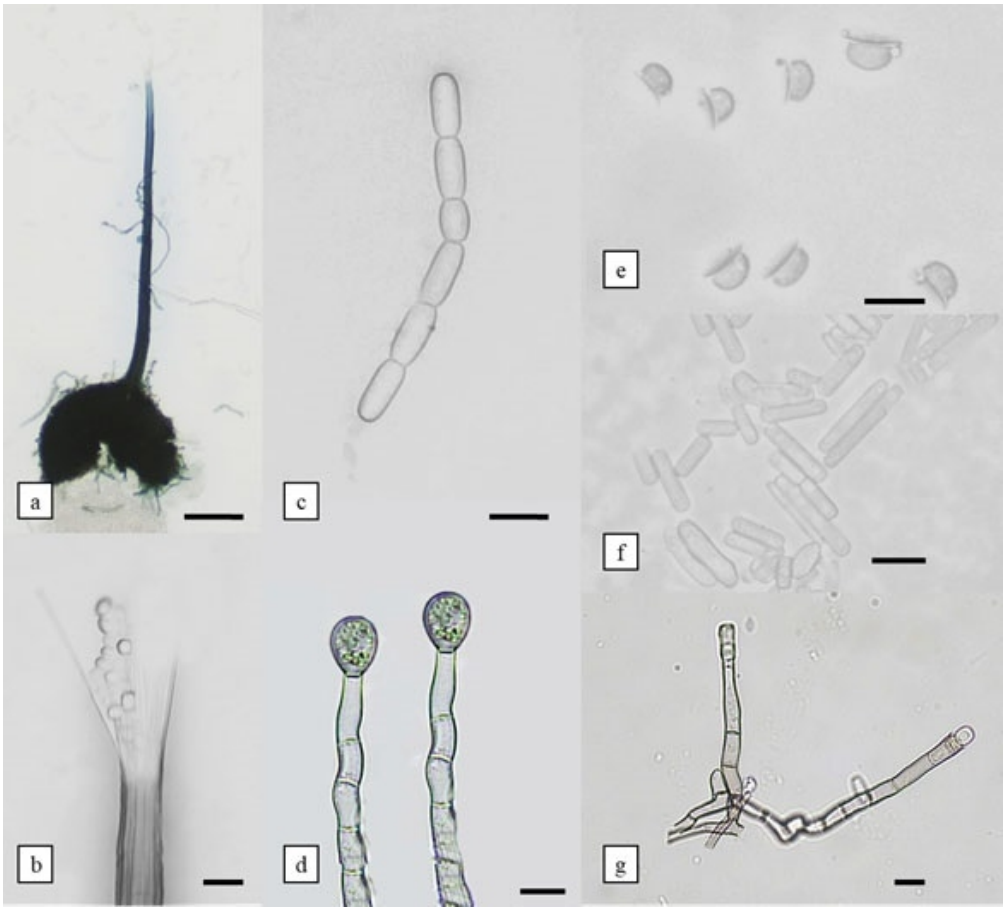

Fig. 3. Morphological characteristics of Ceratocystis fimbriata isolated from Lansium domesticum stem lesion: a. globose ascomata with a long neck, b. divergent ostiolar hyphae, c. barrel-shaped conidia, d. chlamydospores, e. hat-shaped ascospores, f. cylindrical conidia g. conidiophore/phialide, —Scale bars: a = 100 μm; b,c,d,e = 10 μm; f = 5 μm.

14x13mm (999 x 999 DPI)

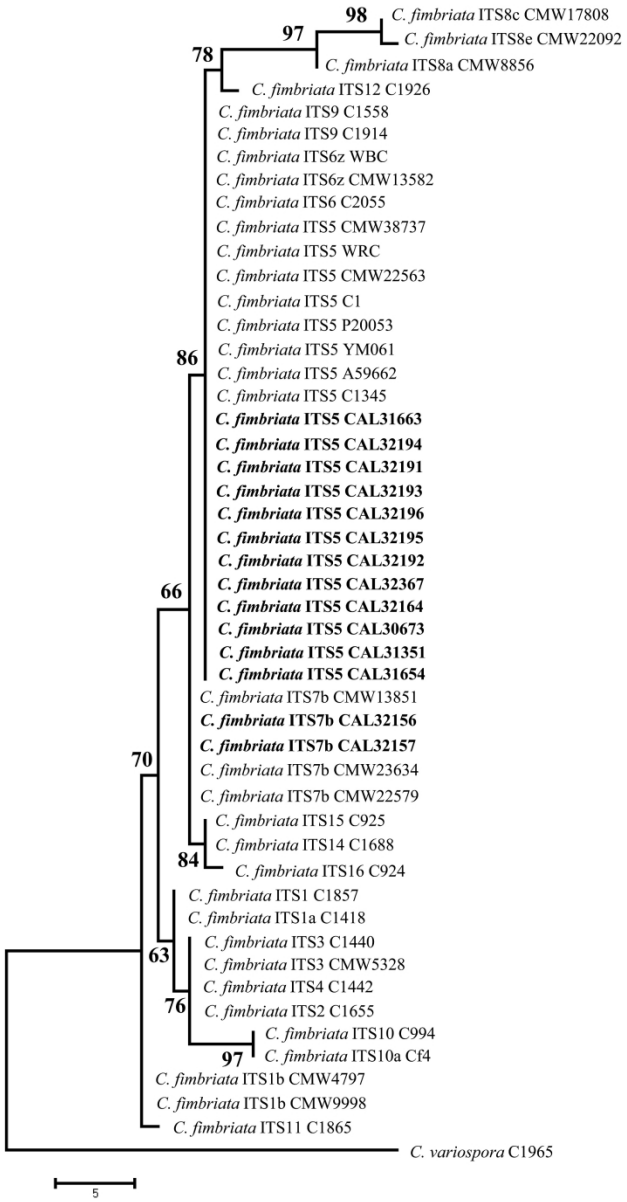

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between Ceratocystis fimbriata from the Lansium tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the C. fimbriata species complex. C. variospora is used as an outgroup.

62x90mm (999 x 999 DPI)

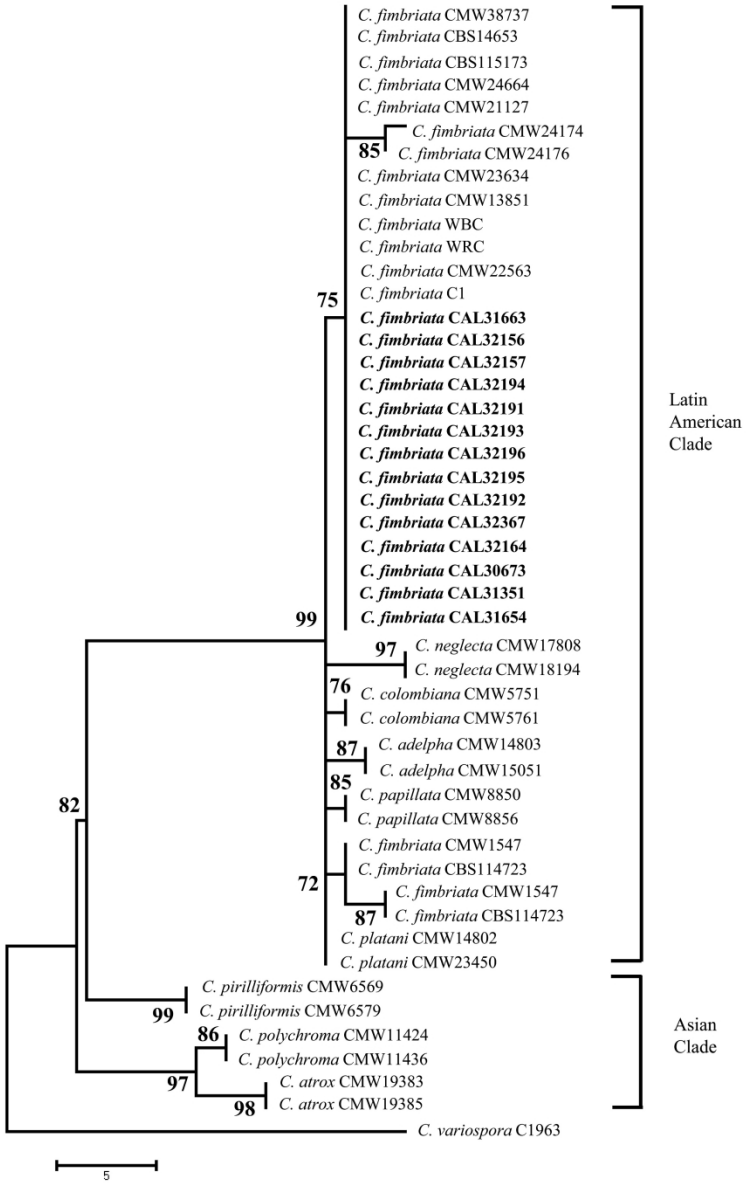

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in Ceratocystis fimbriata sensu stricto. Isolates from Lansium domesticum in Indonesia are marked in bold. The ITS haplotypes of C. fimbriata are numbered following the numerical designation of Harrington et al. (2014). C. variospora is used as an outgroup taxon.

62x90mm (999 x 999 DPI)

Fig. 6. Symptoms of mycelial plug inoculation with Ceratocystis fimbriata isolates (CAL32194 and CAL32159) from Lansium domesticum 45 days after inoculation. a. Symptoms on 2-year-old duku seedlings (L. domesticum) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). b. The formation of an upward lesion from the inoculation site (red arrow) on duku plants after being inoculated by CAL32194 (II) and CAL32159 (III). c. d. 4-month-old Acacia plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (II) and CAL32159 (III). e. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old Eucalyptus, at 45 days of observation did not show any signs of wilting. f. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old Acacia crassicarpa, at 45 days of observation did not show any signs of wilting.

10x12mm (999 x 999 DPI)

6.Bukti konfirmasi review, hasil review kedua dan bukti konfirmasi artikel accepted (15 Februari 2022)

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

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The Plant Pathology Journal <onbehalfof@manuscriptcentral.com> Tue, Feb 15, 2022 at 4:39 PM
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15-Feb-2022

Dear Dr. Ahmad Muslim:

It is a pleasure to accept your manuscript entitled "Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, Indonesia" in its current form for publication in The Plant Pathology Journal. The comments of the reviewer(s) who reviewed your manuscript may be included at the foot of this letter.

Here is a few things I would point out as minor comments.
In Fig. 2, no mention about yellow arrow in the legend.
In Table 3, no mark of superscript "a", "b"
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Sincerely,

Prof. Jungkwan Lee
Editor In Chief
The Plant Pathology Journal
jungle@dau.ac.kr

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

Comments to the Author
Thank you very much for your revision.

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On behalf of Associate Editors of The Plant Pathology Journal, we thank you for your fine contribution and we look forward to your continued participation in the Plant Pathology Journal.

Sincerely,

Prof. Jungkwan Lee
Editor In Chief
The Plant Pathology Journal
jungle@dau.ac.kr

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

Comments to the Author
Thank you very much for your revision.

Date Sent: 15-Feb-2022

File 1: * PPJ-copytight-transfer-form.pdf

Close Window

7.Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit (17 Februari 2022)

122K

a. muslim unsri <a_muslim@unsri.ac.id> Thu, Feb 17, 2022 at 6:17 PM
To: paper@kspp.org
Cc: hyuck1857@dau.ac.kr

Dear Editor in Chief
The Plant Pathology Journal

Thank you very much for your email regarding our paper entitled “Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia” for publishing in the Journal of Forestry Research”.

We are really happy to hear that our paper has been accepted for publishing in your Journal " The Plant Pathology Journal". We have made corrections and some modification according to reviewer’s and editor’s revisions. Below is a summary of our changes made in response to the reviewer’s and editor’s comments.

Reviewer’s comment: In Fig. 2, no mention about yellow arrow in the legend.

Our response:

We agree and the sentence has been changed to be “**Fig. 2.** Symptoms of wilt and die-back on *Lansium domesticum*. a, b. Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. c, d. Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrow). e. Squirrel bite caused peeled-off bark on diseased tree (yellow arrow). f. a beetle hole on affected diseased wood (yellow arrow). g. *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis”*.

Reviewer’s comment: In Table 3, no mark of superscript "a", "b"

Our response: We agree and mark the superscript "a", "b". The mark had inserted to sentence.

Reviewer’s comment: Lastly, it is better to move Table 4 to supplementary Table. Moreover, another Table can be move to supplementary. Too many Tables in main manuscript. Please consider about that.

Our response: We agree and have moved Table 4 to supplementary Table. Our revision have been changed in our manuscript. Another tables are important data should be showed in our manuscript.

Editor’s comments: Please complete the attached copyright form and indicate your acceptance by signing and returning it to The Korean Society of Plant Pathology (paper@kspp.org).

Our response: Thank you very much, we agree and complete the attached copyright form and signed the copyright form (The signed form enclosed).

Editor’s comments: Authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing services, such as the ones described at the following web sites.

http://www.prof-editing.com
http://www.bostonbioedit.com
http://www.asiascienceediting.com
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http://www.anitaksnyder.com
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Our response: Our manuscripts have been edited by London Proofreaders. The certificate are enclosed.

Here, we enclosed the edited manuscript in attachment file.
We are waiting for the galley proofs of our manuscript.
Thank you very much for your kindness and excellent cooperation

Best Regard
Ahmad Muslim
Sriwijaya University
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한국식물병리학회 편집위원회 <paper@kspp.org> Fri, Feb 18, 2022 at 7:58 AM
Reply-To: "\"한국식물병리학회 편집위원회\"" <paper@kspp.org>
To: "a. muslim unsri" <a_muslim@unsri.ac.id>

Dear Prof. Ahmad Muslim

This is Yoonjin Kim from the PPJ editorial office.

Would it be possible to send us the revised figures in separate files?
I will update the manuscript file and the supplementary files at Manuscript Central so that our editors can edit with the new files.

Thank you for your contribution to PPJ.

Best regards,
Yoonjin Kim

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

> ----------Original Message----------
> **Subject :** Re: The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182.R1
> **Date :** 2022-02-17 20:22:17
> **From :** "a. muslim unsri" <a_muslim@unsri.ac.id>

To : paper@kspp.org

Cc : hyuck1857@dau.ac.kr

[Quoted text hidden]

---

a. muslim unsri <a_muslim@unsri.ac.id> Fri, Feb 18, 2022 at 10:43 AM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Yoonjin Kim,

Thank you very much for your email regarding our paper entitled "Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia" for publishing in the Journal of Forestry Research".
We have sent the figure files separately in the attachment of this email. We have tagged the files by name in the manuscript.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

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

- Fig. 1.png
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- Fig. 3g.jpg
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Fig. 5e.jpg
Fig. 5f.jpg

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한국식물병리학회 편집위원회 <paper@kspp.org> Wed, Feb 23, 2022 at 8:51 AM
Reply-To: "\"한국식물병리학회 편집위원회\"" <paper@kspp.org>
To: "a. muslim unsri" <a_muslim@unsri.ac.id>

Dear Prof. Ahmad Muslim

Thank you for sending the figure files.
However, it seems that I cannot access and download the files as of now (access is denied).
Could you send it in a zip file?

Thank you.
Best regards,
Yoonjin Kim

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

> ----------Original Message----------
> **Subject :** Re: Re: The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182.R1
> **Date :** 2022-02-18 12:43:34
> **From :** "a. muslim unsri" <a_muslim@unsri.ac.id>
> **To :** 한국식물병리학회 편집위원회 <paper@kspp.org>
> **Cc :**
> [Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Feb 23, 2022 at 12:57 PM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email regarding our paper entitled “Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia” for publishing in the Journal of Forestry Research”.
We have sent the figure files with zip file format. We have tagged the files by name in the manuscript. We send in separate files because campus email domain policy does not allow sharing google drive with domains other than google.com
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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Feb 23, 2022 at 12:59 PM
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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Feb 23, 2022 at 1:03 PM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email regarding our paper entitled “Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia” for publishing in the Journal of Forestry Research”.
We have sent the figure files with zip file format. We have tagged the files by name in the manuscript. We send in separate files because campus email domain policy does not allow sharing google drive with domains other than google.com

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,

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

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To: "a. muslim unsri" <a_muslim@unsri.ac.id>

Dear Prof. Ahmad Muslim

I have sent a request for access to Google drive. The email that I used to request is yjkim@infolumi.co.kr

It would be appreciated if you could grant access to yjkim@infolumi.co.kr account for all files.

Thank you.

Best ,
Yoonjin Kim

사단법인 한국식물병리학회 Korean Society of Plant Pathology

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> ----------Original Message----------
> **Subject :** Re: Re: Re: The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182.R1
> **Date :** 2022-02-23 15:03:29
> **From :** "a. muslim unsri" <a_muslim@unsri.ac.id>
> **To :** 한국식물병리학회 편집위원회 <paper@kspp.org>
> **Cc :**
> Prof. Yoonjin Kim
> [Quoted text hidden]
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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Feb 23, 2022 at 2:28 PM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email regarding our paper entitled “Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia” for

publishing in the Journal of Forestry Research".
We have sent the file in the form of a zip file to avoid google drive. The policy set by the administrator of Sriwijaya University prohibits sharing items with yjkim@infolumi.co.kr, because it is not a Google Account in a compatible domain in the whitelist. if the file cannot be accessed, is there an email domain in the form of a Google Account or we use another email account to respond to this email.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id
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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Feb 23, 2022 at 2:28 PM
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Fig. 6.rar
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a. muslim unsri <a_muslim@unsri.ac.id> Mon, Mar 14, 2022 at 3:55 PM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>, jungle@dau.ac.kr
Cc: hyuck1857@dau.ac.kr

Prof. Jungkwan Lee
Editor In Chief
The Plant Pathology Journal
jungle@dau.ac.kr

Dear Prof. Jungkwan Lee

We are really appreciated and many thank for your kindness to accept our manuscript (Accepted letter on February 15, 2022) entitled "Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt

on *Lansium domesticum* in South Sumatra, Indonesia" for publishing in plant pathology journal".

We have revised the figure in a separate file and also sent the figure files with zip file format.

Anyway, would you please let me know the process of galley proofs/publication of our manuscript?

We are really happy and hopefully if our manuscript can be published in the next issue on April 1, 2022.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your kindness and excellent cooperation.

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

On Tue, Feb 15, 2022 at 4:39 PM The Plant Pathology Journal <onbehalfof@manuscriptcentral.com> wrote:
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이정관 <jungle@dau.ac.kr> Mon, Mar 14, 2022 at 4:11 PM
To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: 한국식물병리학회 편집위원회 <paper@kspp.org>

Dear Ahmad Muslim,

I am sorry for the late process.
Your paper will be published on April and PPJ managing editor will contact you soon.

I appreciate your contribution to PPJ, and have a great day.

Best regards,

보낸 사람: a. muslim unsri <a_muslim@unsri.ac.id>
보낸 날짜: 2022년 3월 14일 월요일 17:55
받는 사람: 한국식물병리학회 편집위원회 <paper@kspp.org>; 이정관 <jungle@dau.ac.kr>
참조: 최기혁 <hyuck1857@dau.ac.kr>
제목: Re: The Plant Pathology Journal - Decision on Manuscript ID PPJ-OA-12-2021-0182.R1

[Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Mon, Mar 14, 2022 at 8:58 PM
To: 이정관 <jungle@dau.ac.kr>
Cc: 한국식물병리학회 편집위원회 <paper@kspp.org>

Dear Prof. Jungkwan Lee

Thank you very much for your quick response regarding the process of our paper being published in Plant Pathology Journal.
We are waiting for your PPJ managing editor to contact us for processing our paper.

Thank you very much for your kindness and excellent cooperation.

Sincerely,
Ahmad Muslim
Senior Lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih, Km. 32, Inderalaya, Palembang, Indonesia
E-mail : a_muslim@unsri.ac.id

[Quoted text hidden]

Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

Running title: Ceratocystis Wilt on *Lansium domesticum*

Ahmad Muslim*, Rahmat Pratama, Suwandi Suwandi, Harman Hamidson

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

*Corresponding author : Ahmad Muslim (Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra, 30662, Indonesia, +62 811-7826-119, a_muslim@unsri.ac.id, https://orcid.org/0000-0002-3973-7443)

Abstract

Ceratocystis wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on ITS and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately

pathogenic to *Acacia carsicarpa, Eucalyptus urophylla,* and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata, Hevea brasiliensis,* and *Alstonia scholaris.* Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.

Keywords: agroforestry plants, canker, *Certocystis fimbriata,* die-back disease.

Introduction

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8, 14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum Corr.* has also been reported to have benefits as larvicides (Ni'mah et al., 2015: Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria spp.* (Nurhayati et al., 2016),

organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Tilaar et al., 2008; Subandrate et al., 2016).

Previous studies conducted in 2014 to 2017 (Suwandi et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in 3 locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis* and *Chukrasia tabularis* (Chi et al., 2019a; Chi et al., 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Alstonia scholaris, Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Material and Methods

Diseases incidence, Sample collection, and Fungal isolation

Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Figure 1). In each

plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Suwandi et al., 2021; Pratama et al., 2021a). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *Lansium domesticum* which showed symptoms of branch wilting, discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of Ceratocystis was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 minutes, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on Malt Extract Agar (MEA) media at room temperature (25 °C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% malt extract agar (MEA, 20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization

The morphological characteristics of the observed fungi were represented by isolates originating from 8 regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture

To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of 8 isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30 °C with an interval of 5 °C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses

The pure cultures used for the DNA extraction were fourteen isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193,

CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, USA).

Amplification and PCR sequencing were obtained from two gene regions, namely Beta tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Germany) (25 mM MgCl2, 0.06 U/µl Taq-DNA-Polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch™ thermal cycler (Bio-Rad, USA). The parameters were initial denaturation for 3 minutes at 94°C, 30 cycles for 30 seconds at 94°C for 30 seconds, for 30 seconds at 52°C, and 1 minute at 72 °C for. Amplification was completed at 72°C for 10 minutes and the PCR product was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For maximum parsimony analysis, the metrics calculated included tree length (TL), retention index (RI), and consistency index (CI). Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials

These studies were conducted using ten isolates of *C. fimbriata.* The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2–3 cm and a height of 50–60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *Acacia mangium, Acacia carsicarpa, Eucalyptus urophylla, Dyera costulata, Hevea brasiliensis, Alstonia scholaris,* and *Melaleuca cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2–3 cm and a height of 70–80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the

same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package. Furthermore, the Analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey'sHSD) test was used to determine the significant differences in the mean comparisons of the different treatments.

Results and discussion

Diseases incidence, Sample collection, and Fungal isolation

Ceratocystis wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu district, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Figure 1). It has spread widely in other plantations in the Ogan Komering Ulu district covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in

Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya.

From 2020 to 2021, there were similar disease incidences on the duku plantations in Ogan Komering Ilir (OKI), within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively. Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Figure 2 a and b). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Figures 2c and d). There is a wound on the diseased tree caused by a squirrel scratch (Figure 2e). In general, holes will appear on the infected duku stem caused by *Hypocryphalus mangiferae* (Figure 2 f) which is a vector insect for *Ceratocystis* (Figure 2g).

225 Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct
226 planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East
227 Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi
228 Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this
229 disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each
230 region was 65%, 53.3%, 56%, 80%, 64%, 80 %, 53.3%, and 60% for Ogan Komering Ulu,
231 Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas,
232 Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).
233 Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and
234 there include (CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192) from Ogan
235 Komering Ulu, (CAL32159, CAL32156, CAL32157, and CAL32158) from Musi Banyuasin,
236 CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu,
237 CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan
238 Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study
239 were preserved in the Culture Collection (CMW), Laboratory of Phytopathology, Department
240 of Plant Protection, Faculty of Agriculture, Sriwijaya University.
241 **Morphological characterization and Growth in culture**
242 The isolates obtained had similar morphological characteristics when grown on MEA
243 media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black
244 ascomata bases that were globose to subglobose (Figure 3a) and produced an ascomata neck
245 with divergent ostiolar hyphae at the ends (Figure 3b). This fungus also produced chained
246 barrel-shaped conidia (Figure 3c), and chlamydospores (Figure 3d), it also had hat-shaped
247 ascospores (Figure 3e). Cylindrical conidia (Figure 3g) were generated from the primary
248 phialidic conidiophore (Figure 3f).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *Mangifera indica* (van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *Acacia mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. Fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Figure 4).

DNA extraction, amplification, sequencing, and phylogenetic analyses

For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences. Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*.

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the maximum parsimony (MP) method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis, Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figures 5 and 6). The phylogeny was assessed and analyzed using bootstrap analysis with 1000 replications, as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic

274 characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in
275 Indonesia is classified as *C. fimbriata*.
276
277 **Inoculation trials**
278 *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the
279 bundle vessels, whereby 90% and 100% of it dies 45, as well as 70 days after pathogen
280 inoculation respectively (Fig. 6a; b). Analysis of variance for lesion length in duku showed that
281 there was no significant difference among all isolates inoculated to this host. All inoculated
282 isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4).
283 Statistical analysis showed a significant difference in lesion length between inoculated *L.*
284 *domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata*
285 and no fungus was found in the control nurseries.
286 The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of
287 wilt disease, which include extensive vascular discoloration in all inoculated seedlings, and
288 wilt was noted to reach 100% of all seedlings at day 70 after inoculation (figure 6c;d). There
289 was no significant difference in the length of lesion produced by the *Ceratocystis* isolate used
290 in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata*
291 inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis*
292 fungus was not discovered in the control seedlings after re-isolation.
293 The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death
294 and infection in plants which were characterized by the formation of significant lesions. In *A.*
295 *crassicarpa, E. urophylla,* and *M. leucadendra* seedlings, all isolates caused moderately
296 pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm,
297 respectively. However, in *D. costulata, H. brasiliensis,* and *A. scholaris* plants, these isolates
298 caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51

cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The reisolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006; Al Adawi et al., 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by

323 wind (Harrington, 2007; Tarigan, 2011). *Ceratocystis* is also transmitted from infected wild
324 acacia around duku plantations or other plants that are hosts of this pathogen.

325 Field observations show that attacks from this disease occur from the trunk or branches
326 at the top and go down to the stem, which is spread by squirrels and insects. This disease also
327 occur from the root and continues up to the base of the stem. The infection from these roots is
328 caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some
329 locations in a district affected by the disease, the plants were able to grow healthy, while in
330 other places the attacks were very severe. The variety of disease severity at each location and
331 district is probably due to the various levels of resistance offered by the planted varieties of
332 duku and the degree of soil fertility, which affects the growth and resistance of the plants. There
333 was no correlation between the polyculture and monoculture systems of duku with the attack
334 rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both
335 polyculture and monoculture.

336 The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum*
337 was determined based on morphological characteristics and a comparison of DNA sequences
338 which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192,
339 CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference
340 isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates
341 CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were
342 identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South
343 Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*.
344 Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities
345 to *C. fimbriata* which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et
346 al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were 2 variations of the ITS rDNA sequence from 2 isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin district. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Tarigan et al., 2011; Pratama et al., 2021a; Pratama et al., 2021b). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *Lansium domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010; Tarigan et al., 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by

372 *Colocasia esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum*
373 (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A.*
374 *heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the
375 perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting
376 other trees not previously mentioned.

377 Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees
378 like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test
379 on duku showed that a very high attack intensity of 100% causes wilting and death of plants.
380 Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata*
381 derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021),
382 moderately pathogenic to *A. carsicarpa, E. urophylla,* and *M. cajuputi,* as well as weakly
383 pathogenic to *D. costulata, A. scholaris,* and *H. brasiliensis*. This was shown by the formation
384 of lesions on the stems which leads to the death of the inoculated seedlings.

385 The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of
386 seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants
387 showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue
388 until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis*
389 is a very serious economical disease that has attacked *L. domesticum* in all duku production
390 centers in South Sumatra hence it damages the income sources of farmers in this province.
391 Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes
392 death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the
393 attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

394 Sudden wilt disease on *Lansium domesticum* caused by *Ceratocystis Fimbriata* has
395 spread widely to duku production centers in various districts of South Sumatra. Furthermore,
396 the population consisted of individuals with uniform morphology dominated by ITS5 and

ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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520 **Table 1.** Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (*n* = 89)	53.9	64	85.4
Saleman (*n* = 74)	41.9	58.1	95.9
Singapura (*n* = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (*n* = 91)	59.3	72.5	84.6
Tebat Agung (*n* = 67)	10.5	16.4	31.3
Padang Bindu (*n* = 71)	5.6	15.5	19.7
Kepayang (*n* = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5

Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

527 **Table 2.** Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood
528 onto the MEA from samples collected from dying *Lansium domesticum* trees in Ogan
529 Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*
Ogan Komering Ulu	Kartamulya	2019	2/5 (40 %)
	Saleman	2019	5/5 (100 %)
	Singapura	2019	2/5 (40 %)
	Pengaringan	2020	5/5 (100 %)
	Reksa Jiwa	2020	2/5 (40 %)
	Tebat Agung	2020	3/5 (60 %)
	Padang Bindu	2020	2/5 (40 %)
	Kepayang	2020	5/5 (100 %)
		Total	26/40 (65%)
East Ogan Komering Ulu	Bantan Pelita	2021	4/5 (80%)
		Total	4/5 (80%)
South Ogan Komering Ulu	Simpang	2021	4/5 (80%)
	Tanjung Sari	2021	2/5 (40%)
	Tanjung	2021	4/5 (80%)
	Beringin	2021	2/5 (40%)
	Kisau	2021	2/5 (40%)
		Total	14/25 (56%)
Ogan Komering Ilir	Penyandingan	2020	3/5 (60%)
	Ulak Kemang	2020	3/5 (60%)
	Tanjung Lubuk	2020	2/5 (40%)
		Total	8/15 (53.3%)

Musi Banyuasin	Kasmaran	2021		1/5 (20 %)
	Babat Toman	2021		2/5 (40 %)
	Beruge	2021		1/5 (20 %)
	Sereka	2021		2/5 (40 %)
	Sanga Desa	2021		5/5 (100 %)
	Tanjung Raya	2021		5/5 (100 %)
			Total	16/30 (53.3 %)
Musi Rawas	Tuah Negri	2021		4/5 (80%)
	Mambang	2021		5/5 (100%)
	Lubuk Tuo	2021		3/5 (60%)
			Total	12/15 (80%)
North Musi Rawas	Beringin Jaya	2021		3/5 (60%)
	Lawang Agung	2021		5/5 (100%)
	Karang Waru	2021		3/5 (60%)
	Rantau Kadam	2021		3/5 (60%)
	Lesung Batu	2021		2/5 (40%)
			Total	16/25 (64%)
Muara Enim	Ujan mas	2020		3/5 (60%)
			Total	3/5 (60%)

533 **Table 3.** Morphology of selected *Ceratocystis Fimbriata* isolates from a different district in South Sumatra

534

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3 to 312.4	122.9 to 291.4	135.7 to 325.2	141.3 to 317.1	137.9 to 321.1	132.1 to 334.9	137.9 to 346.1	122.1 to 316.9
Ascomatal base (l)	153.1 to 404.4	131 to 315.4	148.1 to 398.4	151.1 to 411.4	143.1 to 398.4	152.4 to 394.1	139.1 to 421.8	157.1 to 412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4 to 768.4	354.9 to 677.7	413.7 to 798.8	439.9 to 736.4	475.8 to 813.6	484.6 to 790.9	463.8 to 723.6	484.6 to 780.9
Neck (w) top	11.5 to 26.8	7.06 to 18.4	11.3 to 21.9	11.1 to 25.4	10.1 to 17.9	11.3 to 21.7	11.1 to 22.9	11.3 to 21.7

Neck (w) bottom	24.8 to 47.9	20.3 to 39.7	23.6 to 42.6	22.6 to 51.2	23.7 to 43.8	22.67 to 42.9	23.7 to 43.6	22.67 to 44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2 to 43.5	30.4 to 40.1	32.7 to 44.7	32.7 to 42.2	33.5 to 43.9	33.7 to 44.8	33.5 to 42.9	31.7 to 44.8
Ascospores								
Hat-shaped ascospores (l)	3.4 to 5.7	3.3 to 5.2	3.2 to 5.4	3.4 to 4.9	3.2 to 4.4	3.1 to 5.1	3.1 to 4.3	3.3 to 4.9
Ascospores (w) without sheath	3.4 to 5.1	3.1 to 4.1	3.3 to 4.7	3.4 to 4.4	3.3 to 4.1	3.4 to 4.5	3.3 to 4.1	3.5 to 4.4
Ascospores (w) with sheath	5 to 6.8	4.1 to 6.1	5.1 to 6.7	5.3 to 6.4	5.2 to 6.5	5.5 to 6.7	5.2 to 6.3	5.4 to 6.6
Primary conidia (l)	12.1 to 27.5	10.6 to 18.9	13.8 to 23.8	12.2 to 29.3	13.2 to 25.7	14.9 to 24.8	12.5 to 21.6	13.7 to 24.6
Primary conidia (w)	3.5 to 7.4	3.2 to 4.3	3.1 to 5.1	3.4 to 4.1	3.2 to 5.1	3.4 to 4.4	3.4 to 4.1	3.5 to 4.7
Secondary Conidia (l)	6.3 to 11.6	5.7 to 10.1	6.6 to 11.8	7.9 to 11.8	6.7 to 11.9	6.8 to 11.5	6.5 to 11.5	6.2 to 11.3

Secondary Conidia (w)	4.5 to 7.6	4.1 to 7.4	4.7 to 7.5	5.6 to 7.9	4.3 to 7.8	4.3 to 7.8	4.3 to 7.1	4.1 to 7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7 to 15.1	8.7 to 15.1	11.3 to 15.6	9.7 to 17.8	10.7 to 15.4	10.1 to 16.5	10.3 to 14.6	10.4 to 14.5
Chlamydospores (w)	7.9 to 13.9	8.3 to 11.1	6.9 to 14.2	6.8 to 13.6	7.6 to 11.8	7.7 to 12.5	7.6 to 11.8	7.6 to 12.9
Culture growth rate at[b]								
10 °C	0	0	0	0	0	0	0	0
15 °C	3.3 to 3.5	2.2 to 2.5	3.2 to 3.5	2.2 to 2.7	3.2 to 3.4	2.2 to 2.8	2.3 to 2.9	2.4 to 2.8
20 °C	3.2 to 3.7	3.1 to 2.9	3.2 to 3.9	3.3 to 3.9	4.2 to 4.4	3.2 to 3.5	4.2 to 4.4	3.2 to 3.5
25 °C	5.1 to 5.3	4.1 to 4.5	4.7 to 5.1	4.4 to 4.7	4.4 to 4.9	4.1 to 4.5	4.4 to 4.9	4.1 to 4.5
30 °C	3.3 to 3.6	3.1 to 3.9	3.5 to 4.6	3.5 to 4.2	3.8 to 4.2	3.1 to 3.4	3.8 to 4.2	3.1 to 3.4

535 [a] All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm

536 [b] Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after fourteen days

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538 **Table 4.** Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery
539 condition.
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Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35f	7/10	10/10
CAL32157	10	15.49ef	7/10	8/10
CAL32158	10	12.29cd	5/10	5/10
CAL32159	10	11.02c	2/10	5/10
CAL32191	10	11.73cd	2/10	3/10
CAL32192	10	13.83def	7/10	8/10
CAL32193	10	19.81g	9/10	10/10
CAL32194	10	6.86b	2/10	2/10
CAL32195	10	12.89cde	5/10	6/10
CAL32196	10	11.19cde	5/10	7/10
Control (MEA)	10	0.01a	0/10	0/10
P		<0.001		

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542 Values followed by the same letters in a column are not different among isolates at P=0.05
543 according to Tukey's HSD multiple range test.
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547 **Table 5.** Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition.

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Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*		
		Lesion length (cm)	Wilting and death at 45 dpi*	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25ef	10/10	10/10	9.86de	0/10	1/10	11.32b	0/10	1/10
CAL32157	10	16.32de	10/10	10/10	10.16de	0/10	2/10	11.81b	0/10	1/10
CAL32158	10	14.49cde	8/10	10/10	9.39cd	0/10	1/10	9.33b	0/10	0/10
CAL32159	10	13.59bcd	8/10	10/10	8.26bcd	0/10	1/10	9.86b	0/10	0/10
CAL32191	10	11.73bc	7/10	10/10	7.96bcd	0/10	0/10	9.82b	0/10	0/10
CAL32192	10	15.54cde	10/10	10/10	6.57bc	0/10	0/10	10.59b	0/10	0/10
CAL32193	10	20.93f	10/10	10/10	12.59e	0/10	5/10	11.92b	0/10	3/10
CAL32194	10	9.943b	5/10	10/10	5.97b	0/10	0/10	8.80b	0/10	0/10

CAL32195	10	15.39cde	9/10	10/10	7.82bcd	0/10	2/10	11.20b	0/10	2/10
CAL32196	10	14.64cde	8/10	10/10	8.64bcd	0/10	1/10	11.15b	0/10	1/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001		

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550 Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test. *

551 dpi=days post inoculation.

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561 **Table 5.** (Continued)

Isolates	Host test	*Dyera costulata*			*Hevea brasiliensis*			*Alstonia scholaris*			*Melaleuca leucadendra*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	4.25b	0/10	0/10	5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10
CAL32157	10	3.91b	0/10	0/10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10
CAL32158	10	3.63b	0/10	0/10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10
CAL32159	10	3.83b	0/10	0/10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10
CAL32191	10	3.57b	0/10	0/10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10
CAL32192	10	5.15b	0/10	0/10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10
CAL32193	10	5.39b	0/10	0/10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10
CAL32194	10	3.05b	0/10	0/10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10

CAL32195	10	4.02b	0/10	0/10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10
CAL32196	10	3.60b	0/10	0/10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10
P		<0.001			<0.001			<0.001			<0.001		

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's HSD multiple range test.

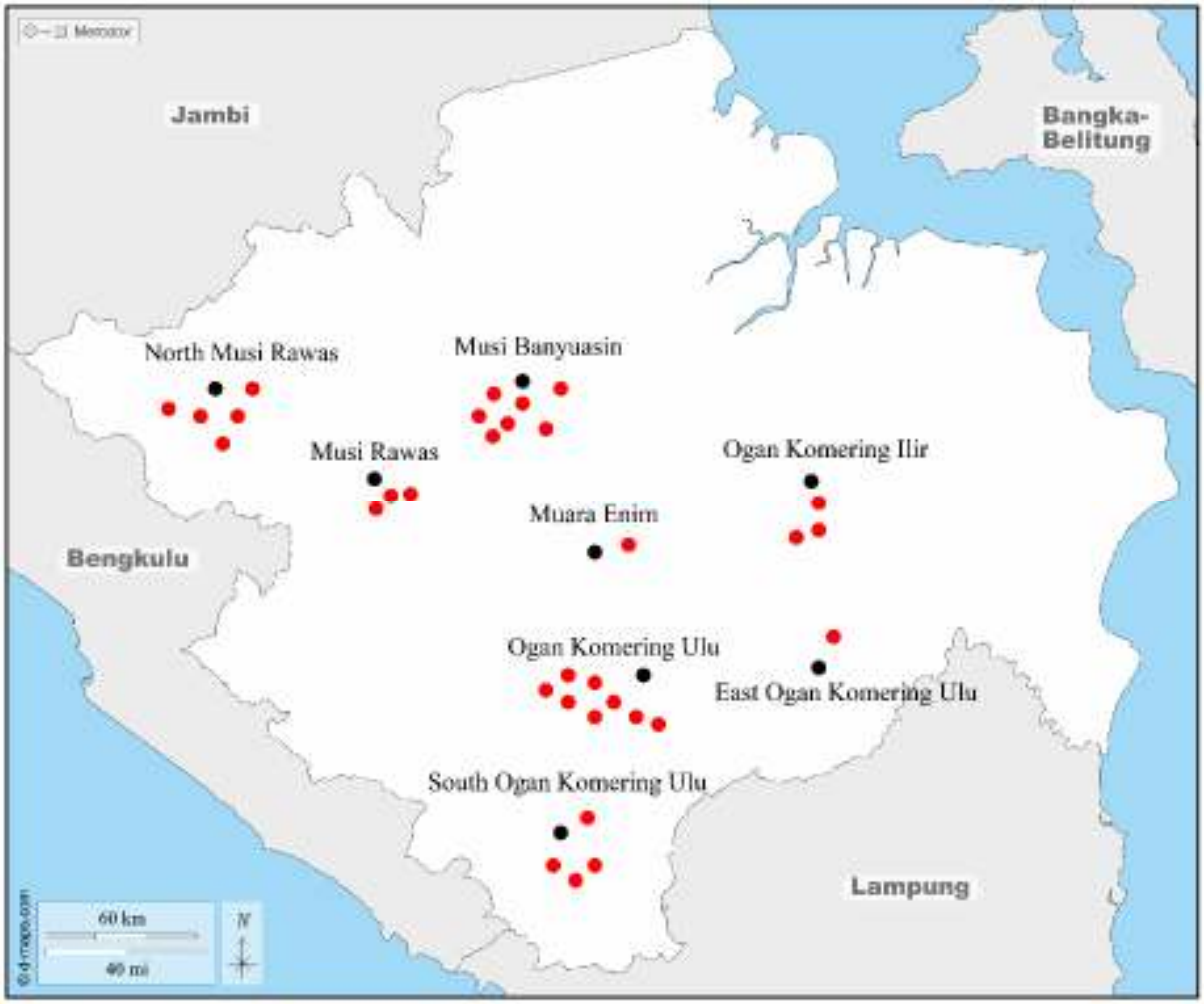

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

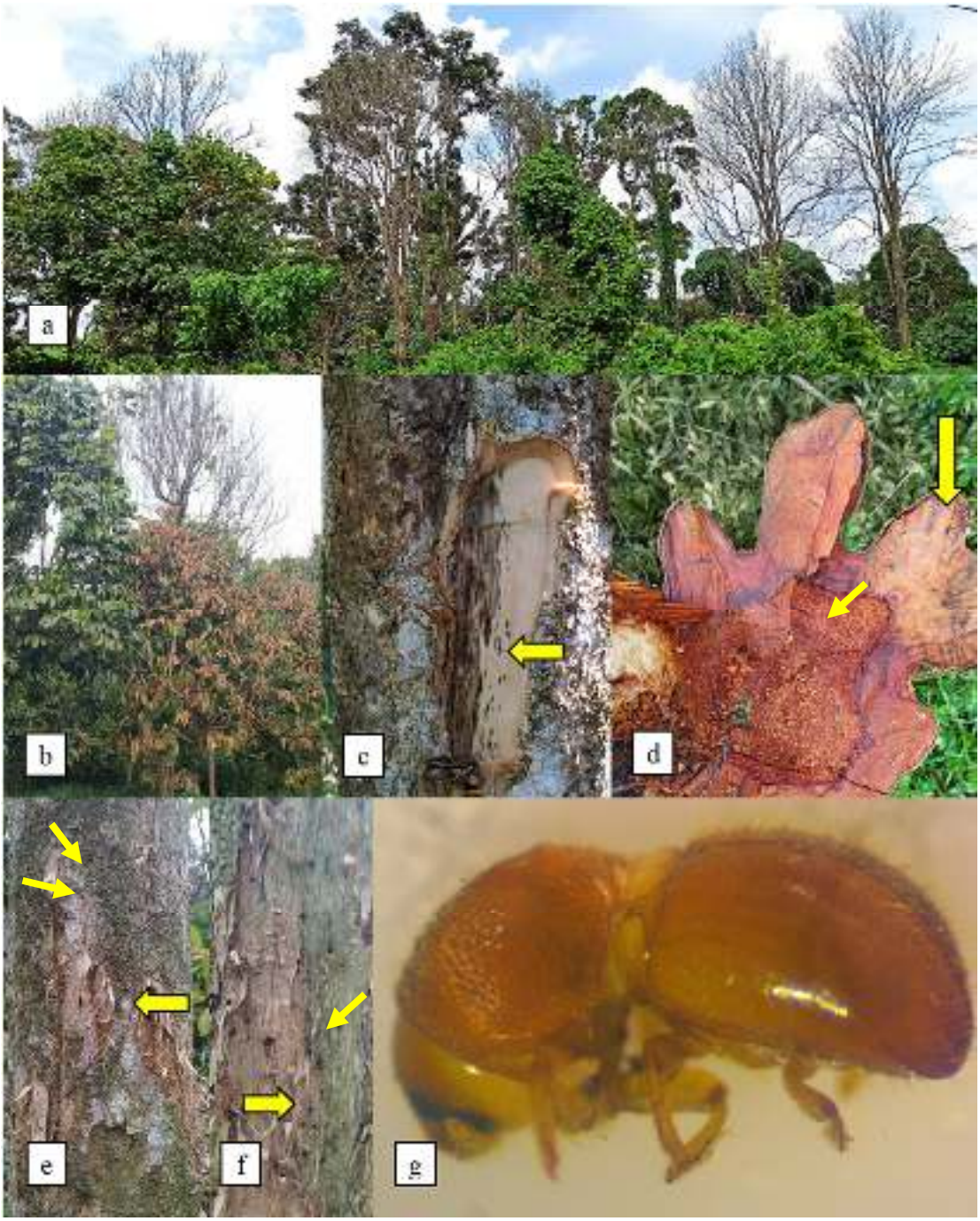

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. a, b. Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. c, d. Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrow). e. Squirrel bite caused peeled-off bark on diseased tree (yellow arrow). f. a beetle hole on affected diseased wood (yellow arrow). g. *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

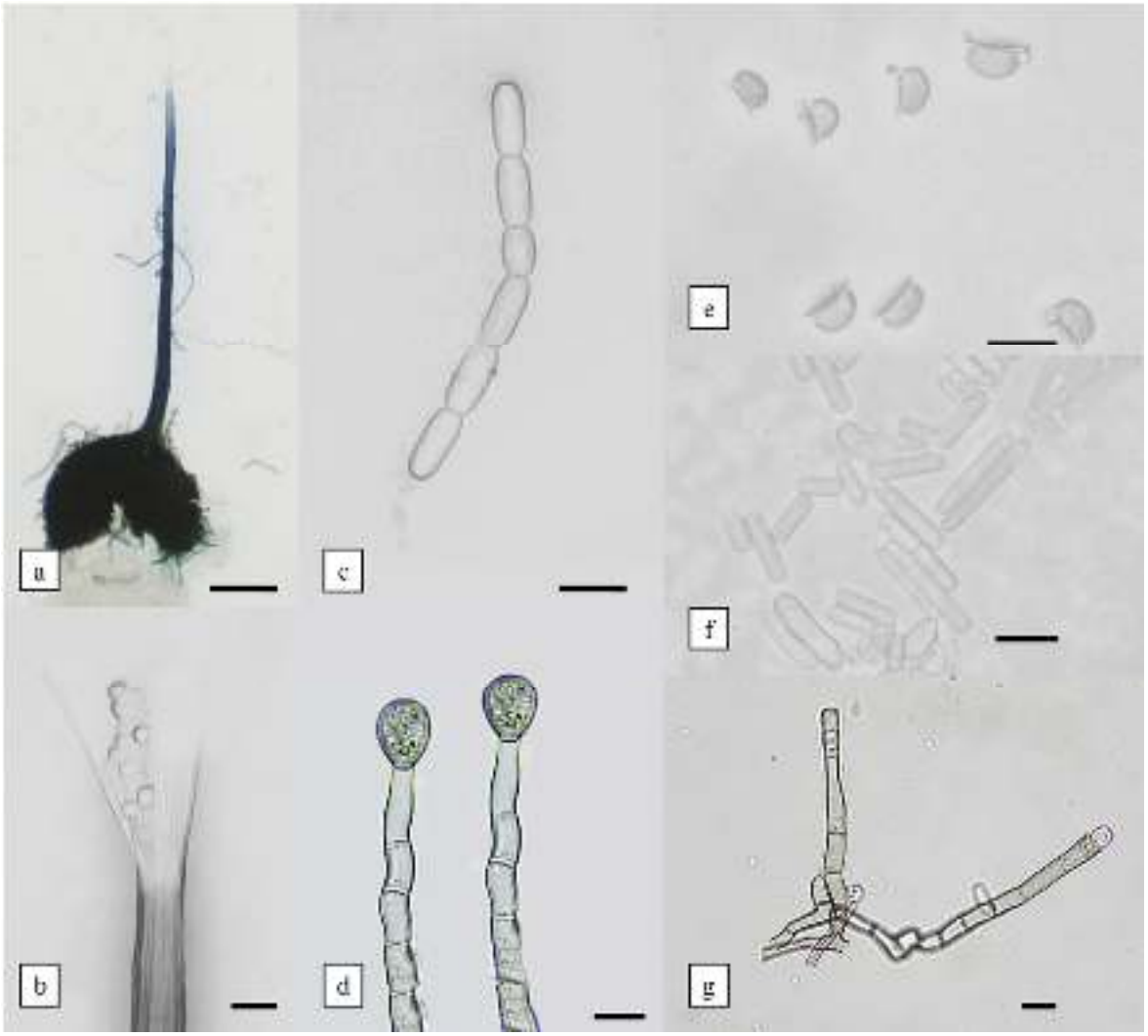

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: a. globose ascomata with a long neck, b. divergent ostiolar hyphae, c. barrel-shaped conidia, d. chlamydospores, e. hat-shaped ascospores, f. cylindrical conidia g. conidiophore/phialide, ——— Scale bars: a = 100 µm; b,c,d,e = 10 µm; f = 5 µm.

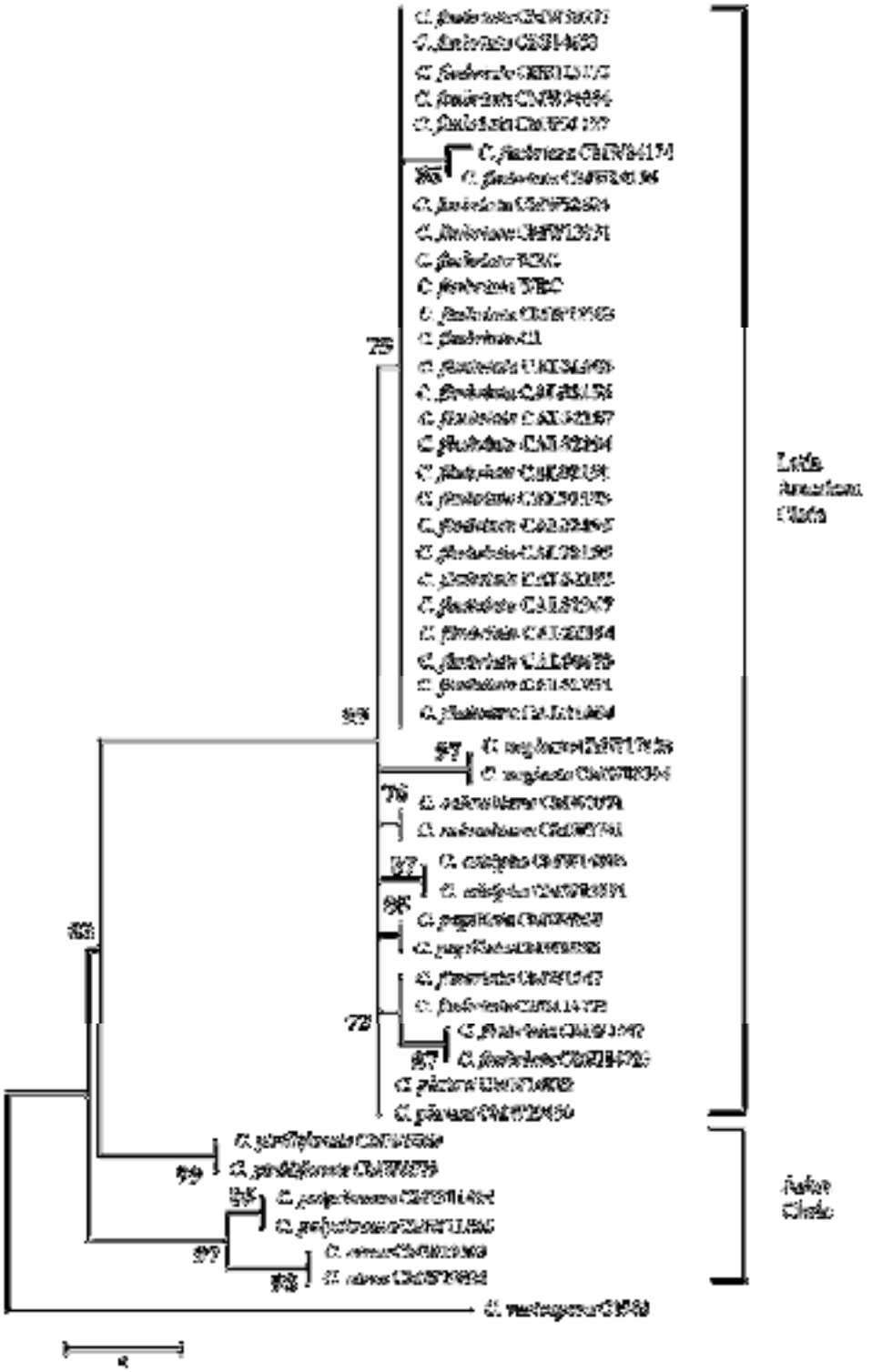

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

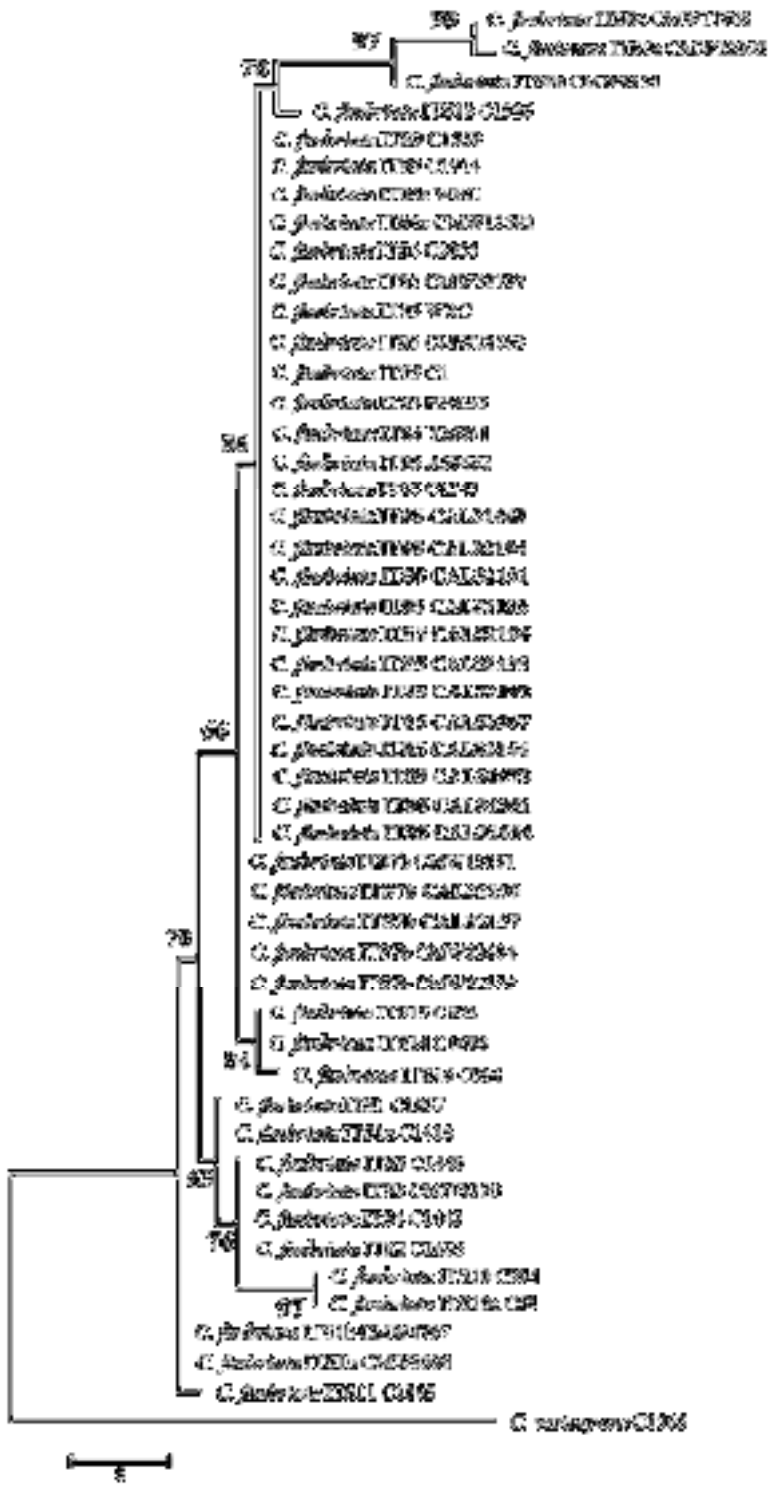

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

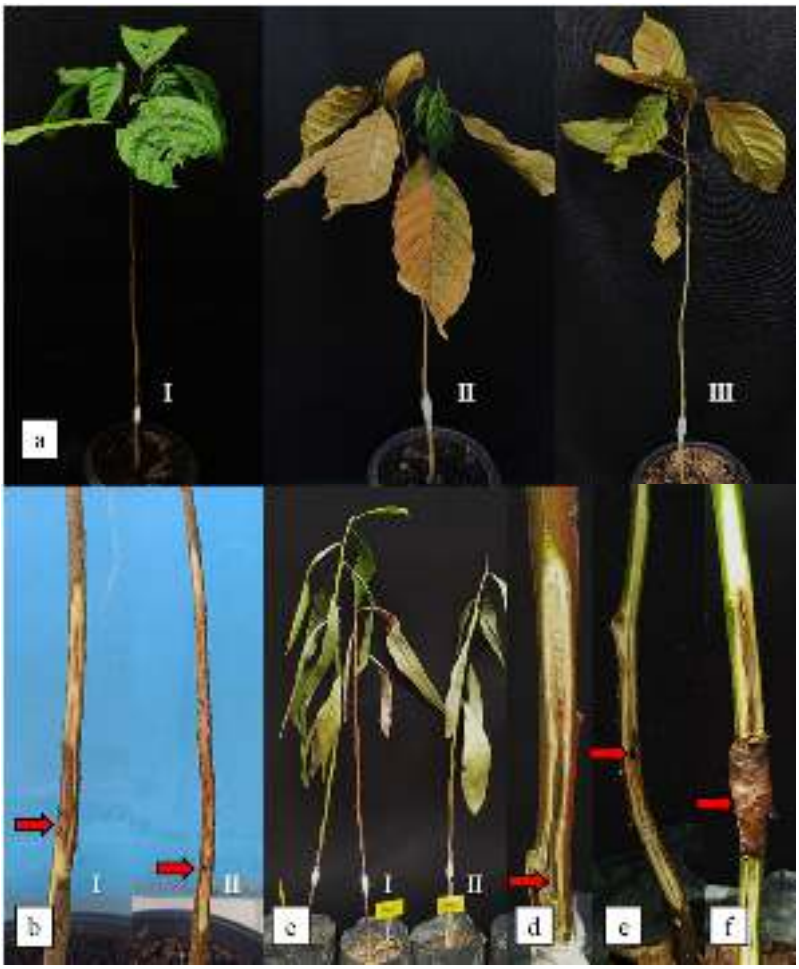

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. a. Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). b. The formation of an upward lesion from the inoculation site (red arrow) on duku plants after being inoculated by CAL32194 (II) and CAL32159 (III). c. d. 4-month-old *Acacia* plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (II) and CAL32159 (III). e. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. f. The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

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Title (Referred to as the "Article"): Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on Lansium domesticum in South Sumatra, Indonesia

Complete List of Author(s): Ahmad Muslim, Rahmat Pratama, Suwandi Suwandi, Harman Hamidson

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Supplementary 1. *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. Grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	CAL32194	*Lansium domesticum*	Indonesia	MT373418	MW752140

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS5	CAL32191	*L. domesticum*	Indonesia	MT373420	MW752141
	ITS5	CAL32193	*L. domesticum*	Indonesia	MT373417	MW752142
	ITS5	CAL32196	*L. domesticum*	Indonesia	MT373419	MW752144
	ITS5	CAL32195	*L. domesticum*	Indonesia	MT373416	MW752145
	ITS5	CAL32192	*L. domesticum*	Indonesia	MT373415	MW752146
	ITS5	CAL31663	*L. domesticum*	Indonesia	MT373422	-
	ITS5	CAL32367	*L. domesticum*	Indonesia	MT373421	-
	ITS5	CAL32164	*L. domesticum*	Indonesia	-	-
	ITS5	CAL30673	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31351	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31654	*L. domesticum*	Indonesia	-	-
	ITS5	CMW38737	*E. Grandis*	Zimbabwe	KF878326	KF878335

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus Mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CAL32156	*L. domesticum*	Indonesia	-	MW752143
	ITS7b	CAL32157	*L. domesticum*	Indonesia	-	MW752147
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina Arborea*	Brazil	AY157967	-
	ITS16	C924	*G. Arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin America n clade (LAC)	CMW17808	*E. Grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillate	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina Arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

8.Bukti konfirmasi dan hasil proof corrections pertama (17 Maret 2022)

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PPJ 2021-0182: Final Proof Corrections

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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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***Ceratocystis* wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.**

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Keywords **:** agroforestry plants, canker, *Certocystis fimbriata*, die-back disease

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8,14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum* Corr. has also been reported to have benefits as larvicides (Ni'mah et al., 2015; Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria* spp. (Norhayati et al., 2016), organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Subandrate et al., 2016; Tilaar et al., 2008).

Previous studies conducted from 2014 to 2017 (Suwandi

et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in three locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis,* and *Chukrasia tabularis* (Chi et al., 2019a, 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa*, *Eucalyptus urophylla*, *Dyera costulata*, *Alstonia scholaris*, *Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Material and Methods

Diseases incidence, sample collection, and fungal isolation. Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Fig. 1). In each plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Pratama et al., 2021a; Suwandi et al., 2021). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *L. domesticum* which showed symptoms of branch wilting,

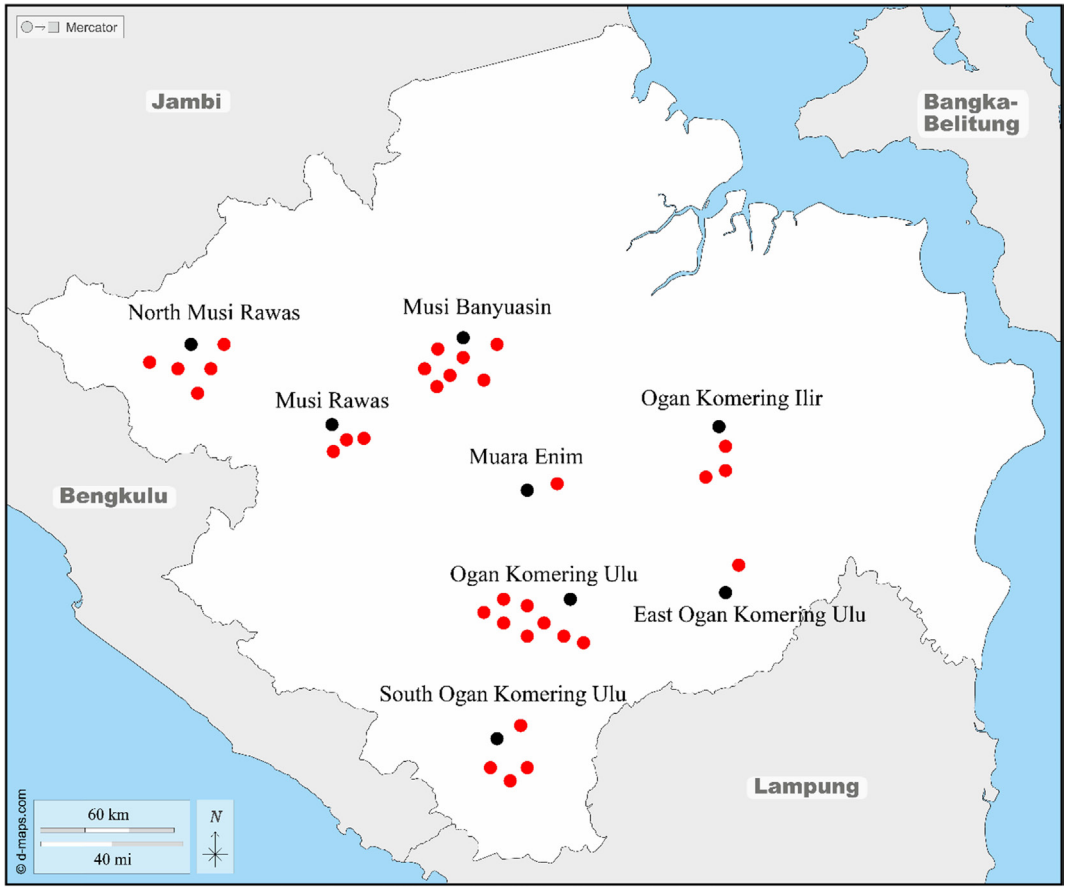

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of Ceratocystis was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 min, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on malt extract agar (MEA) media at room temperature (25°C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% MEA (20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization. The morphological characteristics of the observed fungi were represented by isolates originating from eight regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture. To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of eight isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30°C with an interval of 5°C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses. The pure cultures used for the DNA extraction were 14 isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

Amplification and PCR sequencing were obtained from two gene regions, namely beta-tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq-DNA-polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were initial denaturation for 3 min at 94°C, 30 cycles for 30 seconds at 94°C for 30 s, for 30 s at 52°C, and 1 min at 72°C for. Amplification was completed at 72°C for 10 min and the PCR product was stored at 10°C. The PCR

amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For MP analysis, the metrics calculated included tree length, retention index, and consistency index. Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials. These studies were conducted using ten isolates of *C. fimbriata*. The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2-3 cm and a height of 50-60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *A. mangium*, *A. carsicarpa*, *E. urophylla*, *D. costulata*, *H. brasiliensis*, *A. scholaris*, and *M. cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2-3 cm and a height of 70-80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on MEA plates or placed between two carrot slices.

Table 1. Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (n = 89)	53.9	64	85.4
Saleman (n = 74)	41.9	58.1	95.9
Singapura (n = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (n = 91)	59.3	72.5	84.6
Tebat Agung (n = 67)	10.5	16.4	31.3
Padang Bindu (n = 71)	5.6	15.5	19.7
Kepayang (n = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

Pathogenicity test data were analyzed using the SAS university edition software package (SAS Institute Inc., Cary, NC, USA). Furthermore, the Analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey's honestly significant difference) test was used to determine the significant differences in the mean comparisons of the different treatments.

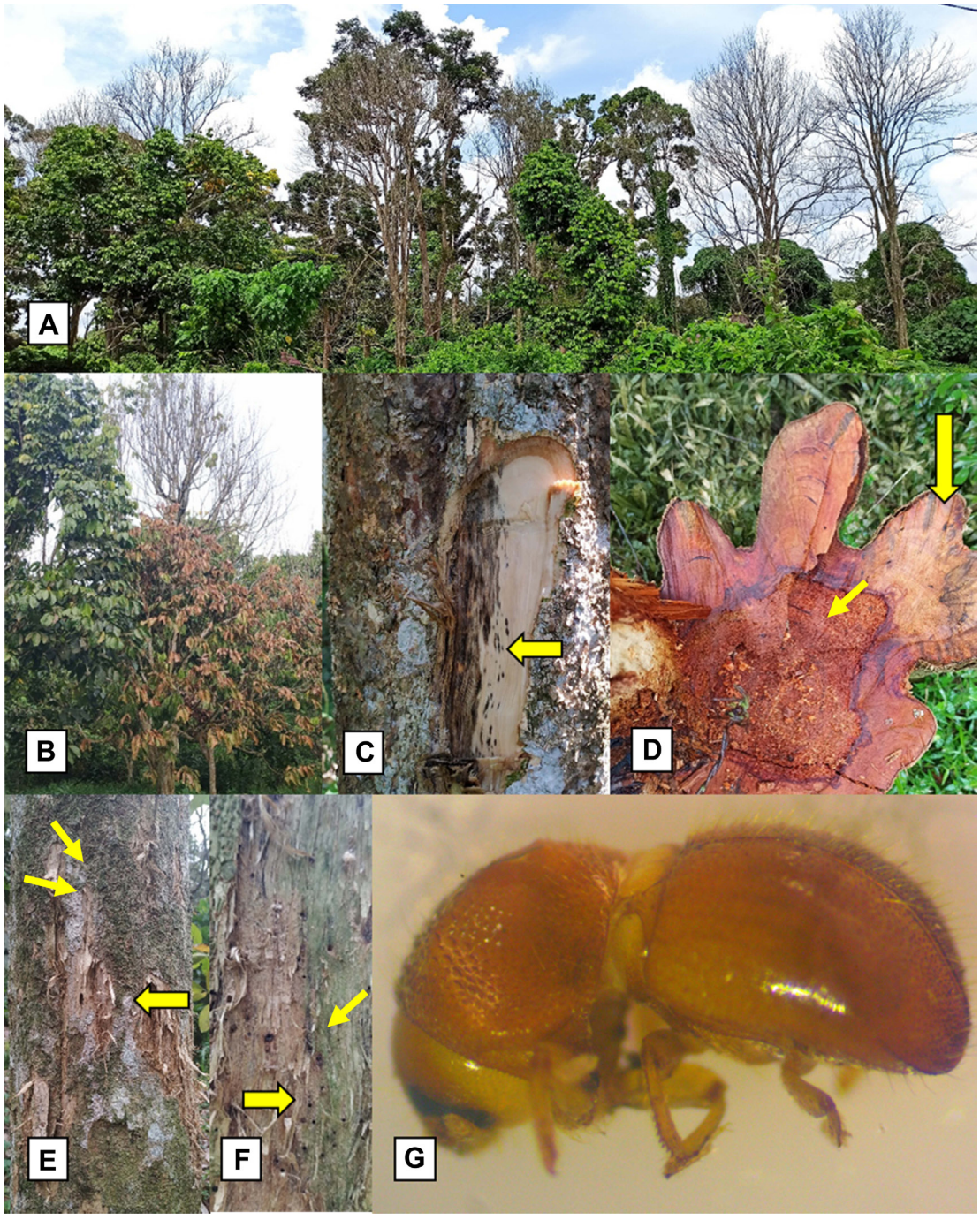

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. (A, B) Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. (C, D) Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrows). (E) Squirrel bite caused peeled-off bark on diseased tree (yellow arrows). (F) A beetle hole on affected diseased wood (yellow arrow). (G) *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

Results

Diseases incidence, Sample collection, and fungal isolation. *Ceratocystis* wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu District, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Fig. 1). It has spread widely in other plantations in the Ogan Komering Ulu District covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya.

From 2020 to 2021, there were similar disease incidences

Table 2. Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood onto the MEA from samples collected from dying *Lansium domesticum* trees in Ogan Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*, *n* (%)
Ogan Komering Ulu (26/40, 65%)	Kartamulya	2019	2/5 (40)
	Saleman	2019	5/5 (100)
	Singapura	2019	2/5 (40)
	Pengaringan	2020	5/5 (100)
	Reksa Jiwa	2020	2/5 (40)
	Tebat Agung	2020	3/5 (60)
	Padang Bindu	2020	2/5 (40)
	Kepayang	2020	5/5 (100)
East Ogan Komering Ulu (4/5, 80%)	Bantan Pelita	2021	4/5 (80)
South Ogan Komering Ulu (14/25, 56%)	Simpang	2021	4/5 (80)
	Tanjung Sari	2021	2/5 (40)
	Tanjung Beringin	2021	4/5 (80)
		2021	2/5 (40)
	Kisau	2021	2/5 (40)
Ogan Komering Ilir (8/15, 53.3%)	Penyandingan	2020	3/5 (60)
	Ulak Kemang	2020	3/5 (60)
	Tanjung Lubuk	2020	2/5 (40)
Musi Banyuasin (16/30, 53.3%)	Kasmaran	2021	1/5 (20)
	Babat Toman	2021	2/5 (40)
	Beruge	2021	1/5 (20)
	Sereka	2021	2/5 (40)
	Sanga Desa	2021	5/5 (100)
	Tanjung Raya	2021	5/5 (100)
Musi Rawas (12/15, 80%)	Tuah Negri	2021	4/5 (80)
	Mambang	2021	5/5 (100)
	Lubuk Tuo	2021	3/5 (60)
North Musi Rawas (16/25, 64%)	Beringin Jaya	2021	3/5 (60)
	Lawang Agung	2021	5/5 (100)
	Karang Waru	2021	3/5 (60)
	Rantau Kadam	2021	3/5 (60)
	Lesung Batu	2021	2/5 (40)
Muara Enim (3/5, 60%)	Ujan mas	2020	3/5 (60)

MEA, malt extract agar.

on the duku plantations in Ogan Komering Ilir, within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively. Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Fig. 2A and B). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Fig. 2C and D). There is a wound on the diseased tree caused by a squirrel scratch (Fig. 2E). In general, holes will appear on the infected duku stem caused by *Hypocryphalus mangiferae*

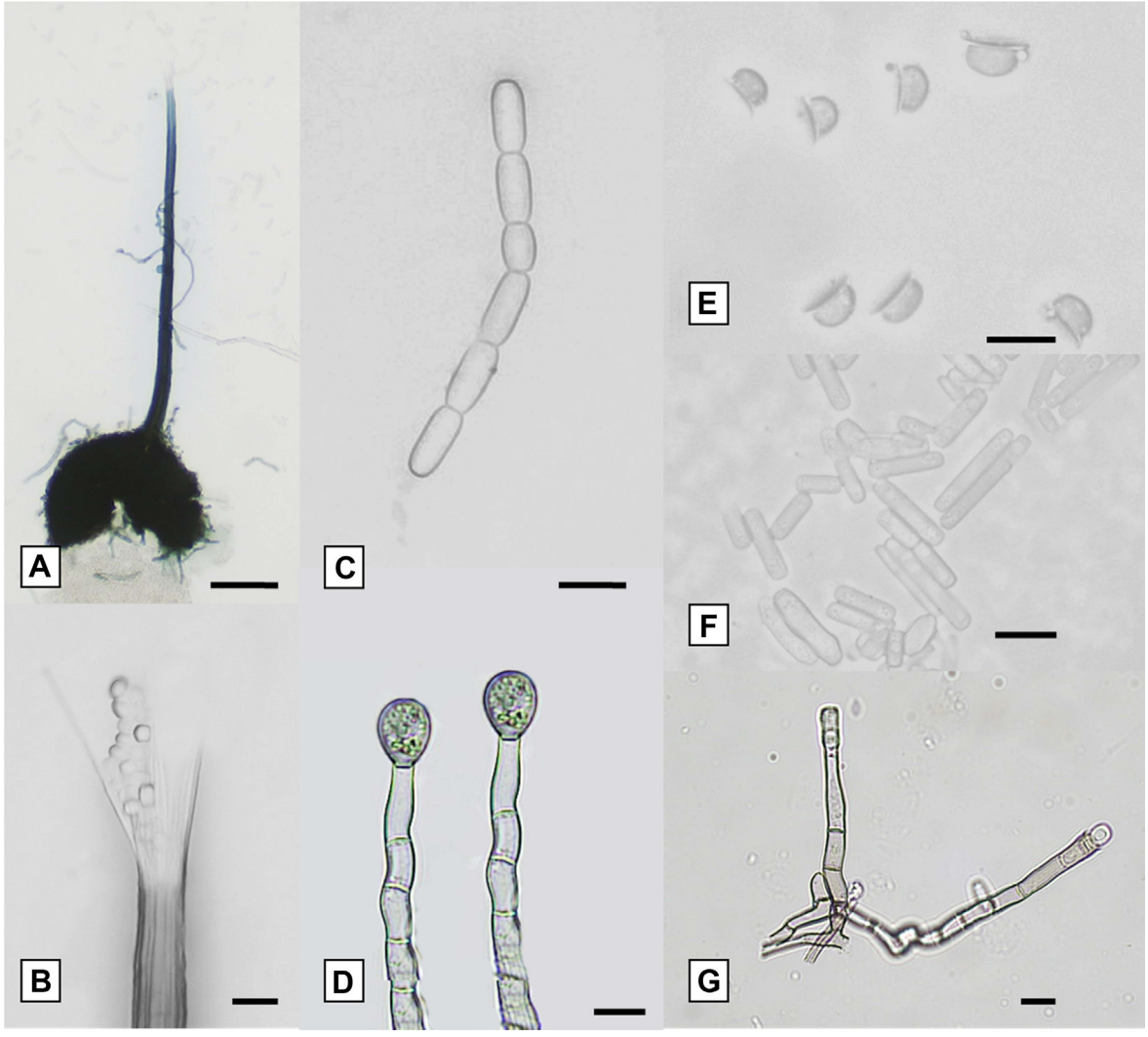

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: (A) globose ascomata with a long neck, (B) divergent ostiolar hyphae, (C) barrel-shaped conidia, (D) chlamydospores, (E) hat-shaped ascospores, (F) cylindrical conidia, (G) conidiophore/phialide. Scale bars: A = 100 µm, B-E = 10 µm, F = 5 µm.

(Fig. 2F) which is a vector insect for *Ceratocystis* (Fig. 2G).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80%, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include (CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192) from Ogan Komering Ulu, (CAL32159, CAL32156, CAL32157, and CAL32158) from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the Culture Collection (CMW), Laboratory of

Table 3. Morphology of selected *Ceratocystis fimbriata* isolates from a different district in South Sumatra

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3-312.4	122.9-291.4	135.7-325.2	141.3-317.1	137.9-321.1	132.1-334.9	137.9-346.1	122.1-316.9
Ascomatal base (l)	153.1-404.4	131-315.4	148.1-398.4	151.1-411.4	143.1-398.4	152.4-394.1	139.1-421.8	157.1-412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4-768.4	354.9-677.7	413.7-798.8	439.9-736.4	475.8-813.6	484.6-790.9	463.8-723.6	484.6-780.9
Neck (w) top	11.5-26.8	7.06-18.4	11.3-21.9	11.1-25.4	10.1-17.9	11.3-21.7	11.1-22.9	11.3-21.7
Neck (w) bottom	24.8-47.9	20.3-39.7	23.6-42.6	22.6-51.2	23.7-43.8	22.67-42.9	23.7-43.6	22.67-44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2-43.5	30.4-40.1	32.7-44.7	32.7-42.2	33.5-43.9	33.7-44.8	33.5-42.9	31.7-44.8
Ascospores								
Hat-shaped ascospores (l)	3.4-5.7	3.3-5.2	3.2-5.4	3.4-4.9	3.2-4.4	3.1-5.1	3.1-4.3	3.3-4.9
Ascospores (w) without sheath	3.4-5.1	3.1-4.1	3.3-4.7	3.4-4.4	3.3-4.1	3.4-4.5	3.3-4.1	3.5-4.4
Ascospores (w) with sheath	5-6.8	4.1-6.1	5.1-6.7	5.3-6.4	5.2-6.5	5.5-6.7	5.2-6.3	5.4-6.6
Primary conidia (l)	12.1-27.5	10.6-18.9	13.8-23.8	12.2-29.3	13.2-25.7	14.9-24.8	12.5-21.6	13.7-24.6
Primary conidia (w)	3.5-7.4	3.2-4.3	3.1-5.1	3.4-4.1	3.2-5.1	3.4 -4.4	3.4-4.1	3.5-4.7
Secondary conidia (l)	6.3-11.6	5.7-10.1	6.6-11.8	7.9-11.8	6.7-11.9	6.8-11.5	6.5-11.5	6.2-11.3
Secondary conidia (w)	4.5-7.6	4.1-7.4	4.7-7.5	5.6-7.9	4.3-7.8	4.3-7.8	4.3-7.1	4.1-7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7-15.1	8.7-15.1	11.3-15.6	9.7-17.8	10.7-15.4	10.1-16.5	10.3-14.6	10.4-14.5
Chlamydospores (w)	7.9-13.9	8.3-11.1	6.9-14.2	6.8-13.6	7.6-11.8	7.7-12.5	7.6-11.8	7.6-12.9
Culture growth rate[b]								
10°C	0	0	0	0	0	0	0	0
15°C	3.3-3.5	2.2-2.5	3.2-3.5	2.2-2.7	3.2-3.4	2.2-2.8	2.3-2.9	2.4-2.8
20°C	3.2-3.7	3.1-2.9	3.2-3.9	3.3-3.9	4.2-4.4	3.2-3.5	4.2-4.4	3.2-3.5
25°C	5.1-5.3	4.1-4.5	4.7-5.1	4.4-4.7	4.4-4.9	4.1-4.5	4.4-4.9	4.1-4.5
30°C	3.3-3.6	3.1-3.9	3.5-4.6	3.5-4.2	3.8-4.2	3.1-3.4	3.8-4.2	3.1-3.4

[a]All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm.
[b]Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after 14 days.

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Morphological characterization and growth in culture. The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Fig. 3A) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Fig. 3B). This fungus also produced chained barrel-shaped conidia (Fig. 3C), and chlamydospores (Fig. 3D), it also had hat-shaped ascospores (Fig. 3E). Cylindrical conidia (Fig. 3G) were generated from the primary phialidic conidiophore (Fig. 3F).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *M. indica* (Van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *A. mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Fig. 4).

DNA extraction, amplification, sequencing, and phylogenetic analyses. For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary Table 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences. Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*.

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the MP method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis*, *Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figs. 5 and 6). The phylogeny was assessed and analyzed using bootstrap analysis with 1,000 replications, as well as β-tubulin sequence respectively, and

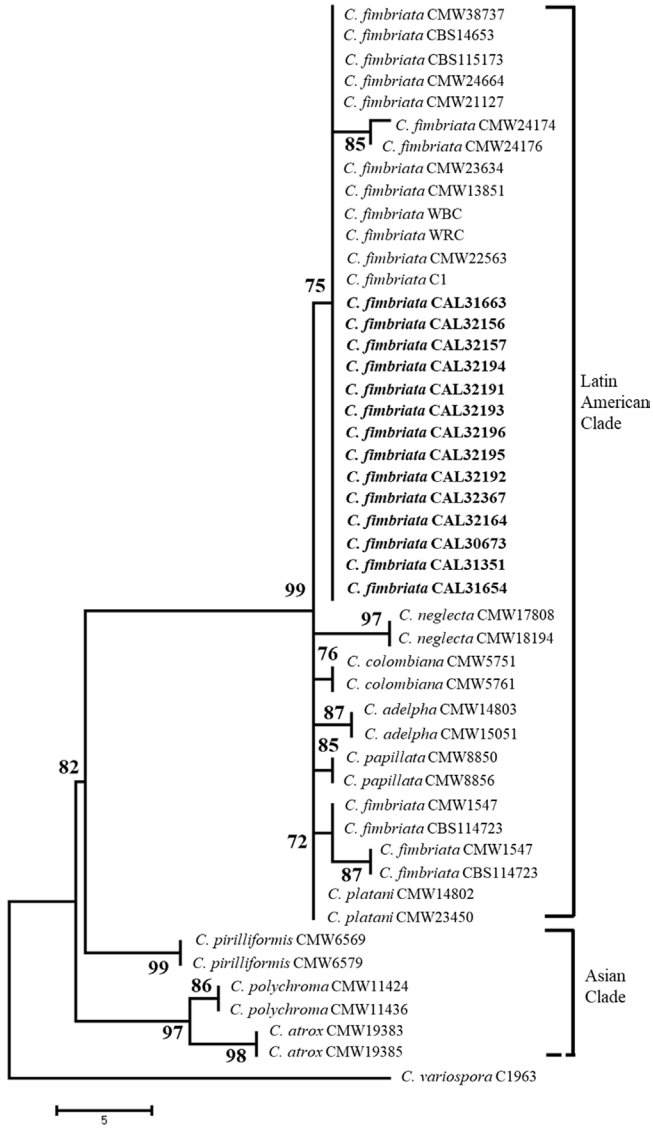

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials. *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the bundle vessels, whereby 90% and 100% of it dies 45, as well as 70

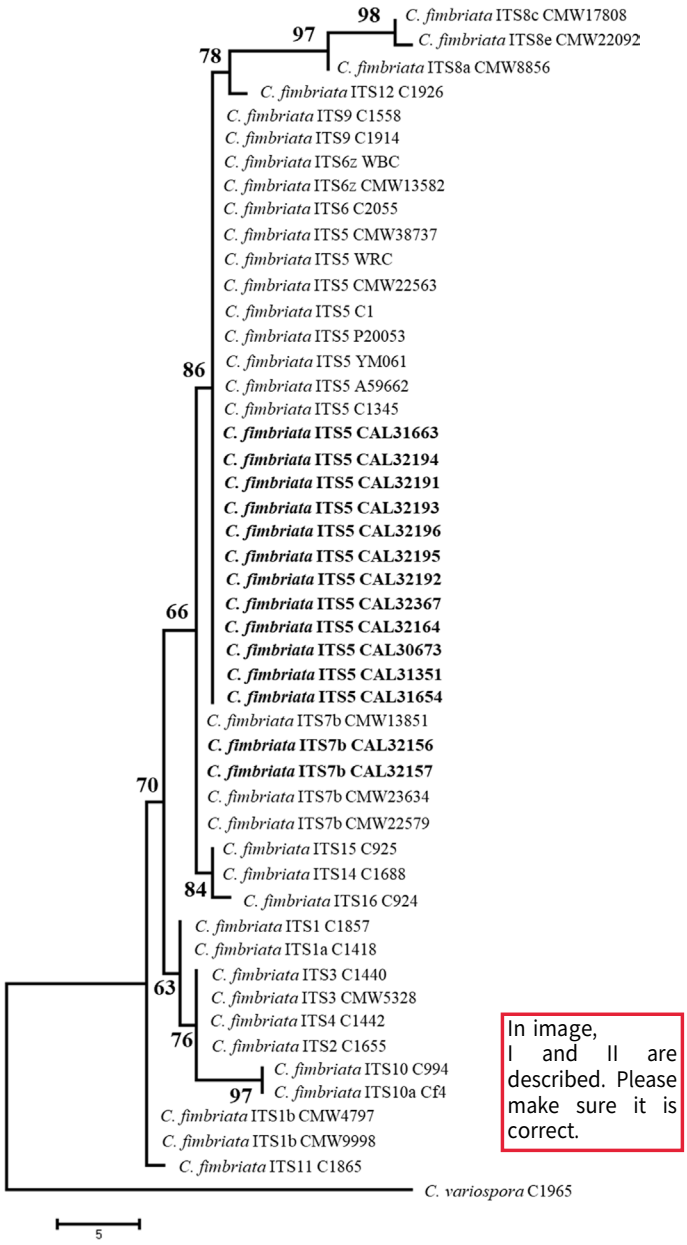

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

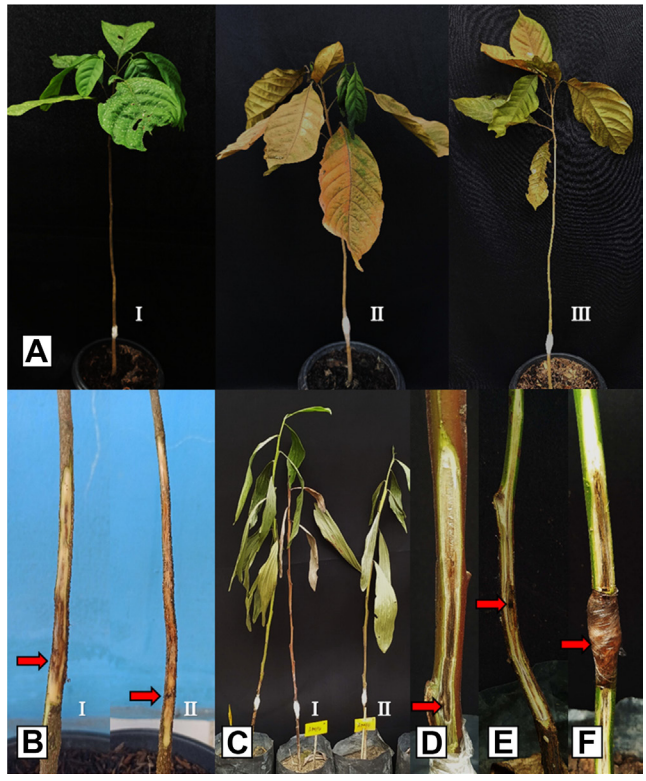

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. (A) Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). (B) The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (II) and CAL32159 (III). (C, D) 4-month-old *Acacia* plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (II) and CAL32159 (III). (E) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. (F) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

days after pathogen inoculation respectively (Fig. 6A and B). ANOVA for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings, and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (Fig. 6C and D). There was no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of

Table 4. Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery condition

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35 f	7/10	10/10
CAL32157	10	15.49 ef	7/10	8/10
CAL32158	10	12.29 cd	5/10	5/10
CAL32159	10	11.02 c	2/10	5/10
CAL32191	10	11.73 cd	2/10	3/10
CAL32192	10	13.83 def	7/10	8/10
CAL32193	10	19.81 g	9/10	10/10
CAL32194	10	6.86 b	2/10	2/10
CAL32195	10	12.89 cde	5/10	6/10
CAL32196	10	11.19 cde	5/10	7/10
Control (MEA)	10	0.01 a	0/10	0/10
P-value		<0.001		

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.

lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa*, *E. urophylla*, and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata*, *H. brasiliensis*, and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The re-isolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006, 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007; Tarigan et al., 2011). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Please check correction.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to the stem, which is spread by squirrels and insects. This dis-

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Table 5. Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*			*Dyera costulata*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25 ef	10/10	10/10	9.86 de	0/10	1/10	11.32 b	0/10	1/10	4.25b	0/10	0/10
CAL32157	10	16.32 de	10/10	10/10	10.16 de	0/10	2/10	11.81 b	0/10	1/10	3.91b	0/10	0/10
CAL32158	10	14.49 cde	8/10	10/10	9.39 cd	0/10	1/10	9.33 b	0/10	0/10	3.63b	0/10	0/10
CAL32159	10	13.59 bcd	8/10	10/10	8.26 bcd	0/10	1/10	9.86 b	0/10	0/10	3.83b	0/10	0/10
CAL32191	10	11.73 bc	7/10	10/10	7.96 bcd	0/10	0/10	9.82 b	0/10	0/10	3.57b	0/10	0/10
CAL32192	10	15.54 cde	10/10	10/10	6.57 bc	0/10	0/10	10.59 b	0/10	0/10	5.15b	0/10	0/10
CAL32193	10	20.93 f	10/10	10/10	12.59 e	0/10	5/10	11.92 b	0/10	3/10	5.39b	0/10	0/10
CAL32194	10	9.943 b	5/10	10/10	5.97 b	0/10	0/10	8.80 b	0/10	0/10	3.05b	0/10	0/10
CAL32195	10	15.39 cde	9/10	10/10	7.82 bcd	0/10	2/10	11.20 b	0/10	2/10	4.02b	0/10	0/10
CAL32196	10	14.64 cde	8/10	10/10	8.64 bcd	0/10	1/10	11.15 b	0/10	1/10	3.60b	0/10	0/10
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01a	0/10	0/10
P-value		<0.001			<0.001			<0.001			<0.001		
		5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10			
		Hevea brasiliensis			*Alstonia scholaris*			*Melaleuca leucadendra*					
CAL32156	10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10			
CAL32157	10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10			
CAL32158	10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10			
CAL32159	10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10			
CAL32191	10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10			
CAL32192	10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10			
CAL32193	10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10			
CAL32194	10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10			
CAL32195	10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10			
CAL32196	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10			
Control (MEA)	10	<0.001			<0.001			<0.001					
P-value													

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's honestly significant difference multiple range test.
dpi, days post inoculation.

ease also occur from the root and continues up to the base of the stem. The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene

sequences in each isolate obtained showed similarities to *C. fimbriata* which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were two variations of the ITS rDNA sequence from two isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin District. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Pratama et al., 2021a, 2021b; Tarigan et al., 2011). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *L. domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010, 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *C. esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa*, *E. urophylla*, and *M. cajuputi*, as well as weakly pathogenic to *D. costulata*, *A. scholaris*, and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *L. domesticum* caused by *C. fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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Supplementary Table 1. *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolate no.	Host plant	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	CAL32194	*Lansium domesticum*	Indonesia	MT373418	MW752140
	ITS5	CAL32191	*L. domesticum*	Indonesia	MT373420	MW752141
	ITS5	CAL32193	*L. domesticum*	Indonesia	MT373417	MW752142
	ITS5	CAL32196	*L. domesticum*	Indonesia	MT373419	MW752144
	ITS5	CAL32195	*L. domesticum*	Indonesia	MT373416	MW752145
	ITS5	CAL32192	*L. domesticum*	Indonesia	MT373415	MW752146
	ITS5	CAL31663	*L. domesticum*	Indonesia	MT373422	-
	ITS5	CAL32367	*L. domesticum*	Indonesia	MT373421	-
	ITS5	CAL32164	*L. domesticum*	Indonesia	-	-
	ITS5	CAL30673	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31351	*L. domesticum*	Indonesia	-	-
	ITS5	CAL31654	*L. domesticum*	Indonesia	-	-
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus Mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CAL32156	*L. domesticum*	Indonesia	-	MW752143
	ITS7b	CAL32157	*L. domesticum*	Indonesia	-	MW752147
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-

	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillate	LAC	CMW8850	*Citrus* × *Tangelo* hybrid	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus* hybrid	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus* hybrid	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus* hybrid	China	-	JQ862720
	LAC	CBS115173	*Gmelina Arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

9. Bukti konfirmasi submit proof corrections, respon kepada editor, dan artikel yang proof corrections pertama (19 Maret 2022)

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

PPJ 2021-0182: Final Proof Corrections

a. muslim unsri <a_muslim@unsri.ac.id> Sat, Mar 19, 2022 at 6:45 PM
To: 한국식물병리학회 편집위원회 <paper@kspp.org>

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email regarding our manuscript is to be published in The Plant Pathology Journal, 38 (2), April issue, 2022.

We have checked and revised our galley proofs as Editor's comment, indicated by highlighting each revisions made in PDF file.

Beside Editor's comment/correction, we also revise some minor mistake and add Table/Figure numbers which explain the sentence.

All Revisions of our changes made in response to the editor's comments and also our minor revision summarized in cover letter and indicated by highlighting in PDF File (attachment file).

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
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Faculty of Agriculture, Sriwijaya University
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E-mail: a_muslim@unsri.ac.id
[Quoted text hidden]

2 attachments

Revision of Galley Proofs.pdf
109K

Revisions of PPJ 2021-0182.pdf
7036K

Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email regarding our manuscript is to be published in The Plant Pathology Journal, 38 (2), April issue, 2022.

We have checked and revised our galley proofs indicated by highlighting each revisions made in PDF file.

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

1. **Editor's comment:** Please provide FAX (Page 1).

Our response: We agree and FAX mail is (0711) 580276. We would like also to change Our Telephone number (+62 811-7826-119) to office telephone number (0711) 580059.

2. **Editor's comment:** In Fig. 6, I and II are described. Please make sure it is correct (Page 10).

Our response: Thank you very much. We agree and the sentence has been changed to be "(B) The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (I) and CAL32159 (II). (C, D) 4-month-old *Acacia* plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (I) and CAL32159 (II)".

3. **Editor's comment:** Please check correction "which is also caused by wind (Harrington, 2007; Tarigan et al., 2011)". (Page 11).

Our response: We agree and deleted reference of Tarigan et al., 2011. The sentence has been changed to be "which is also caused by wind (Harrington, 2007)".

4. **Editor's comment:** Please check corrected table 5. (Page 12)

Our response: There are small mistake in table 5. The data of 5.23e; 0/10; 0/10; 5.21b; 0/10; 0/10; 5.81e; 0/10; 2/10 is belong to CAL32156. Therefore, the data should be moved to below of *Hevea brasiliensis*; *Alstonia scholaris*; *Melaleuca leucadendra* in row of CAL32156.

Beside Editor's comment/correction above. We would like also to revise some minor mistake and add Table/Figure numbers which explain the sentence.

1. In page 3: Isolation of Ceratocystis was carried out. Ceratocystis should be written in italic.
   The correct sentence to be: Isolation of *Ceratocystis* was carried out….
2. In page 6 : ……. in South Sumatra with varying levels of disease incidence (Fig 1). "(Fig 1)" should be changed with "(Table 1)".
   The correct sentence to be : ….. in South Sumatra with varying levels of disease incidence (Table 1).
3. In page 6 : after the sentence ….in Sanga Desa and Tanjung Raya, we add "(Table 1)".
   The correct sentence to be : ……in Sanga Desa and Tanjung Raya (Table 1).
4. In page 7: after the sentence …… and Lawang Agung with a percentage of 56.1% and 43.6%, respectively, we add "(Table 1)".

The correct sentence to be : ……and Lawang Agung with a percentage of 56.1% and 43.6%, respectively (Table 1).

5. In Page 9. In the sentence of They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Fig.4). "(Fig 4)" should be changed to "(Table 3)".
   The correct sentence to be: They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Table 3).
6. In page 9: in the sub title: DNA extraction, amplification, sequencing, and phylogenetic analyses.
   a. After the sentence ……..*C. fimbriata* sensu stricto were grouped with 99% identical sequences, we add "(Fig. 4)".
      The correct sentence to be : ……..*C. fimbriata* sensu stricto were grouped with 99% identical sequences (Fig. 4).
   b. After the sentence ……previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*, we add "(Fig. 5)".
      The correct sentence to be: ….previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata* (Fig. 5).
   c. In the sentence …….as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figs. 5 and 6). We delete "(Figs. 5 and 6)".
      The correct sentence to be : …….as well as *Mangifera indica* in Oman, Pakistan, and Indonesia.
7. In page 9 : the sentence ……..whereby 90% and 100% of it dies 45. After 45, we add "days"
   The correct sentence to be : ……..whereby 90% and 100% of it dies 45 days,
8. In page 10 : ….vascular discoloration in all inoculated seedlings. After inoculated seedlings, we add "(Fig. 6C–F)"
   The correct sentence to be : …….. vascular discoloration in all inoculated seedlings (Fig. 6C–F),.
9. In page 10 : ….and wilt was noted to reach 100% of all seedling at day 70 after inoculation (Fig. 6C and D). The "(Fig. 6C and D)" should be changed to "(Table 5)".
   The correct sentence to be : ….and wilt was noted to reach 100% of all seedling at day 70 after inoculation (Table 5).
10. In page 11 : After this sentence …..to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation), we add "(Table 5)".
    The correct sentence to be : …to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation) (Table 5).

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Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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(Received on December 30, 2021; Revised on January 30, 2022; Accepted on February 15, 2022)

***Ceratocystis* wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.**

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Handling Editor : Kihyuck Choi

Keywords **:** agroforestry plants, canker, *Certocystis fimbriata*, die-back disease

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8,14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum* Corr. has also been reported to have benefits as larvicides (Ni'mah et al., 2015; Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria* spp. (Norhayati et al., 2016), organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Subandrate et al., 2016; Tilaar et al., 2008).

Previous studies conducted from 2014 to 2017 (Suwandi

et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in three locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis,* and *Chukrasia tabularis* (Chi et al., 2019a, 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa*, *Eucalyptus urophylla*, *Dyera costulata*, *Alstonia scholaris*, *Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Material and Methods

Diseases incidence, sample collection, and fungal isolation. Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Fig. 1). In each plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Pratama et al., 2021a; Suwandi et al., 2021). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *L. domesticum* which showed symptoms of branch wilting,

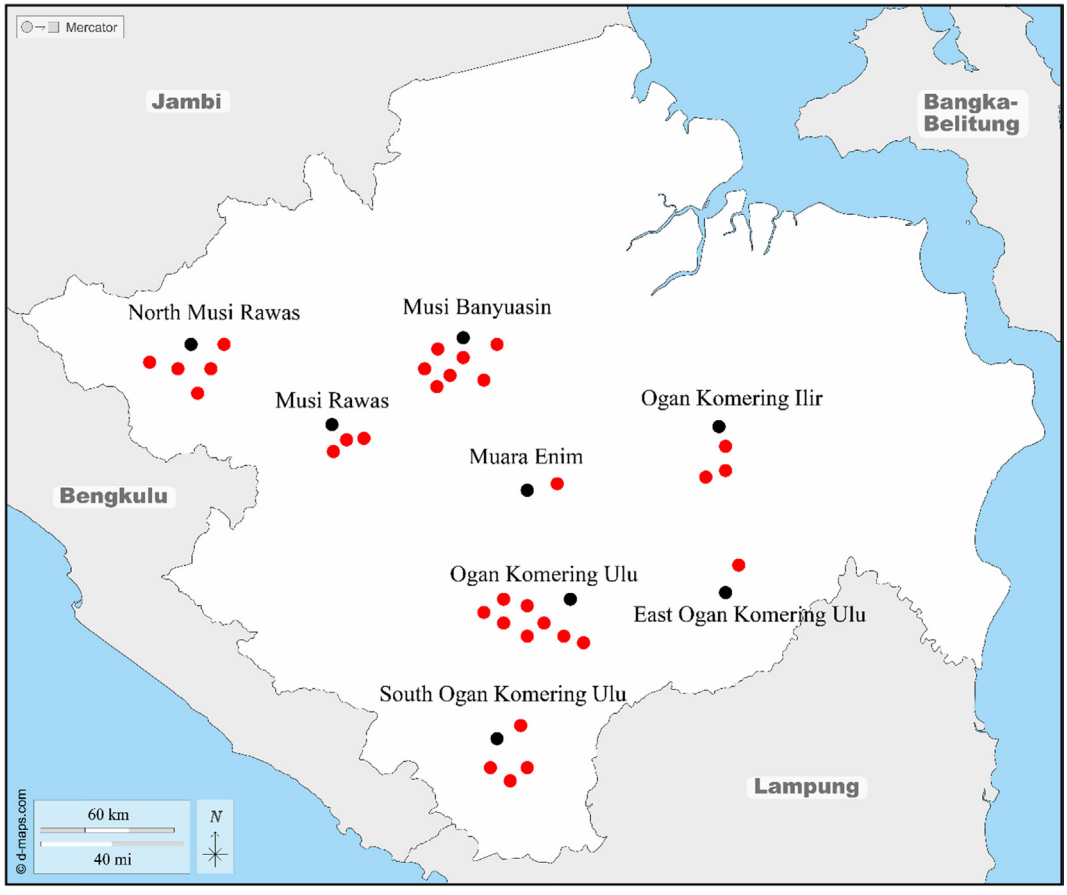

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of Ceratocystis was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 min, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on malt extract agar (MEA) media at room temperature (25°C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% MEA (20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization. The morphological characteristics of the observed fungi were represented by isolates originating from eight regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture. To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of eight isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30°C with an interval of 5°C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses. The pure cultures used for the DNA extraction were 14 isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

Amplification and PCR sequencing were obtained from two gene regions, namely beta-tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq-DNA-polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were initial denaturation for 3 min at 94°C, 30 cycles for 30 seconds at 94°C for 30 s, for 30 s at 52°C, and 1 min at 72°C for. Amplification was completed at 72°C for 10 min and the PCR product was stored at 10°C. The PCR

amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For MP analysis, the metrics calculated included tree length, retention index, and consistency index. Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials. These studies were conducted using ten isolates of *C. fimbriata*. The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2-3 cm and a height of 50-60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *A. mangium*, *A. carsicarpa*, *E. urophylla*, *D. costulata*, *H. brasiliensis*, *A. scholaris*, and *M. cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2-3 cm and a height of 70-80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on MEA plates or placed between two carrot slices.

Table 1. Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (n = 89)	53.9	64	85.4
Saleman (n = 74)	41.9	58.1	95.9
Singapura (n = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (n = 91)	59.3	72.5	84.6
Tebat Agung (n = 67)	10.5	16.4	31.3
Padang Bindu (n = 71)	5.6	15.5	19.7
Kepayang (n = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

Pathogenicity test data were analyzed using the SAS university edition software package (SAS Institute Inc., Cary, NC, USA). Furthermore, the Analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey's honestly significant difference) test was used to determine the significant differences in the mean comparisons of the different treatments.

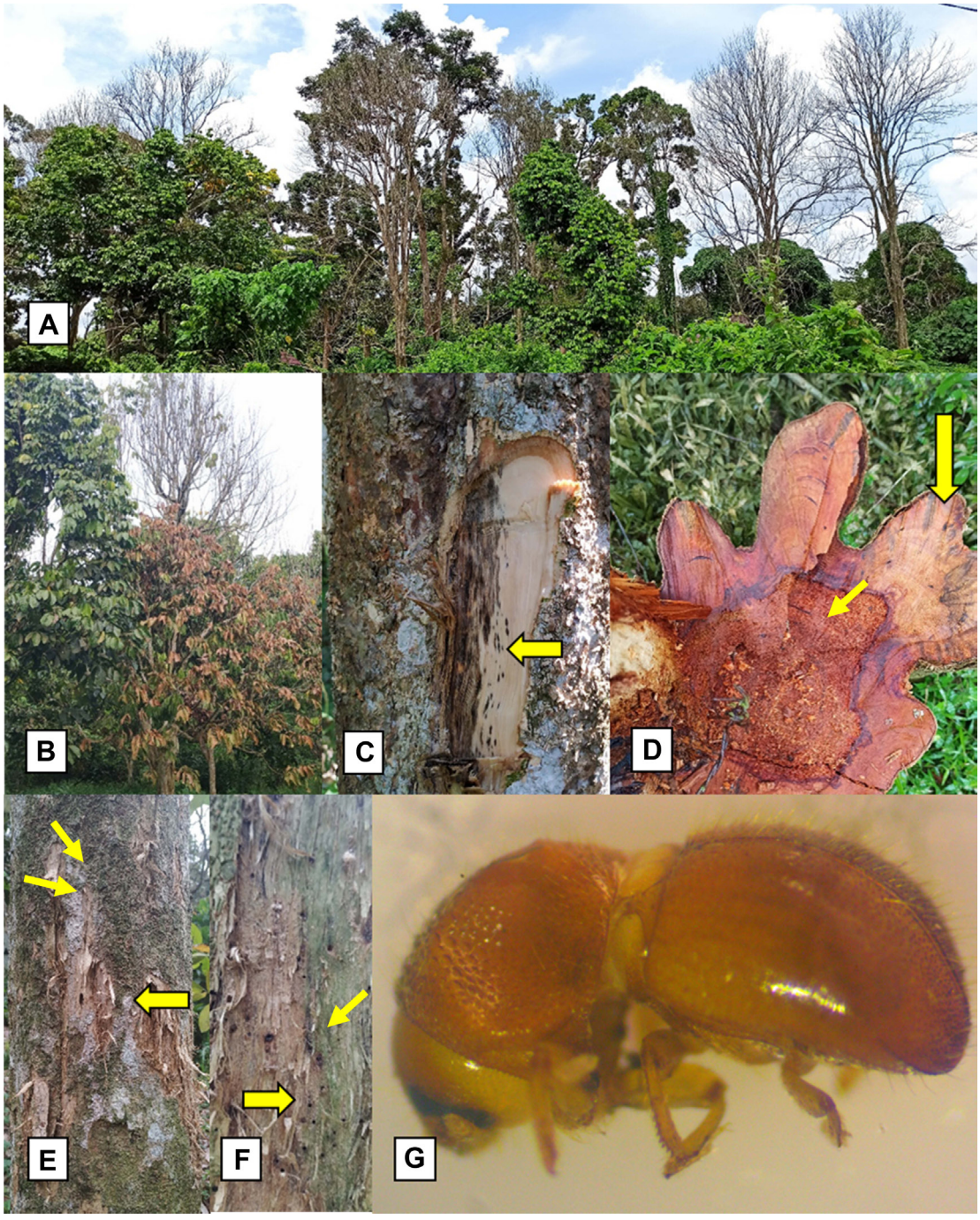

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. (A, B) Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. (C, D) Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrows). (E) Squirrel bite caused peeled-off bark on diseased tree (yellow arrows). (F) A beetle hole on affected diseased wood (yellow arrow). (G) *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

Results

Diseases incidence, Sample collection, and fungal isolation. *Ceratocystis* wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu District, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Fig. 1). It has spread widely in other plantations in the Ogan Komering Ulu District covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya.

From 2020 to 2021, there were similar disease incidences

Table 2. Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood onto the MEA from samples collected from dying *Lansium domesticum* trees in Ogan Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*, *n* (%)
Ogan Komering Ulu (26/40, 65%)	Kartamulya	2019	2/5 (40)
	Saleman	2019	5/5 (100)
	Singapura	2019	2/5 (40)
	Pengaringan	2020	5/5 (100)
	Reksa Jiwa	2020	2/5 (40)
	Tebat Agung	2020	3/5 (60)
	Padang Bindu	2020	2/5 (40)
	Kepayang	2020	5/5 (100)
East Ogan Komering Ulu (4/5, 80%)	Bantan Pelita	2021	4/5 (80)
South Ogan Komering Ulu (14/25, 56%)	Simpang	2021	4/5 (80)
	Tanjung Sari	2021	2/5 (40)
	Tanjung Beringin	2021	4/5 (80)
		2021	2/5 (40)
	Kisau	2021	2/5 (40)
Ogan Komering Ilir (8/15, 53.3%)	Penyandingan	2020	3/5 (60)
	Ulak Kemang	2020	3/5 (60)
	Tanjung Lubuk	2020	2/5 (40)
Musi Banyuasin (16/30, 53.3%)	Kasmaran	2021	1/5 (20)
	Babat Toman	2021	2/5 (40)
	Beruge	2021	1/5 (20)
	Sereka	2021	2/5 (40)
	Sanga Desa	2021	5/5 (100)
	Tanjung Raya	2021	5/5 (100)
Musi Rawas (12/15, 80%)	Tuah Negri	2021	4/5 (80)
	Mambang	2021	5/5 (100)
	Lubuk Tuo	2021	3/5 (60)
North Musi Rawas (16/25, 64%)	Beringin Jaya	2021	3/5 (60)
	Lawang Agung	2021	5/5 (100)
	Karang Waru	2021	3/5 (60)
	Rantau Kadam	2021	3/5 (60)
	Lesung Batu	2021	2/5 (40)
Muara Enim (3/5, 60%)	Ujan mas	2020	3/5 (60)

MEA, malt extract agar.

on the duku plantations in Ogan Komering Ilir, within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively. Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Fig. 2A and B). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Fig. 2C and D). There is a wound on the diseased tree caused by a squirrel scratch (Fig. 2E). In general, holes will appear on the infected duku stem caused by *Hypocryphalus mangiferae*

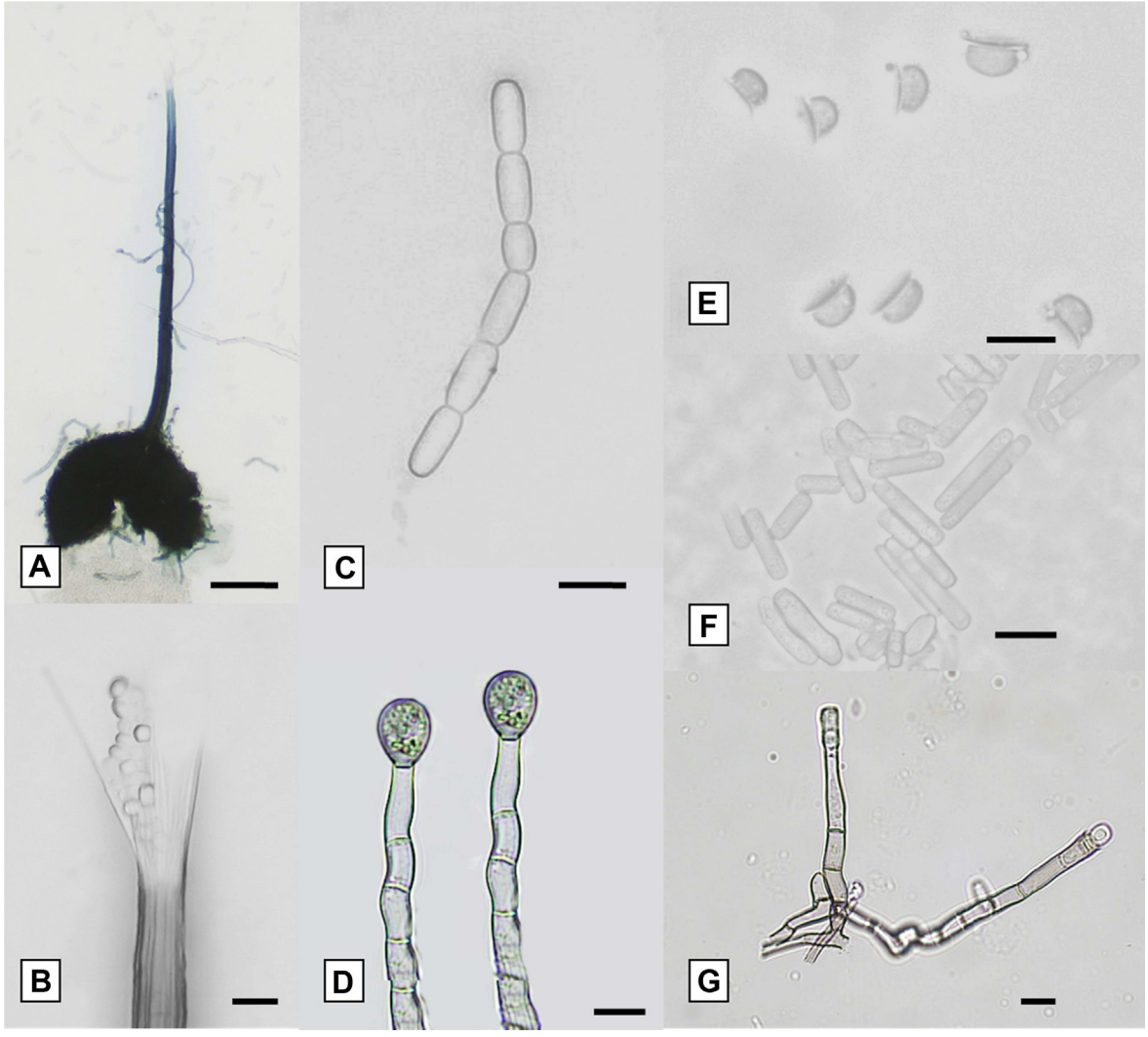

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: (A) globose ascomata with a long neck, (B) divergent ostiolar hyphae, (C) barrel-shaped conidia, (D) chlamydospores, (E) hat-shaped ascospores, (F) cylindrical conidia, (G) conidiophore/phialide. Scale bars: A = 100 µm, B-E = 10 µm, F = 5 µm.

(Fig. 2F) which is a vector insect for *Ceratocystis* (Fig. 2G).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80%, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include (CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192) from Ogan Komering Ulu, (CAL32159, CAL32156, CAL32157, and CAL32158) from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the Culture Collection (CMW), Laboratory of

Table 3. Morphology of selected *Ceratocystis fimbriata* isolates from a different district in South Sumatra

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3-312.4	122.9-291.4	135.7-325.2	141.3-317.1	137.9-321.1	132.1-334.9	137.9-346.1	122.1-316.9
Ascomatal base (l)	153.1-404.4	131-315.4	148.1-398.4	151.1-411.4	143.1-398.4	152.4-394.1	139.1-421.8	157.1-412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4-768.4	354.9-677.7	413.7-798.8	439.9-736.4	475.8-813.6	484.6-790.9	463.8-723.6	484.6-780.9
Neck (w) top	11.5-26.8	7.06-18.4	11.3-21.9	11.1-25.4	10.1-17.9	11.3-21.7	11.1-22.9	11.3-21.7
Neck (w) bottom	24.8-47.9	20.3-39.7	23.6-42.6	22.6-51.2	23.7-43.8	22.67-42.9	23.7-43.6	22.67-44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2-43.5	30.4-40.1	32.7-44.7	32.7-42.2	33.5-43.9	33.7-44.8	33.5-42.9	31.7-44.8
Ascospores								
Hat-shaped ascospores (l)	3.4-5.7	3.3-5.2	3.2-5.4	3.4-4.9	3.2-4.4	3.1-5.1	3.1-4.3	3.3-4.9
Ascospores (w) without sheath	3.4-5.1	3.1-4.1	3.3-4.7	3.4-4.4	3.3-4.1	3.4-4.5	3.3-4.1	3.5-4.4
Ascospores (w) with sheath	5-6.8	4.1-6.1	5.1-6.7	5.3-6.4	5.2-6.5	5.5-6.7	5.2-6.3	5.4-6.6
Primary conidia (l)	12.1-27.5	10.6-18.9	13.8-23.8	12.2-29.3	13.2-25.7	14.9-24.8	12.5-21.6	13.7-24.6
Primary conidia (w)	3.5-7.4	3.2-4.3	3.1-5.1	3.4-4.1	3.2-5.1	3.4 -4.4	3.4-4.1	3.5-4.7
Secondary conidia (l)	6.3-11.6	5.7-10.1	6.6-11.8	7.9-11.8	6.7-11.9	6.8-11.5	6.5-11.5	6.2-11.3
Secondary conidia (w)	4.5-7.6	4.1-7.4	4.7-7.5	5.6-7.9	4.3-7.8	4.3-7.8	4.3-7.1	4.1-7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7-15.1	8.7-15.1	11.3-15.6	9.7-17.8	10.7-15.4	10.1-16.5	10.3-14.6	10.4-14.5
Chlamydospores (w)	7.9-13.9	8.3-11.1	6.9-14.2	6.8-13.6	7.6-11.8	7.7-12.5	7.6-11.8	7.6-12.9
Culture growth rate[b]								
10°C	0	0	0	0	0	0	0	0
15°C	3.3-3.5	2.2-2.5	3.2-3.5	2.2-2.7	3.2-3.4	2.2-2.8	2.3-2.9	2.4-2.8
20°C	3.2-3.7	3.1-2.9	3.2-3.9	3.3-3.9	4.2-4.4	3.2-3.5	4.2-4.4	3.2-3.5
25°C	5.1-5.3	4.1-4.5	4.7-5.1	4.4-4.7	4.4-4.9	4.1-4.5	4.4-4.9	4.1-4.5
30°C	3.3-3.6	3.1-3.9	3.5-4.6	3.5-4.2	3.8-4.2	3.1-3.4	3.8-4.2	3.1-3.4

[a]All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm.
[b]Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after 14 days.

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Morphological characterization and growth in culture. The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Fig. 3A) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Fig. 3B). This fungus also produced chained barrel-shaped conidia (Fig. 3C), and chlamydospores (Fig. 3D), it also had hat-shaped ascospores (Fig. 3E). Cylindrical conidia (Fig. 3G) were generated from the primary phialidic conidiophore (Fig. 3F).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *M. indica* (Van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *A. mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Fig. 4).

DNA extraction, amplification, sequencing, and phylogenetic analyses. For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary Table 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences. Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata*.

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the MP method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis*, *Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia (Figs. 5 and 6). The phylogeny was assessed and analyzed using bootstrap analysis with 1,000 replications, as well as β-tubulin sequence respectively, and

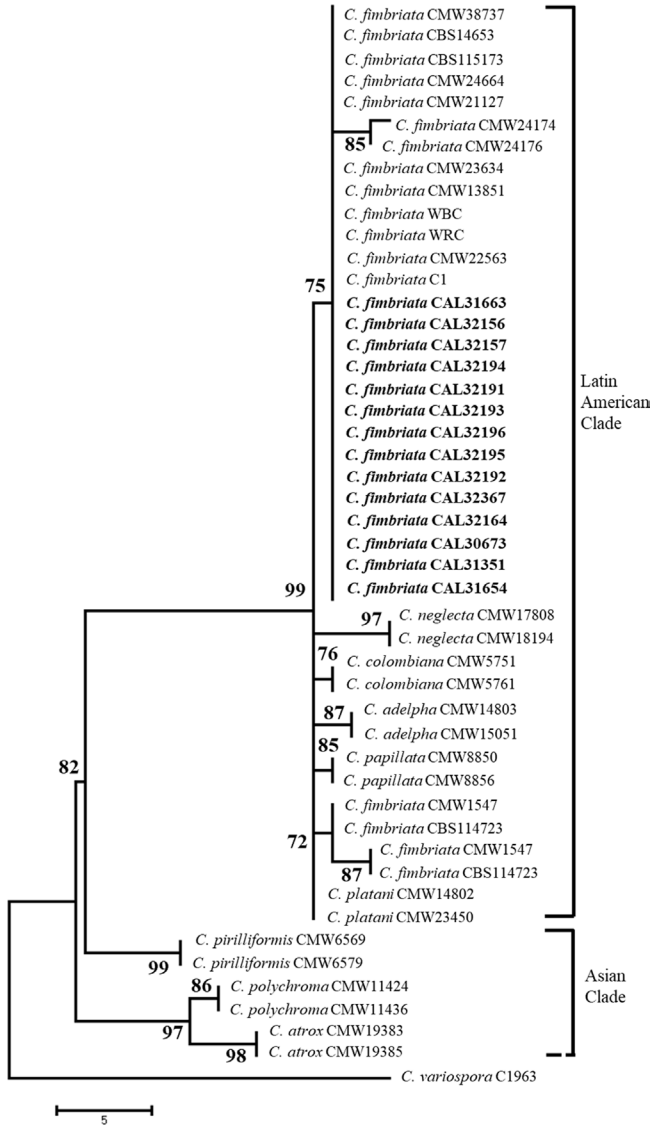

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials. *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the bundle vessels, whereby 90% and 100% of it dies 45, as well as 70

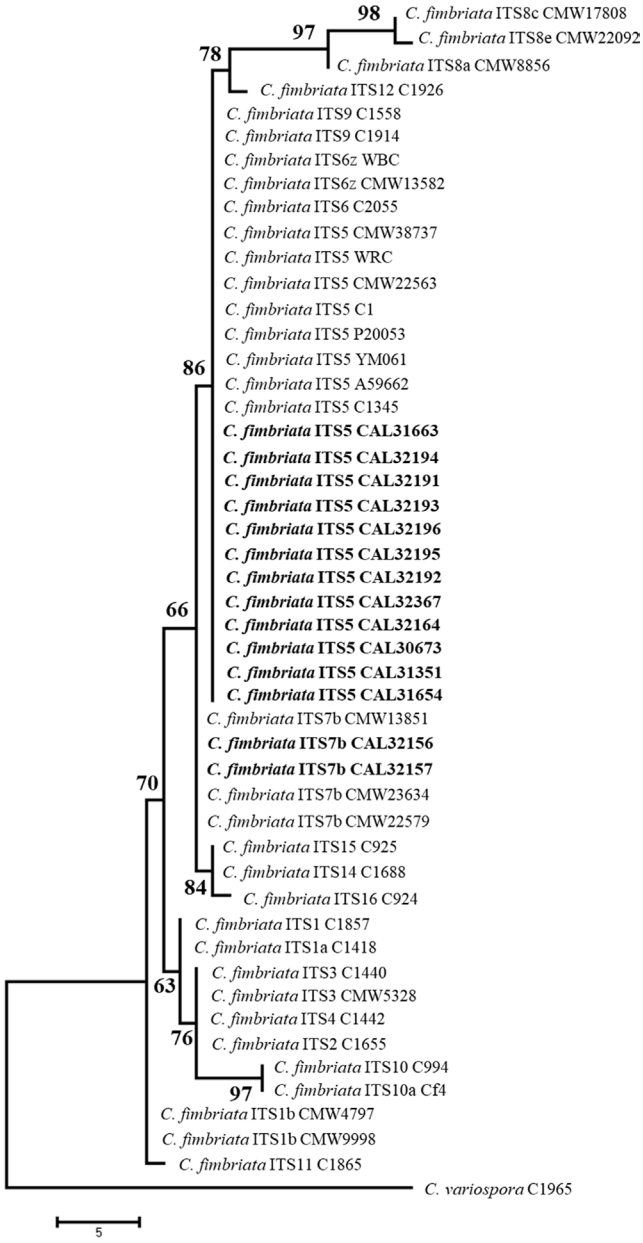

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

days after pathogen inoculation respectively (Fig. 6A and B). ANOVA for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4). Statistical analysis showed a significant difference in le-

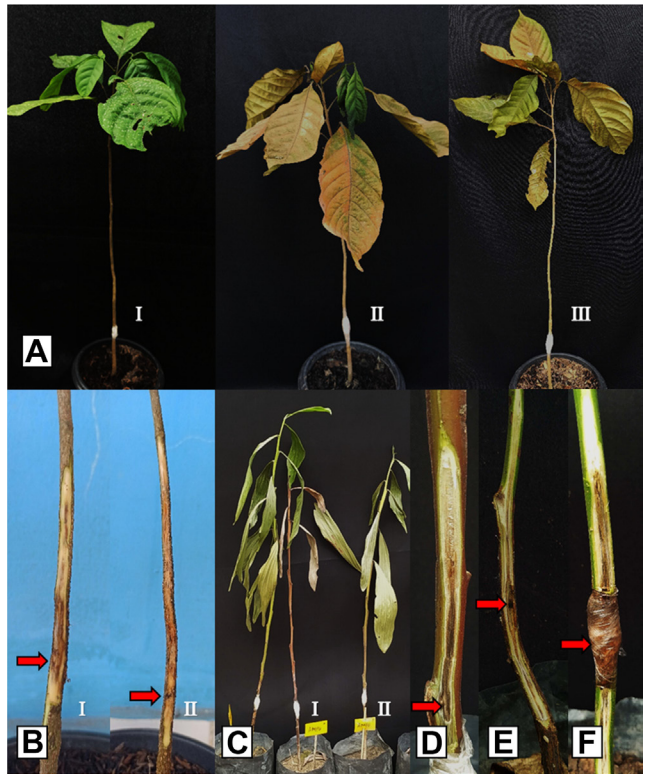

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. (A) Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). (B) The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (II) and CAL32159 (III). (C, D) 4-month-old *Acacia* plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (II) and CAL32159 (III). (E) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. (F) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

sion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings, and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (Fig. 6C and D). There was no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of

Table 4. Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery condition

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35 f	7/10	10/10
CAL32157	10	15.49 ef	7/10	8/10
CAL32158	10	12.29 cd	5/10	5/10
CAL32159	10	11.02 c	2/10	5/10
CAL32191	10	11.73 cd	2/10	3/10
CAL32192	10	13.83 def	7/10	8/10
CAL32193	10	19.81 g	9/10	10/10
CAL32194	10	6.86 b	2/10	2/10
CAL32195	10	12.89 cde	5/10	6/10
CAL32196	10	11.19 cde	5/10	7/10
Control (MEA)	10	0.01 a	0/10	0/10
P-value		<0.001		

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.

lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa*, *E. urophylla*, and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata*, *H. brasiliensis*, and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The re-isolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006, 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007; Tarigan et al., 2011). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to the stem, which is spread by squirrels and insects. This dis-

Table 5. Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*			*Dyera costulata*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25 ef	10/10	10/10	9.86 de	0/10	1/10	11.32 b	0/10	1/10	4.25b	0/10	0/10
CAL32157	10	16.32 de	10/10	10/10	10.16 de	0/10	2/10	11.81 b	0/10	1/10	3.91b	0/10	0/10
CAL32158	10	14.49 cde	8/10	10/10	9.39 cd	0/10	1/10	9.33 b	0/10	0/10	3.63b	0/10	0/10
CAL32159	10	13.59 bcd	8/10	10/10	8.26 bcd	0/10	1/10	9.86 b	0/10	0/10	3.83b	0/10	0/10
CAL32191	10	11.73 bc	7/10	10/10	7.96 bcd	0/10	0/10	9.82 b	0/10	0/10	3.57b	0/10	0/10
CAL32192	10	15.54 cde	10/10	10/10	6.57 bc	0/10	0/10	10.59 b	0/10	0/10	5.15b	0/10	0/10
CAL32193	10	20.93 f	10/10	10/10	12.59 e	0/10	5/10	11.92 b	0/10	3/10	5.39b	0/10	0/10
CAL32194	10	9.943 b	5/10	10/10	5.97 b	0/10	0/10	8.80 b	0/10	0/10	3.05b	0/10	0/10
CAL32195	10	15.39 cde	9/10	10/10	7.82 bcd	0/10	2/10	11.20 b	0/10	2/10	4.02b	0/10	0/10
CAL32196	10	14.64 cde	8/10	10/10	8.64 bcd	0/10	1/10	11.15 b	0/10	1/10	3.60b	0/10	0/10
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01a	0/10	0/10
P-value		<0.001			<0.001			<0.001			<0.001		
		5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10			
		Hevea brasiliensis			*Alstonia scholaris*			*Melaleuca leucadendra*					
CAL32156	10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10			
CAL32157	10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10			
CAL32158	10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10			
CAL32159	10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10			
CAL32191	10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10			
CAL32192	10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10			
CAL32193	10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10			
CAL32194	10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10			
CAL32195	10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10			
CAL32196	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10			
Control (MEA)	10	<0.001			<0.001			<0.001					
P-value													

Values followed by the same letters in a column are not different among isolates at P=0.05 according to Tukey's honestly significant difference multiple range test.
dpi, days post inoculation.

ease also occur from the root and continues up to the base of the stem. The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene

sequences in each isolate obtained showed similarities to *C. fimbriata* which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were two variations of the ITS rDNA sequence from two isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin District. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Pratama et al., 2021a, 2021b; Tarigan et al., 2011). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *L. domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010, 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *C. esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa*, *E. urophylla*, and *M. cajuputi*, as well as weakly pathogenic to *D. costulata*, *A. scholaris*, and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *L. domesticum* caused by *C. fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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10.Bukti konfirmasi dan hasil proof corrections kedua (26 Maret 2022)

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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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***Ceratocystis* wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.**

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Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8,14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum* Corr. has also been reported to have benefits as larvicides (Ni'mah et al., 2015; Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria* spp. (Norhayati et al., 2016), organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Subandrate et al., 2016; Tilaar et al., 2008).

Previous studies conducted from 2014 to 2017 (Suwandi

et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in three locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis,* and *Chukrasia tabularis* (Chi et al., 2019a, 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa*, *Eucalyptus urophylla*, *Dyera costulata*, *Alstonia scholaris*, *Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Materials and Methods

Diseases incidence, sample collection, and fungal isolation. Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Fig. 1). In each plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Pratama et al., 2021b; Suwandi et al., 2021). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *L. domesticum* which showed symptoms of branch wilting,

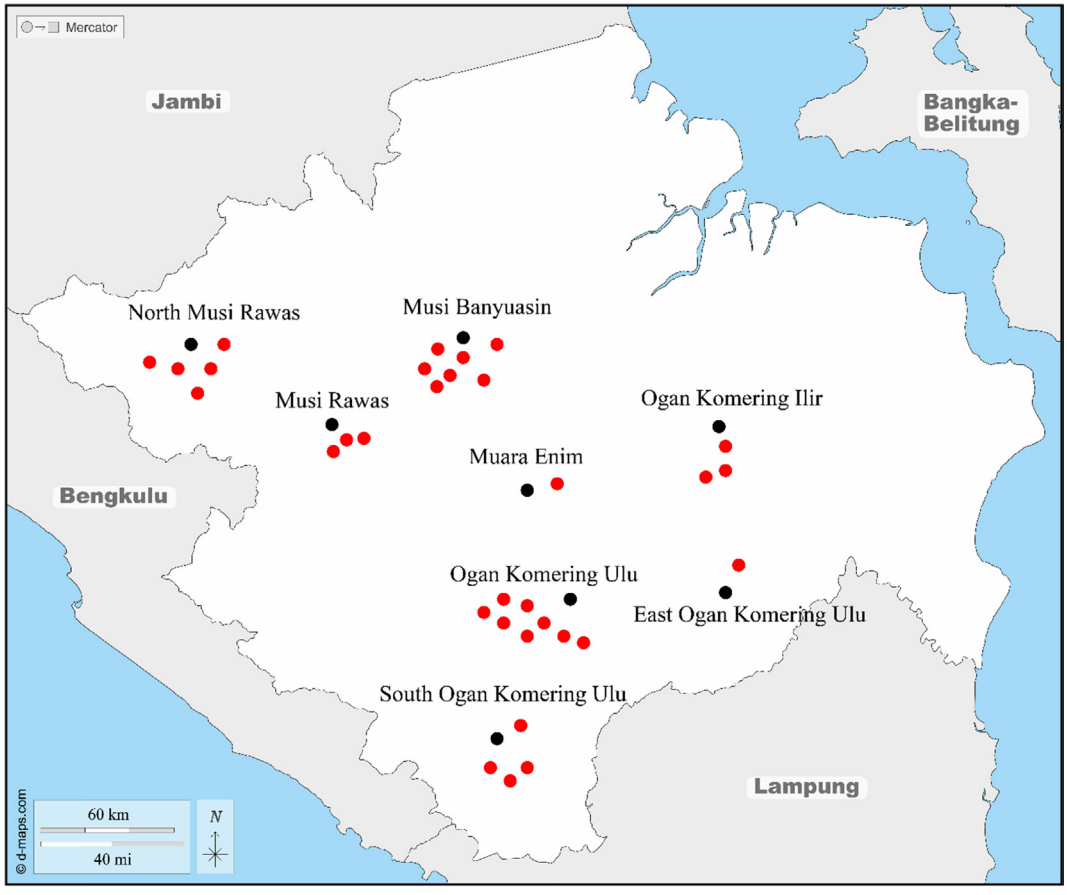

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of *Ceratocystis* was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 min, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on malt extract agar (MEA) media at room temperature (25°C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% MEA (20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization. The morphological characteristics of the observed fungi were represented by isolates originating from eight regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture. To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of eight isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30°C with an interval of 5°C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses. The pure cultures used for the DNA extraction were 14 isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

Amplification and polymerase chain reaction (PCR) sequencing were obtained from two gene regions, namely beta-tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq-DNA-polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were initial denaturation for 3 min at 94°C, 30 cycles for 30 seconds at 94°C for 30 s, for 30 s at 52°C, and 1 min at 72°C for. Amplification was completed at 72°C for 10 min and the PCR prod-

uct was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, MD, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For MP analysis, the metrics calculated included tree length, retention index, and consistency index. Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials. These studies were conducted using ten isolates of *C. fimbriata*. The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2-3 cm and a height of 50-60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *A. mangium*, *A. carsicarpa*, *E. urophylla*, *D. costulata*, *H. brasiliensis*, *A. scholaris*, and *M. cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2-3 cm and a height of 70-80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on

Table 1. Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (*n* = 89)	53.9	64	85.4
Saleman (*n* = 74)	41.9	58.1	95.9
Singapura (*n* = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (*n* = 91)	59.3	72.5	84.6
Tebat Agung (*n* = 67)	10.5	16.4	31.3
Padang Bindu (*n* = 71)	5.6	15.5	19.7
Kepayang (*n* = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package (SAS Institute Inc., Cary, NC, USA). Furthermore, the analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey's honestly significant difference) test was used to determine the significant differences in the mean comparisons of the different treatments.

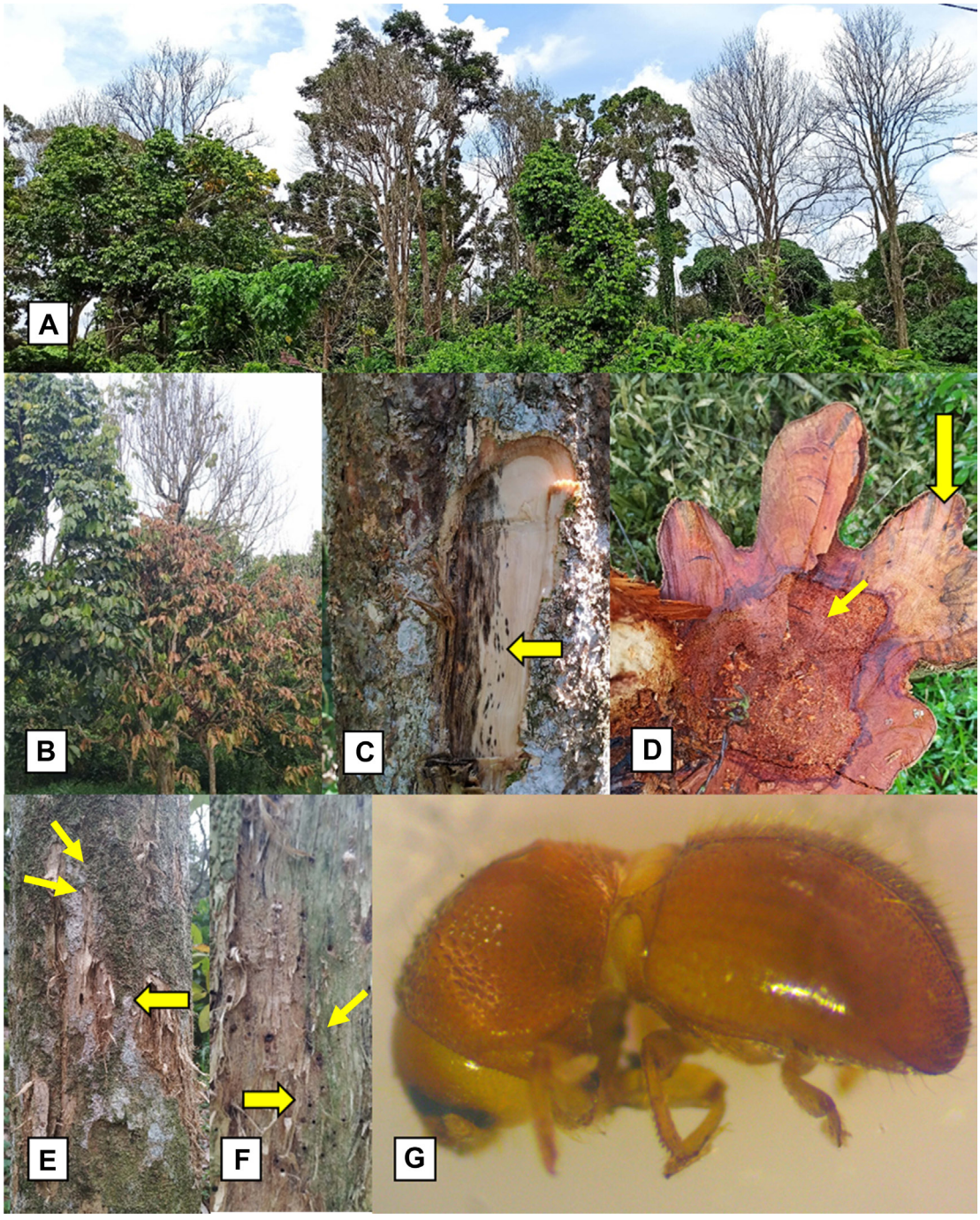

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. (A, B) Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. (C, D) Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrows). (E) Squirrel bite caused peeled-off bark on diseased tree (yellow arrows). (F) A beetle hole on affected diseased wood (yellow arrow). (G) *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

Results and Discussion

Diseases incidence, Sample collection, and fungal isolation. *Ceratocystis* wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu District, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Table 1). It has spread widely in other plantations in the Ogan Komering Ulu District covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya (Table 1).

Table 2. Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood onto the malt extract agar from samples collected from dying *Lansium domesticum* trees in Ogan Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*, *n* (%)
Ogan Komering Ulu (26/40, 65%)	Kartamulya	2019	2/5 (40)
	Saleman	2019	5/5 (100)
	Singapura	2019	2/5 (40)
	Pengaringan	2020	5/5 (100)
	Reksa Jiwa	2020	2/5 (40)
	Tebat Agung	2020	3/5 (60)
	Padang Bindu	2020	2/5 (40)
	Kepayang	2020	5/5 (100)
East Ogan Komering Ulu (4/5, 80%)	Bantan Pelita	2021	4/5 (80)
South Ogan Komering Ulu (14/25, 56%)	Simpang	2021	4/5 (80)
	Tanjung Sari	2021	2/5 (40)
	Tanjung Beringin	2021	4/5 (80)
		2021	2/5 (40)
	Kisau	2021	2/5 (40)
Ogan Komering Ilir (8/15, 53.3%)	Penyandingan	2020	3/5 (60)
	Ulak Kemang	2020	3/5 (60)
	Tanjung Lubuk	2020	2/5 (40)
Musi Banyuasin (16/30, 53.3%)	Kasmaran	2021	1/5 (20)
	Babat Toman	2021	2/5 (40)
	Beruge	2021	1/5 (20)
	Sereka	2021	2/5 (40)
	Sanga Desa	2021	5/5 (100)
	Tanjung Raya	2021	5/5 (100)
Musi Rawas (12/15, 80%)	Tuah Negri	2021	4/5 (80)
	Mambang	2021	5/5 (100)
	Lubuk Tuo	2021	3/5 (60)
North Musi Rawas (16/25, 64%)	Beringin Jaya	2021	3/5 (60)
	Lawang Agung	2021	5/5 (100)
	Karang Waru	2021	3/5 (60)
	Rantau Kadam	2021	3/5 (60)
	Lesung Batu	2021	2/5 (40)
Muara Enim (3/5, 60%)	Ujan mas	2020	3/5 (60)

From 2020 to 2021, there were similar disease incidences on the duku plantations in Ogan Komering Ilir, within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively (Table 1). Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Fig. 2A and B). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Fig. 2C and D). There is a wound on the diseased tree caused by a squirrel scratch (Fig. 2E). In general, holes will appear on the

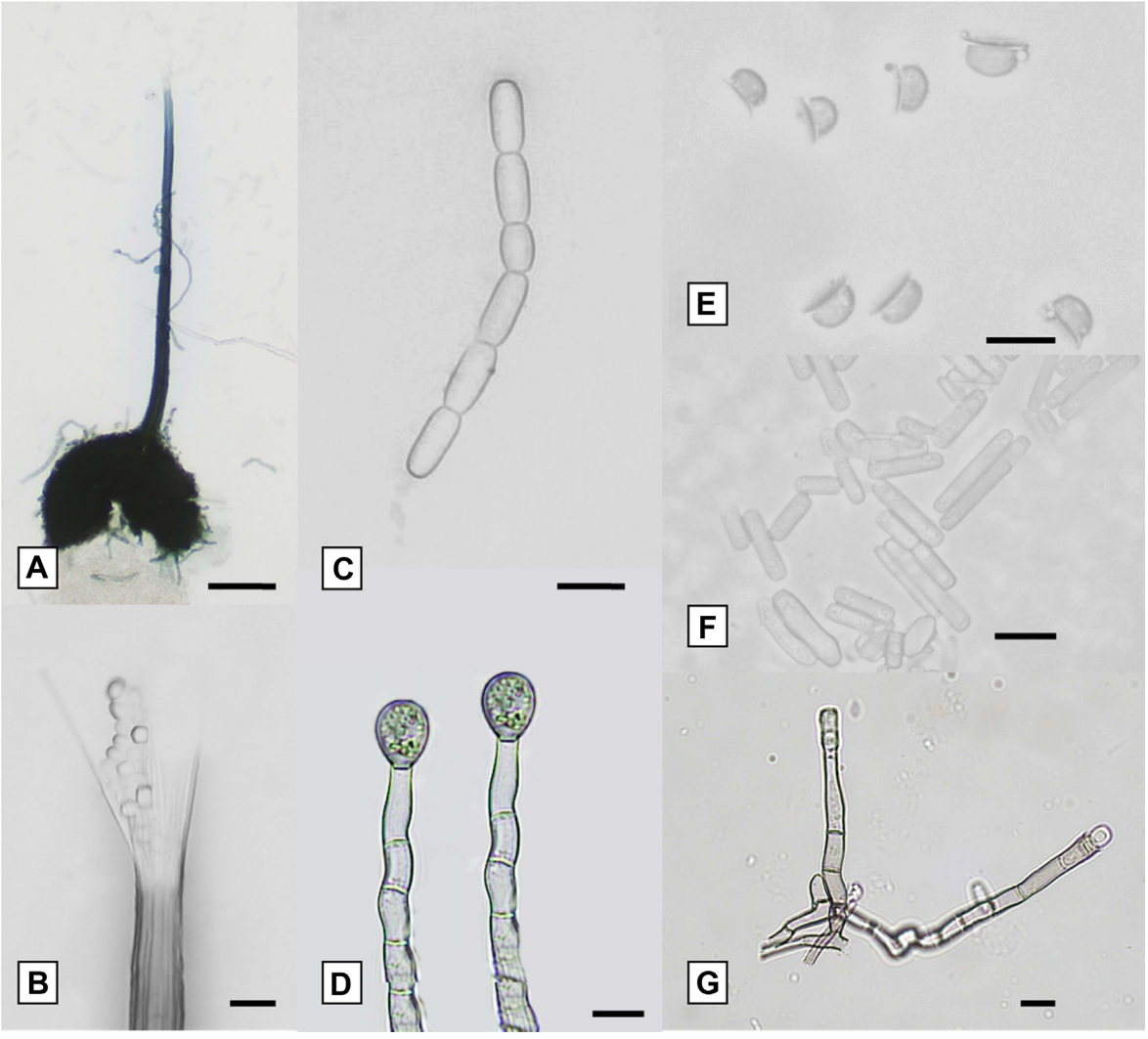

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: (A) globose ascomata with a long neck, (B) divergent ostiolar hyphae, (C) barrel-shaped conidia, (D) chlamydospores, (E) hat-shaped ascospores, (F) cylindrical conidia, and (G) conidiophore/phialide. Scale bars: A = 100 µm, B-E = 10 µm, F = 5 µm.

infected duku stem caused by *Hypocryphalus mangiferae* (Fig. 2F) which is a vector insect for *Ceratocystis* (Fig. 2G).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80%, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192 from Ogan Komering Ulu, CAL32159, CAL32156, CAL32157, and CAL32158 from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the

Table 3. Morphology of selected *Ceratocystis fimbriata* isolates from a different district in South Sumatra

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3-312.4	122.9-291.4	135.7-325.2	141.3-317.1	137.9-321.1	132.1-334.9	137.9-346.1	122.1-316.9
Ascomatal base (l)	153.1-404.4	131-315.4	148.1-398.4	151.1-411.4	143.1-398.4	152.4-394.1	139.1-421.8	157.1-412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4-768.4	354.9-677.7	413.7-798.8	439.9-736.4	475.8-813.6	484.6-790.9	463.8-723.6	484.6-780.9
Neck (w) top	11.5-26.8	7.06-18.4	11.3-21.9	11.1-25.4	10.1-17.9	11.3-21.7	11.1-22.9	11.3-21.7
Neck (w) bottom	24.8-47.9	20.3-39.7	23.6-42.6	22.6-51.2	23.7-43.8	22.67-42.9	23.7-43.6	22.67-44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2-43.5	30.4-40.1	32.7-44.7	32.7-42.2	33.5-43.9	33.7-44.8	33.5-42.9	31.7-44.8
Ascospores								
Hat-shaped ascospores (l)	3.4-5.7	3.3-5.2	3.2-5.4	3.4-4.9	3.2-4.4	3.1-5.1	3.1-4.3	3.3-4.9
Ascospores (w) without sheath	3.4-5.1	3.1-4.1	3.3-4.7	3.4-4.4	3.3-4.1	3.4-4.5	3.3-4.1	3.5-4.4
Ascospores (w) with sheath	5-6.8	4.1-6.1	5.1-6.7	5.3-6.4	5.2-6.5	5.5-6.7	5.2-6.3	5.4-6.6
Primary conidia (l)	12.1-27.5	10.6-18.9	13.8-23.8	12.2-29.3	13.2-25.7	14.9-24.8	12.5-21.6	13.7-24.6
Primary conidia (w)	3.5-7.4	3.2-4.3	3.1-5.1	3.4-4.1	3.2-5.1	3.4 -4.4	3.4-4.1	3.5-4.7
Secondary conidia (l)	6.3-11.6	5.7-10.1	6.6-11.8	7.9-11.8	6.7-11.9	6.8-11.5	6.5-11.5	6.2-11.3
Secondary conidia (w)	4.5-7.6	4.1-7.4	4.7-7.5	5.6-7.9	4.3-7.8	4.3-7.8	4.3-7.1	4.1-7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7-15.1	8.7-15.1	11.3-15.6	9.7-17.8	10.7-15.4	10.1-16.5	10.3-14.6	10.4-14.5
Chlamydospores (w)	7.9-13.9	8.3-11.1	6.9-14.2	6.8-13.6	7.6-11.8	7.7-12.5	7.6-11.8	7.6-12.9
Culture growth rate[b]								
10°C	0	0	0	0	0	0	0	0
15°C	3.3-3.5	2.2-2.5	3.2-3.5	2.2-2.7	3.2-3.4	2.2-2.8	2.3-2.9	2.4-2.8
20°C	3.2-3.7	3.1-2.9	3.2-3.9	3.3-3.9	4.2-4.4	3.2-3.5	4.2-4.4	3.2-3.5
25°C	5.1-5.3	4.1-4.5	4.7-5.1	4.4-4.7	4.4-4.9	4.1-4.5	4.4-4.9	4.1-4.5
30°C	3.3-3.6	3.1-3.9	3.5-4.6	3.5-4.2	3.8-4.2	3.1-3.4	3.8-4.2	3.1-3.4

[a]All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm.
[b]Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after 14 days.

Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Morphological characterization and growth in culture. The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Fig. 3A) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Fig. 3B). This fungus also produced chained barrel-shaped conidia (Fig. 3C), and chlamydospores (Fig. 3D), it also had hat-shaped ascospores (Fig. 3E). Cylindrical conidia (Fig. 3G) were generated from the primary phialidic conidiophore (Fig. 3F).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *M. indica* (Van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *A. mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Table 3).

DNA extraction, amplification, sequencing, and phylogenetic analyses. For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary Table 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences (Fig. 4). Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata* (Fig. 5).

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the MP method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis*, *Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia. The phylogeny was assessed and analyzed using bootstrap analysis with 1,000 replications,

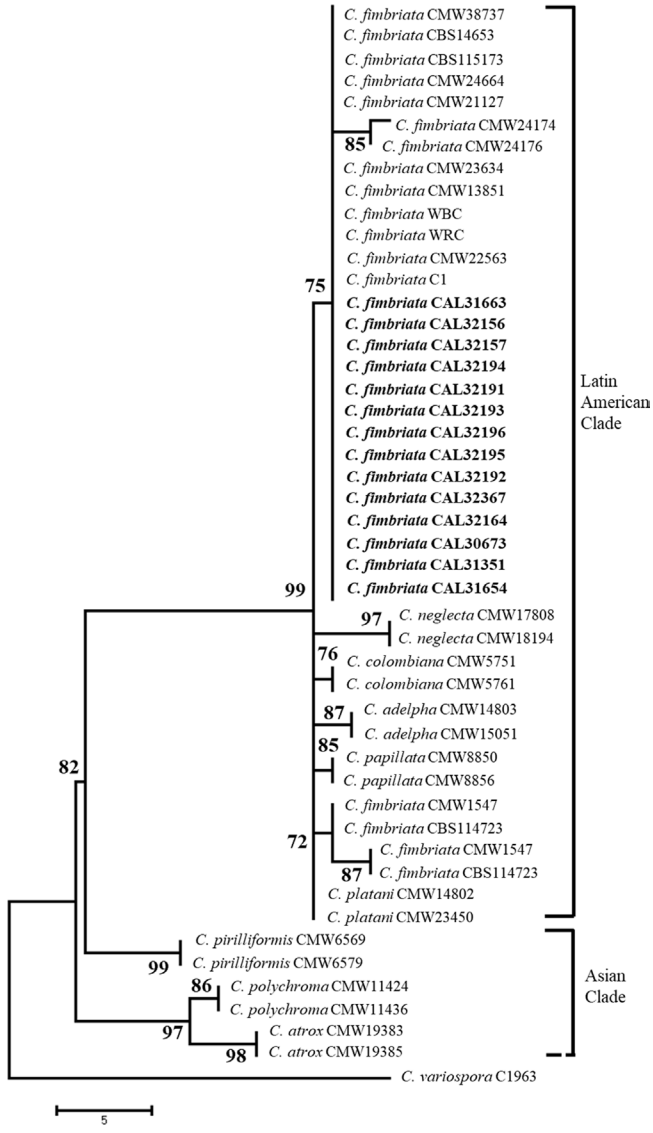

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials. *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the bundle

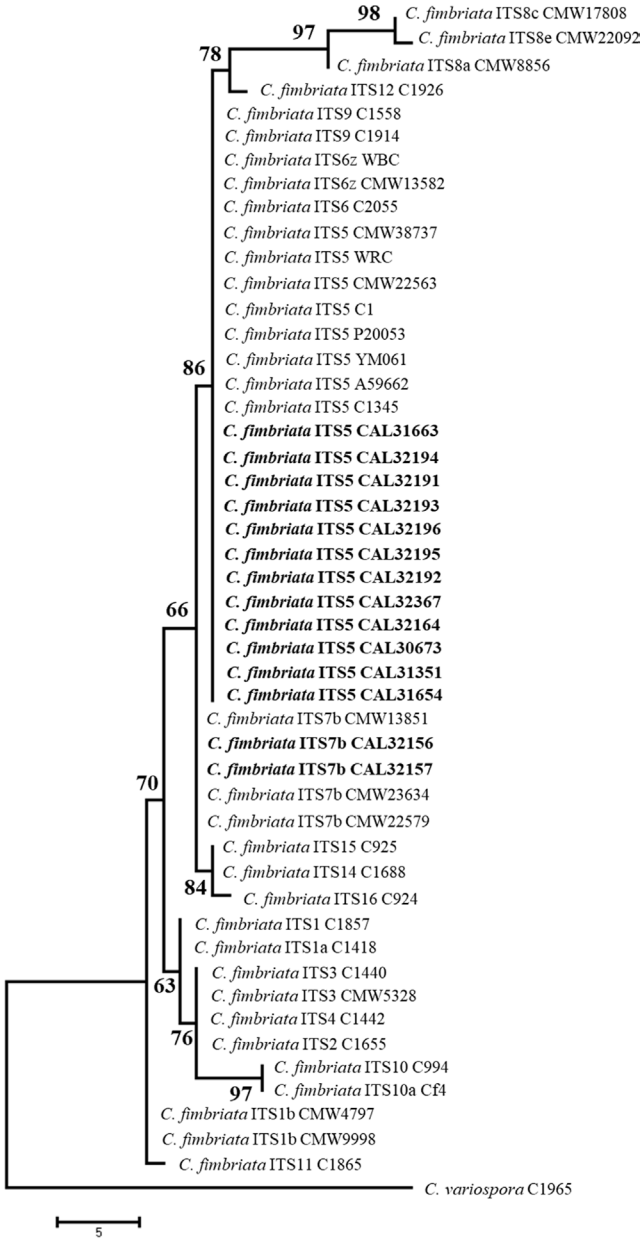

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

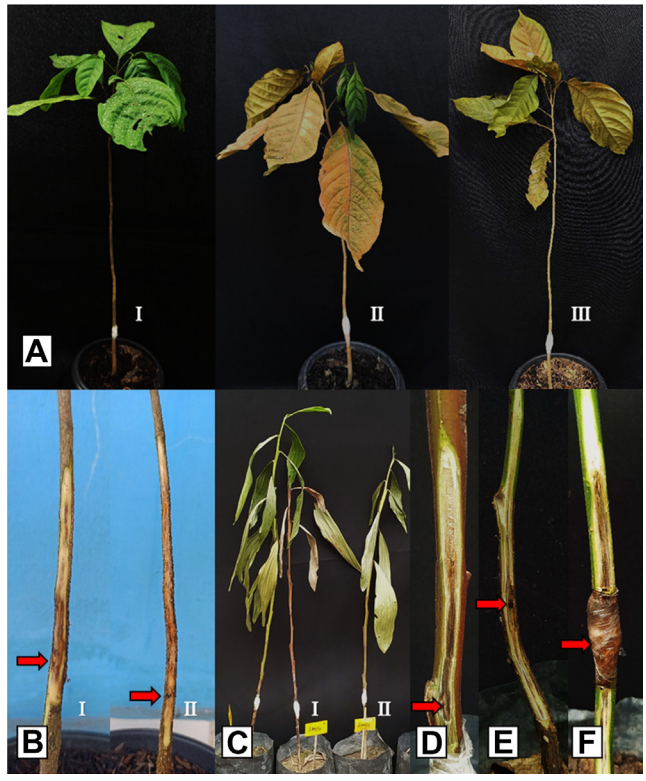

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. (A) Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (I) and CAL32159 (II). (C, D) 4-month-old Acacia plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (I) and CAL32159 (II). (E) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. (F) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

vessels, whereby 90% and 100% of it dies 45 days, as well as 70 days after pathogen inoculation respectively (Fig. 6A and B). ANOVA for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings (Fig. 6C-F), and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (Table 5). There was

Table 4. Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery condition

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35 f	7/10	10/10
CAL32157	10	15.49 ef	7/10	8/10
CAL32158	10	12.29 cd	5/10	5/10
CAL32159	10	11.02 c	2/10	5/10
CAL32191	10	11.73 cd	2/10	3/10
CAL32192	10	13.83 def	7/10	8/10
CAL32193	10	19.81 g	9/10	10/10
CAL32194	10	6.86 b	2/10	2/10
CAL32195	10	12.89 cde	5/10	6/10
CAL32196	10	11.19 cde	5/10	7/10
Control (MEA)	10	0.01 a	0/10	0/10
P-value		<0.001		

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.

no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa*, *E. urophylla*, and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata*, *H. brasiliensis*, and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation) (Table 5).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The re-isolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006, 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to

Table 5. Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*			*Dyera costulata*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25 ef	10/10	10/10	9.86 de	0/10	1/10	11.32 b	0/10	1/10	4.25b	0/10	0/10
CAL32157	10	16.32 de	10/10	10/10	10.16 de	0/10	2/10	11.81 b	0/10	1/10	3.91b	0/10	0/10
CAL32158	10	14.49 cde	8/10	10/10	9.39 cd	0/10	1/10	9.33 b	0/10	0/10	3.63b	0/10	0/10
CAL32159	10	13.59 bcd	8/10	10/10	8.26 bcd	0/10	1/10	9.86 b	0/10	0/10	3.83b	0/10	0/10
CAL32191	10	11.73 bc	7/10	10/10	7.96 bcd	0/10	0/10	9.82 b	0/10	0/10	3.57b	0/10	0/10
CAL32192	10	15.54 cde	10/10	10/10	6.57 bc	0/10	0/10	10.59 b	0/10	0/10	5.15b	0/10	0/10
CAL32193	10	20.93 f	10/10	10/10	12.59 e	0/10	5/10	11.92 b	0/10	3/10	5.39b	0/10	0/10
CAL32194	10	9.943 b	5/10	10/10	5.97 b	0/10	0/10	8.80 b	0/10	0/10	3.05b	0/10	0/10
CAL32195	10	15.39 cde	9/10	10/10	7.82 bcd	0/10	2/10	11.20 b	0/10	2/10	4.02b	0/10	0/10
CAL32196	10	14.64 cde	8/10	10/10	8.64 bcd	0/10	1/10	11.15 b	0/10	1/10	3.60b	0/10	0/10
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01a	0/10	0/10
P-value		<0.001			<0.001			<0.001			<0.001		
		Hevea brasiliensis			*Alstonia scholaris*			*Melaleuca leucadendra*					
CAL32156	10	5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10			
CAL32157	10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10			
CAL32158	10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10			
CAL32159	10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10			
CAL32191	10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10			
CAL32192	10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10			
CAL32193	10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10			
CAL32194	10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10			
CAL32195	10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10			
CAL32196	10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10			
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10			
P-value		<0.001			<0.001			<0.001					

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.
dpi, days post inoculation.

the stem, which is spread by squirrels and insects. This disease also occur from the root and continues up to the base of the stem. The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities to *C.*

fimbriata which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were two variations of the ITS rDNA sequence from two isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin District. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Pratama et al., 2021a, 2021b; Tarigan et al., 2011). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *L. domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010, 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *C. esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa*, *E. urophylla*, and *M. cajuputi*, as well as weakly pathogenic to *D. costulata*, *A. scholaris*, and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *L. domesticum* caused by *C. fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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11. Bukti konfirmasi submit proof corrections, respon kepada editor, dan artikel yang proof corrections kedua

(27 Maret 2022)

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PPJ 2021-0182: Final Proof Corrections

a. muslim unsri <a_muslim@unsri.ac.id> Sun, Mar 27, 2022 at 10:48 PM
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Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email on March 26, 2022 regarding additional editing for figure legends and figure number

We have checked and revised our galley proofs indicated by highlighting each revision including some minor mistake in Phone and FAX number made in PDF file.

Below is a summary of our changes made in response to the editor's comments and Revised Phone and FAX number.

1. **Editor's comment:** p.7: Please provide a description of scale bar in 3G (page 7).
Our response: Thank you very much. We agree and the description of scale bar sentence has been changed to be "Scale bars: A = 100 µm, B-E = 10 µm, F = 5 µm, G = 10 µm."

2. **Editor's comment:** p.9: Please check citation of 3G and 3F.
Our response: Thank you very much. We agree and the sentence has been changed to be "Cylindrical conidia (Fig. 3F) were generated from the primary phialidic conidiophore (Fig. 3G)."

3. **Besides Editor's comment/correction above.** We would like also to revise some minor mistake in Phone number and FAX number. The correct Phone number and FAX number are: Phone) +62-711-580059, FAX) +62-711-580276.

Please address all correspondence concerning this manuscript to me at: a_muslim@unsri.ac.id
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Please feel free to contact me if you need any additional information or clarification. Thank you very much for your excellent cooperation

Sincerely,
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2 attachments

Revision of Galley Proofs.pdf
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Prof. Yoonjin Kim
Editorial Office
The Plant Pathology Journal (PPJ)

Dear Prof. Yoonjin Kim,

Thank you very much for your email on March 26, 2022 regarding additional editing for figure legends and figure number

We have checked and revised our galley proofs indicated by highlighting each revision including some minor mistake in Phone and FAX number made in PDF file.

Below is a summary of our changes made in response to the editor's comments and Revised Phone and FAX number.

1. **Editor's comment:** p.7: Please provide a description of scale bar in 3G (page 7).
Our response: Thank you very much. We agree and the description of scale bar sentence has been changed to be "Scale bars: A = 100 μm, B-E = 10 μm, F = 5 μm, G = 10 μm."

2. **Editor's comment:** p.9: Please check citation of 3G and 3F.
Our response: Thank you very much. We agree and the sentence has been changed to be "Cylindrical conidia (Fig. 3F) were generated from the primary phialidic conidiophore (Fig. 3G)."

3. **Besides Editor's comment/correction above.** We would like also to revise some minor mistake in Phone number and FAX number. The correct Phone number and FAX number are: Phone) +62-711-580059, FAX) +62-711-580276.

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Please feel free to contact me if you need any additional information or clarification. Thank you very much for your excellent cooperation

Sincerely,
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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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***Ceratocystis* wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.**

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Keywords **:** agroforestry plants, canker, *Certocystis fimbriata*, die-back disease

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8,14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum* Corr. has also been reported to have benefits as larvicides (Ni'mah et al., 2015; Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria* spp. (Norhayati et al., 2016), organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Subandrate et al., 2016; Tilaar et al., 2008).

Previous studies conducted from 2014 to 2017 (Suwandi

et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in three locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis,* and *Chukrasia tabularis* (Chi et al., 2019a, 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa*, *Eucalyptus urophylla*, *Dyera costulata*, *Alstonia scholaris*, *Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Materials and Methods

Diseases incidence, sample collection, and fungal isolation. Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Fig. 1). In each plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Pratama et al., 2021b; Suwandi et al., 2021). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *L. domesticum* which showed symptoms of branch wilting,

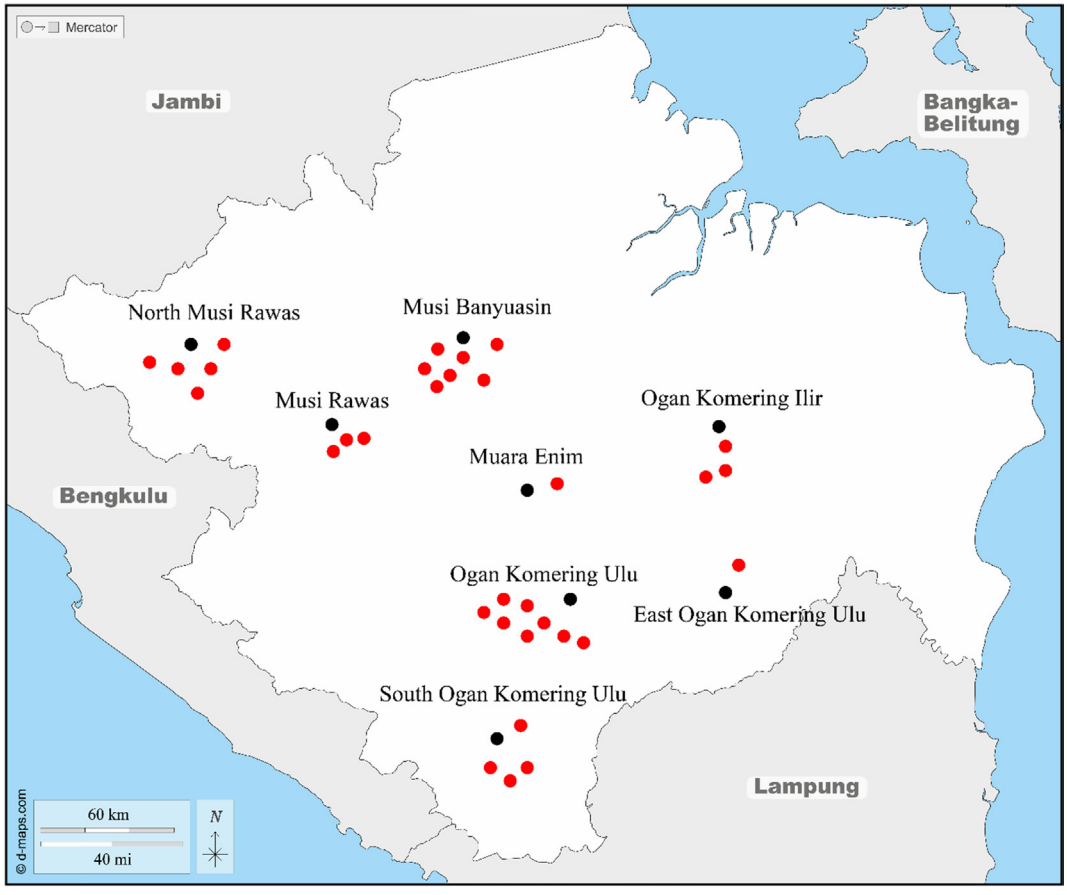

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of *Ceratocystis* was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 min, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on malt extract agar (MEA) media at room temperature (25°C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% MEA (20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization. The morphological characteristics of the observed fungi were represented by isolates originating from eight regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture. To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of eight isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30°C with an interval of 5°C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses. The pure cultures used for the DNA extraction were 14 isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

Amplification and polymerase chain reaction (PCR) sequencing were obtained from two gene regions, namely beta-tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq-DNA-polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were initial denaturation for 3 min at 94°C, 30 cycles for 30 seconds at 94°C for 30 s, for 30 s at 52°C, and 1 min at 72°C for. Amplification was completed at 72°C for 10 min and the PCR prod-

uct was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, MD, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For MP analysis, the metrics calculated included tree length, retention index, and consistency index. Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials. These studies were conducted using ten isolates of *C. fimbriata*. The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2-3 cm and a height of 50-60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *A. mangium*, *A. carsicarpa*, *E. urophylla*, *D. costulata*, *H. brasiliensis*, *A. scholaris*, and *M. cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2-3 cm and a height of 70-80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on

Table 1. Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (*n* = 89)	53.9	64	85.4
Saleman (*n* = 74)	41.9	58.1	95.9
Singapura (*n* = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (*n* = 91)	59.3	72.5	84.6
Tebat Agung (*n* = 67)	10.5	16.4	31.3
Padang Bindu (*n* = 71)	5.6	15.5	19.7
Kepayang (*n* = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package (SAS Institute Inc., Cary, NC, USA). Furthermore, the analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey's honestly significant difference) test was used to determine the significant differences in the mean comparisons of the different treatments.

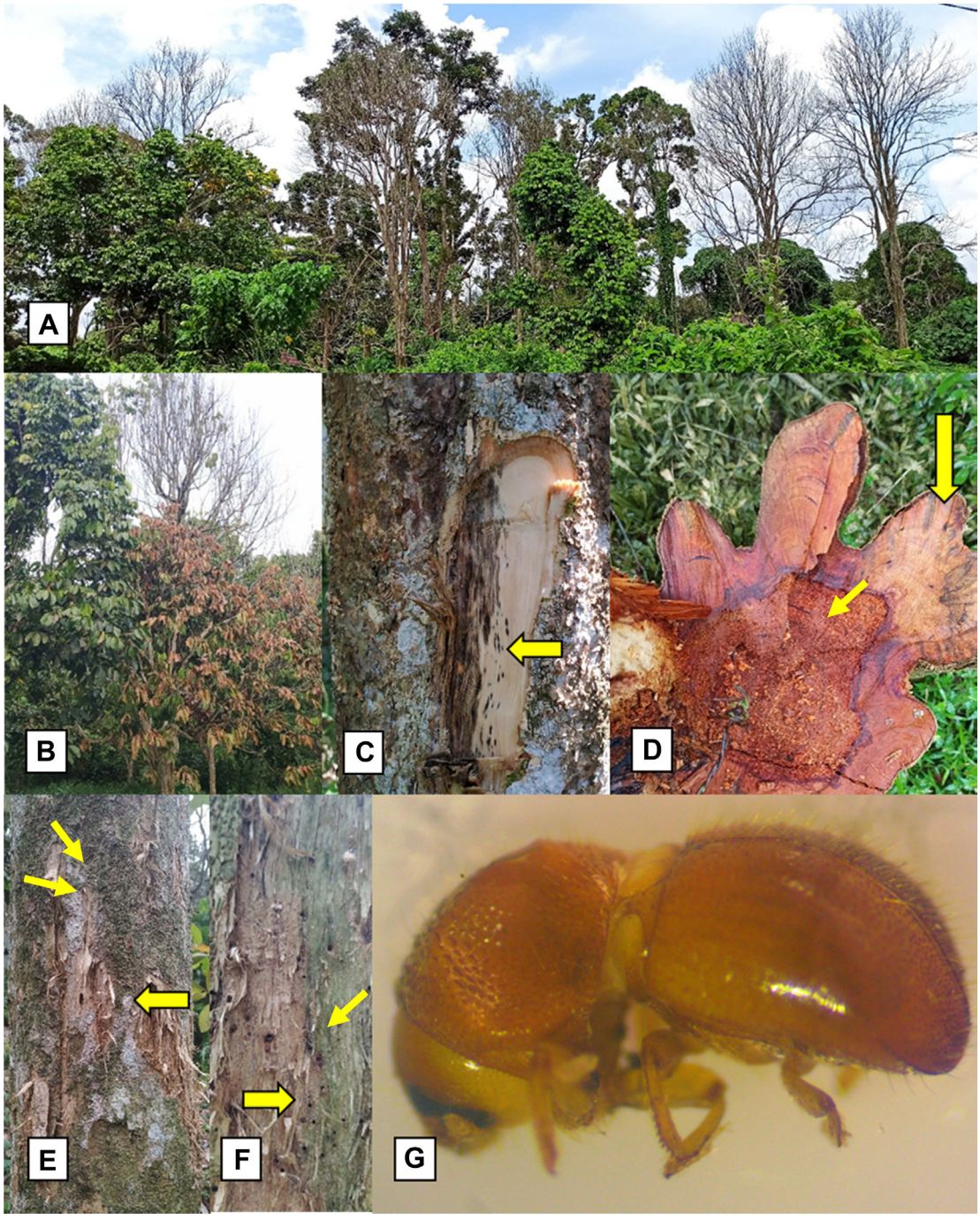

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. (A, B) Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. (C, D) Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrows). (E) Squirrel bite caused peeled-off bark on diseased tree (yellow arrows). (F) A beetle hole on affected diseased wood (yellow arrow). (G) *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

Results and Discussion

Diseases incidence, Sample collection, and fungal isolation. *Ceratocystis* wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu District, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Table 1). It has spread widely in other plantations in the Ogan Komering Ulu District covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya (Table 1).

Table 2. Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood onto the malt extract agar from samples collected from dying *Lansium domesticum* trees in Ogan Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*, *n* (%)
Ogan Komering Ulu (26/40, 65%)	Kartamulya	2019	2/5 (40)
	Saleman	2019	5/5 (100)
	Singapura	2019	2/5 (40)
	Pengaringan	2020	5/5 (100)
	Reksa Jiwa	2020	2/5 (40)
	Tebat Agung	2020	3/5 (60)
	Padang Bindu	2020	2/5 (40)
	Kepayang	2020	5/5 (100)
East Ogan Komering Ulu (4/5, 80%)	Bantan Pelita	2021	4/5 (80)
South Ogan Komering Ulu (14/25, 56%)	Simpang	2021	4/5 (80)
	Tanjung Sari	2021	2/5 (40)
	Tanjung Beringin	2021	4/5 (80)
		2021	2/5 (40)
	Kisau	2021	2/5 (40)
Ogan Komering Ilir (8/15, 53.3%)	Penyandingan	2020	3/5 (60)
	Ulak Kemang	2020	3/5 (60)
	Tanjung Lubuk	2020	2/5 (40)
Musi Banyuasin (16/30, 53.3%)	Kasmaran	2021	1/5 (20)
	Babat Toman	2021	2/5 (40)
	Beruge	2021	1/5 (20)
	Sereka	2021	2/5 (40)
	Sanga Desa	2021	5/5 (100)
	Tanjung Raya	2021	5/5 (100)
Musi Rawas (12/15, 80%)	Tuah Negri	2021	4/5 (80)
	Mambang	2021	5/5 (100)
	Lubuk Tuo	2021	3/5 (60)
North Musi Rawas (16/25, 64%)	Beringin Jaya	2021	3/5 (60)
	Lawang Agung	2021	5/5 (100)
	Karang Waru	2021	3/5 (60)
	Rantau Kadam	2021	3/5 (60)
	Lesung Batu	2021	2/5 (40)
Muara Enim (3/5, 60%)	Ujan mas	2020	3/5 (60)

From 2020 to 2021, there were similar disease incidences on the duku plantations in Ogan Komering Ilir, within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively (Table 1). Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Fig. 2A and B). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Fig. 2C and D). There is a wound on the diseased tree caused by a squirrel scratch (Fig. 2E). In general, holes will appear on the

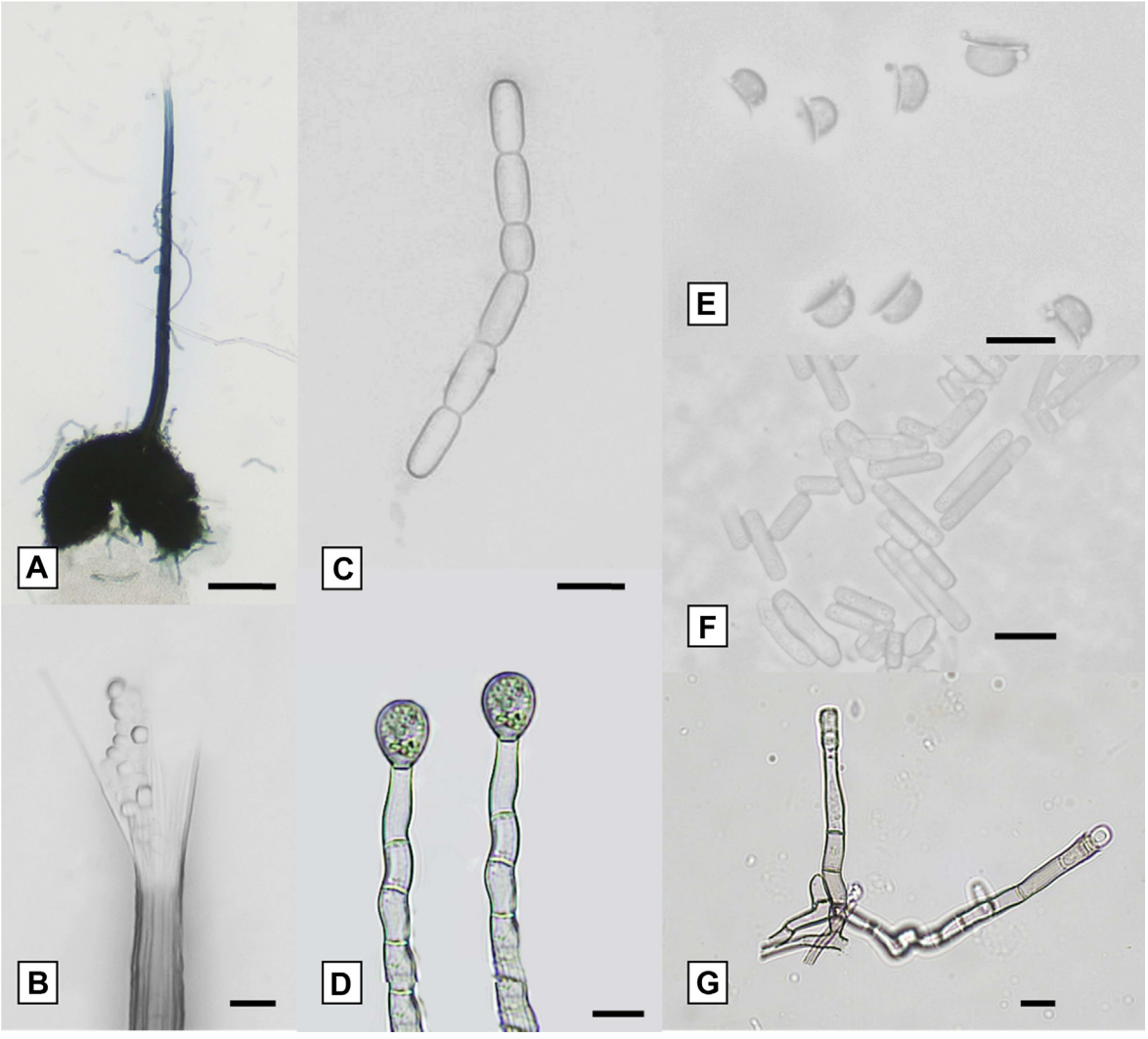

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: (A) globose ascomata with a long neck, (B) divergent ostiolar hyphae, (C) barrel-shaped conidia, (D) chlamydospores, (E) hat-shaped ascospores, (F) cylindrical conidia, and (G) conidiophore/phialide. Scale bars: A = 100 µm, B-E = 10 µm, F = 5 µm.

infected duku stem caused by *Hypocryphalus mangiferae* (Fig. 2F) which is a vector insect for *Ceratocystis* (Fig. 2G).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80%, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192 from Ogan Komering Ulu, CAL32159, CAL32156, CAL32157, and CAL32158 from Musi Banyuasin, CAL32164 from South Ogan Komering Ulu, CAL32367 from East Ogan Komering Ulu, CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas, CAL30673 from Ogan Komering Ilir, and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the

Table 3. Morphology of selected *Ceratocystis fimbriata* isolates from a different district in South Sumatra

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3-312.4	122.9-291.4	135.7-325.2	141.3-317.1	137.9-321.1	132.1-334.9	137.9-346.1	122.1-316.9
Ascomatal base (l)	153.1-404.4	131-315.4	148.1-398.4	151.1-411.4	143.1-398.4	152.4-394.1	139.1-421.8	157.1-412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4-768.4	354.9-677.7	413.7-798.8	439.9-736.4	475.8-813.6	484.6-790.9	463.8-723.6	484.6-780.9
Neck (w) top	11.5-26.8	7.06-18.4	11.3-21.9	11.1-25.4	10.1-17.9	11.3-21.7	11.1-22.9	11.3-21.7
Neck (w) bottom	24.8-47.9	20.3-39.7	23.6-42.6	22.6-51.2	23.7-43.8	22.67-42.9	23.7-43.6	22.67-44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2-43.5	30.4-40.1	32.7-44.7	32.7-42.2	33.5-43.9	33.7-44.8	33.5-42.9	31.7-44.8
Ascospores								
Hat-shaped ascospores (l)	3.4-5.7	3.3-5.2	3.2-5.4	3.4-4.9	3.2-4.4	3.1-5.1	3.1-4.3	3.3-4.9
Ascospores (w) without sheath	3.4-5.1	3.1-4.1	3.3-4.7	3.4-4.4	3.3-4.1	3.4-4.5	3.3-4.1	3.5-4.4
Ascospores (w) with sheath	5-6.8	4.1-6.1	5.1-6.7	5.3-6.4	5.2-6.5	5.5-6.7	5.2-6.3	5.4-6.6
Primary conidia (l)	12.1-27.5	10.6-18.9	13.8-23.8	12.2-29.3	13.2-25.7	14.9-24.8	12.5-21.6	13.7-24.6
Primary conidia (w)	3.5-7.4	3.2-4.3	3.1-5.1	3.4-4.1	3.2-5.1	3.4 -4.4	3.4-4.1	3.5-4.7
Secondary conidia (l)	6.3-11.6	5.7-10.1	6.6-11.8	7.9-11.8	6.7-11.9	6.8-11.5	6.5-11.5	6.2-11.3
Secondary conidia (w)	4.5-7.6	4.1-7.4	4.7-7.5	5.6-7.9	4.3-7.8	4.3-7.8	4.3-7.1	4.1-7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7-15.1	8.7-15.1	11.3-15.6	9.7-17.8	10.7-15.4	10.1-16.5	10.3-14.6	10.4-14.5
Chlamydospores (w)	7.9-13.9	8.3-11.1	6.9-14.2	6.8-13.6	7.6-11.8	7.7-12.5	7.6-11.8	7.6-12.9
Culture growth rate[b]								
10°C	0	0	0	0	0	0	0	0
15°C	3.3-3.5	2.2-2.5	3.2-3.5	2.2-2.7	3.2-3.4	2.2-2.8	2.3-2.9	2.4-2.8
20°C	3.2-3.7	3.1-2.9	3.2-3.9	3.3-3.9	4.2-4.4	3.2-3.5	4.2-4.4	3.2-3.5
25°C	5.1-5.3	4.1-4.5	4.7-5.1	4.4-4.7	4.4-4.9	4.1-4.5	4.4-4.9	4.1-4.5
30°C	3.3-3.6	3.1-3.9	3.5-4.6	3.5-4.2	3.8-4.2	3.1-3.4	3.8-4.2	3.1-3.4

[a]All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm.
[b]Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after 14 days.

Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Morphological characterization and growth in culture. The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Fig. 3A) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Fig. 3B). This fungus also produced chained barrel-shaped conidia (Fig. 3C), and chlamydospores (Fig. 3D), it also had hat-shaped ascospores (Fig. 3E). Cylindrical conidia (Fig. 3G) were generated from the primary phialidic conidiophore (Fig. 3F).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *M. indica* (Van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *A. mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Table 3).

DNA extraction, amplification, sequencing, and phylogenetic analyses. For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary Table 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences (Fig. 4). Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata* (Fig. 5).

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the MP method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis*, *Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia. The phylogeny was assessed and analyzed using bootstrap analysis with 1,000 replications,

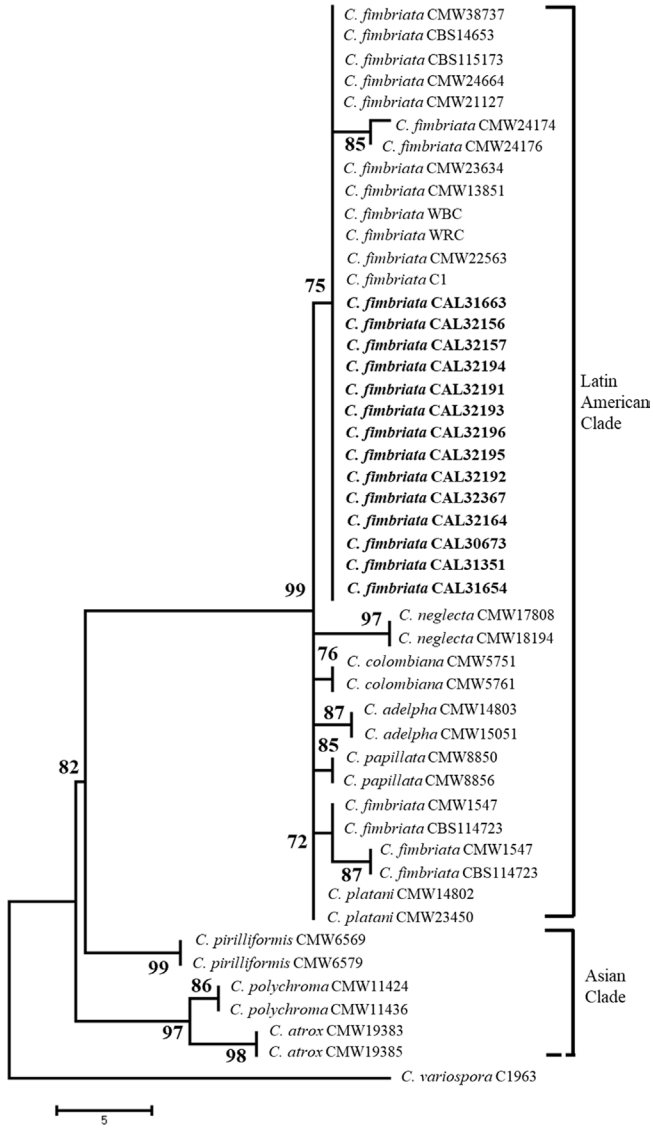

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials. *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the bundle

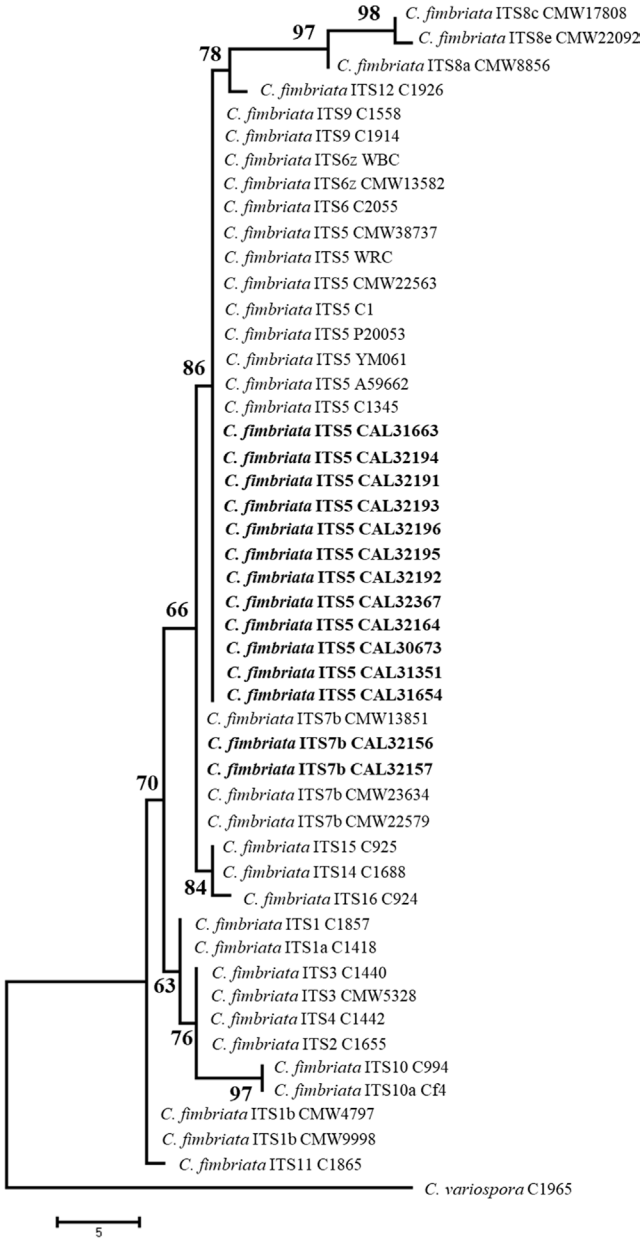

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

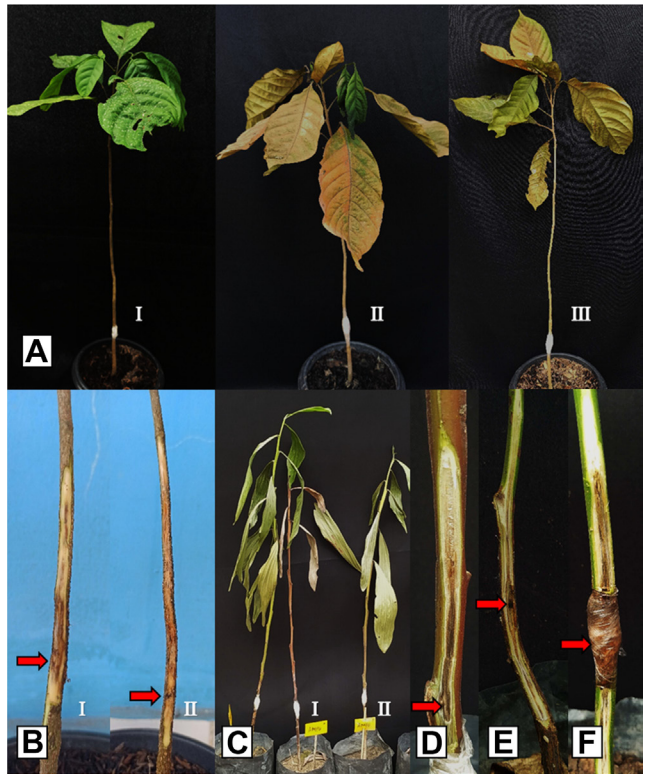

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. (A) Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (I) and CAL32159 (II). (C, D) 4-month-old Acacia plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (I) and CAL32159 (II). (E) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. (F) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

vessels, whereby 90% and 100% of it dies 45 days, as well as 70 days after pathogen inoculation respectively (Fig. 6A and B). ANOVA for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings (Fig. 6C-F), and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (Table 5). There was

Table 4. Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery condition

Isolates	Host test	*Lansium domesticum*		
		Lesion length (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35 f	7/10	10/10
CAL32157	10	15.49 ef	7/10	8/10
CAL32158	10	12.29 cd	5/10	5/10
CAL32159	10	11.02 c	2/10	5/10
CAL32191	10	11.73 cd	2/10	3/10
CAL32192	10	13.83 def	7/10	8/10
CAL32193	10	19.81 g	9/10	10/10
CAL32194	10	6.86 b	2/10	2/10
CAL32195	10	12.89 cde	5/10	6/10
CAL32196	10	11.19 cde	5/10	7/10
Control (MEA)	10	0.01 a	0/10	0/10
P-value		<0.001		

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.

no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa*, *E. urophylla*, and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata*, *H. brasiliensis*, and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation) (Table 5).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The re-isolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006, 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to

Table 5. Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*			*Dyera costulata*		
		Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25 ef	10/10	10/10	9.86 de	0/10	1/10	11.32 b	0/10	1/10	4.25b	0/10	0/10
CAL32157	10	16.32 de	10/10	10/10	10.16 de	0/10	2/10	11.81 b	0/10	1/10	3.91b	0/10	0/10
CAL32158	10	14.49 cde	8/10	10/10	9.39 cd	0/10	1/10	9.33 b	0/10	0/10	3.63b	0/10	0/10
CAL32159	10	13.59 bcd	8/10	10/10	8.26 bcd	0/10	1/10	9.86 b	0/10	0/10	3.83b	0/10	0/10
CAL32191	10	11.73 bc	7/10	10/10	7.96 bcd	0/10	0/10	9.82 b	0/10	0/10	3.57b	0/10	0/10
CAL32192	10	15.54 cde	10/10	10/10	6.57 bc	0/10	0/10	10.59 b	0/10	0/10	5.15b	0/10	0/10
CAL32193	10	20.93 f	10/10	10/10	12.59 e	0/10	5/10	11.92 b	0/10	3/10	5.39b	0/10	0/10
CAL32194	10	9.943 b	5/10	10/10	5.97 b	0/10	0/10	8.80 b	0/10	0/10	3.05b	0/10	0/10
CAL32195	10	15.39 cde	9/10	10/10	7.82 bcd	0/10	2/10	11.20 b	0/10	2/10	4.02b	0/10	0/10
CAL32196	10	14.64 cde	8/10	10/10	8.64 bcd	0/10	1/10	11.15 b	0/10	1/10	3.60b	0/10	0/10
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01a	0/10	0/10
P-value		<0.001			<0.001			<0.001			<0.001		
		Hevea brasiliensis			*Alstonia scholaris*			*Melaleuca leucadendra*					
CAL32156	10	5.23e	0/10	0/10	5.21b	0/10	0/10	5.81e	0/10	2/10			
CAL32157	10	4.05de	0/10	0/10	4.75b	0/10	0/10	5.17de	0/10	2/10			
CAL32158	10	2.83bcd	0/10	0/10	3.70ab	0/10	0/10	3.15bc	0/10	0/10			
CAL32159	10	2.58bcd	0/10	0/10	3.50ab	0/10	0/10	2.63bc	0/10	0/10			
CAL32191	10	1.92bc	0/10	0/10	3.43ab	0/10	0/10	2.32b	0/10	0/10			
CAL32192	10	3.87de	0/10	0/10	3.98ab	0/10	0/10	4.23cde	0/10	1/10			
CAL32193	10	7.56f	0/10	0/10	6.51b	0/10	0/10	5.06de	0/10	4/10			
CAL32194	10	1.62ab	0/10	0/10	3.36ab	0/10	0/10	1.94b	0/10	0/10			
CAL32195	10	3.47cde	0/10	0/10	3.86ab	0/10	0/10	3.79bcd	0/10	1/10			
CAL32196	10	3.19bcd	0/10	0/10	3.83ab	0/10	0/10	3.42bcd	0/10	0/10			
Control (MEA)	10	0.01a	0/10	0/10	0.01a	0/10	0/10	0.01a	0/10	0/10			
P-value		<0.001			<0.001			<0.001					

Values followed by the same letters in a column are not different among isolates at $P=0.05$ according to Tukey's honestly significant difference multiple range test.
dpi, days post inoculation.

the stem, which is spread by squirrels and insects. This disease also occur from the root and continues up to the base of the stem. The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities to *C.*

fimbriata which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021a), and bullet wood (Pratama et al., 2021b) plants.

In a previous study, there were two variations of the ITS rDNA sequence from two isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin District. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Pratama et al., 2021a, 2021b; Tarigan et al., 2011). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *L. domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010, 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021b) and causes a sudden decline in bullet wood disease (Pratama et al., 2021a), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *C. esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021b). This supports the perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa*, *E. urophylla*, and *M. cajuputi*, as well as weakly pathogenic to *D. costulata*, *A. scholaris*, and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *L. domesticum* caused by *C. fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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Abstract

Ceratocystis wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.

Key Words: agroforestry plants, canker, *Certocystis fimbriata*, die-back disease

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Diseases Severity, Genetic Variation, and Pathogenicity of Ceratocystis Wilt on *Lansium domesticum* in South Sumatra, Indonesia

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***Ceratocystis* wilt disease has caused significant mortality in duku (*Lansium domesticum*) since 2014 and has now spread to all districts in South Sumatra, Indonesia. Recently, 16 isolates from duku representing populations from various districts in South Sumatra were isolated. Analysis for the morphological characteristic of the isolate showed that the population has a uniform morphology. Genetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences verified that the population has being dominated by the ITS5 haplotype of *Ceratocystis fimbriata* and a new ITS group, the ITS7b haplotype that was localized in Musi Banyuasin. Both haplotypes were highly pathogenic to duku. Inoculation tests on various forest and agroforestry plant hosts showed that both haplotypes were highly pathogenic to *Acacia mangium*, moderately pathogenic to *Acacia carsicarpa*, *Eucalyptus urophylla*, and *Melaleuca cajuputi*, but weakly pathogenic to *Dyera costulata*, *Hevea brasiliensis*, and *Alstonia scholaris*. Therefore, this pathogen becomes a serious threat to Indonesia's biodiversity due to its ability to infect forest and agroforestry plants, especially the indigenous ones.**

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Keywords **:** agroforestry plants, canker, *Certocystis fimbriata*, die-back disease

Lansium domesticum belongs to the Meliaceae family and is native to Southeast Asia. In Indonesia, this fruit is called *duku* (South Sumatra) and *langsat* (West Kalimantan) (Hanum et al., 2013), *ceroring* (Bali), *dookkoo* (Java, Sumatra), and *duki* (Lim, 2011). Furthermore, it is one of the leading commodity plants and the mascot of flora in South Sumatra, widely known in Indonesia as "*duku Palembang or duku Komering*" (Rupiah et al., 2018). The central production of *L. domesticum* in Indonesia is the province of South Sumatra after which it is distributed to various districts, such as Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Musi Banyuasin, Musi Rawas, and North Musi Rawas.

Additionally, the fruit has high economic value because the selling price is quite expensive and it is liked by the public for its fresh sweet, and very delicious taste. Also, it has other benefits, which include being an ingredient in cancer prevention (Matsumoto and Watanabe, 2020; Tilaar et al., 2008) with the discovery of new compounds in the peel, namely 3-hydroxy-8,14-secogammacera-7, and 14-dien-21-one that exhibits cytotoxic activity that attenuates the MCF-7 breast cancer cell line (Zulfikar et al., 2020). *L. domesticum* Corr. has also been reported to have benefits as larvicides (Ni'mah et al., 2015; Putranta and Wijaya, 2017), antitumor, anticancer (Khalili et al., 2017), antimalarial, antimelanogenesis, antibacterial, antimutagenic (Hanum et al., 2013), prebiotic *Bifidobacteria* spp. (Norhayati et al., 2016), organic catalyst (Nishizawa et al., 2010), and cosmetic ingredient due to its antioxidant properties (Subandrate et al., 2016; Tilaar et al., 2008).

Previous studies conducted from 2014 to 2017 (Suwandi

et al., 2021) showed that a very severe wilt disease of duku was first discovered in Ogan Komering Ulu District in three locations/villages, namely Belatung, Lubuk Batang Baru, and Lubuk Batang Lama. The death symptoms of the disease of *Ceratocystis* are characterized by wilting of part or the whole tree, whereby the branches and eventually the entire plant dies. Therefore, this study aims to examine the spread of this disease from the original area to all duku plantation centers in various districts in South Sumatra and the genetic diversity of the pathogen causing it.

Ceratocystis is a pathogen that attacks various plant species, including *Acacia mangium* and *Acacia crassicarpa* as its original host (Tarigan et al., 2010), *Eucalyptus* spp. (Harrington et al., 2014), *Mangifera indica* (Al Adawi et al., 2013), *Dalbergia tonkinensis,* and *Chukrasia tabularis* (Chi et al., 2019a, 2020), *Albizia lebbeck* (Razzaq et al., 2020), and others. Since the host plant of *Ceratocystis* is widely spread, and the duku is located around the forest, it is very important to consider the host plants of *Ceratocystis* that have economic value, such as *Acacia carsicarpa*, *Eucalyptus urophylla*, *Dyera costulata*, *Alstonia scholaris*, *Hevea brasiliensis*, and *Melaleuca cajuputi*. Therefore, this study aims to determine the distribution of disease in various duku production centers in South Sumatra, genetic variation, and host range in forest and agroforestry plants.

Materials and Methods

Diseases incidence, sample collection, and fungal isolation. Between 2019 to 2021, incidences with disease trees were observed in eight duku plantations in Ogan Komering Ulu District, four in South Ogan Komering Ulu, one in East Ogan Komering Ulu, six in Musi Banyuasin, five in North Musi Rawas, three in Musi Rawas, three in Ogan Komering Ilir, and one in Muara Enim, South Sumatra (Fig. 1). In each plantation, five plots with a size of 10 × 10 m were selected from the center of the diseased tree (Pratama et al., 2021b; Suwandi et al., 2021). Furthermore, the trees are declared infected if some branches or stems show symptoms of the disease. As a result of this, five diseased duku trees were randomly selected from the affected plantations to be isolated in the laboratory.

Isolates were collected from fresh wounds of *L. domesticum* which showed symptoms of branch wilting,

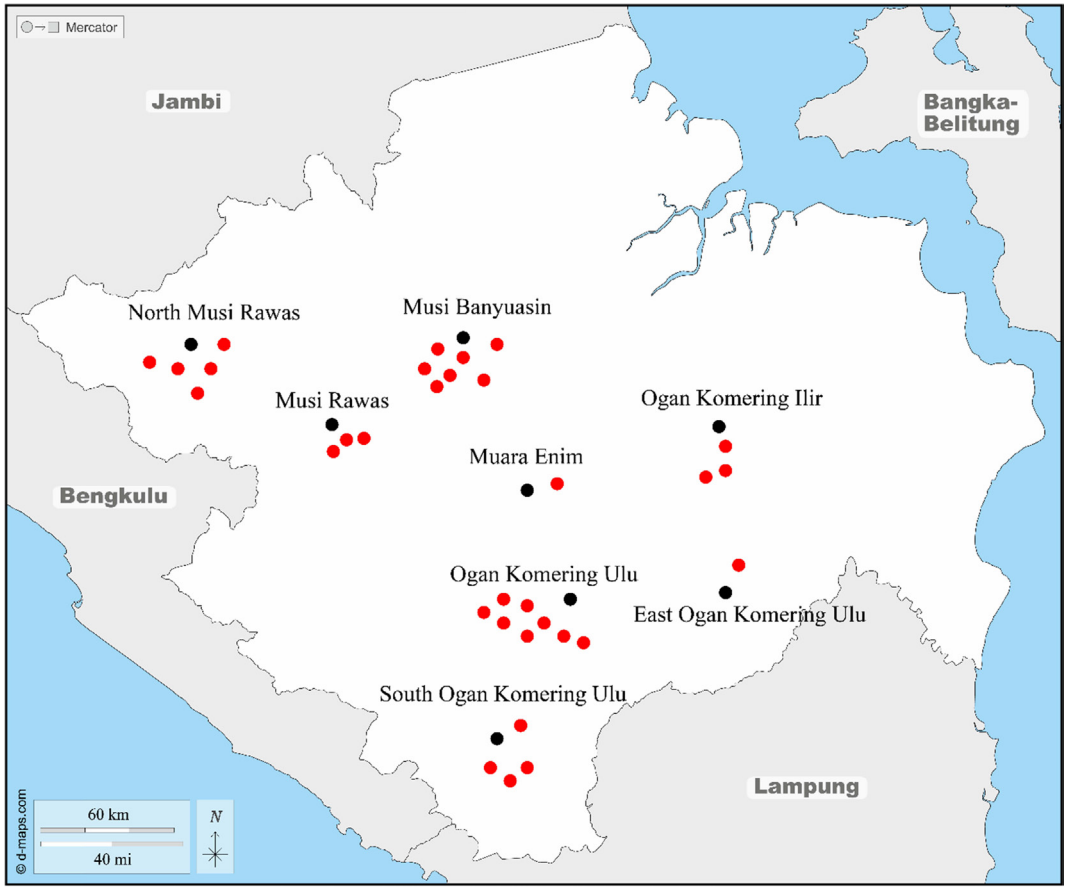

Fig. 1. Map of South Sumatera, red circle showing the collection sites for *Ceratocystis fimbriata.*

discoloration of vascular tissue, and dead plants caused by *Ceratocystis*. Furthermore, the samples were performed by making an incision in the bark and cutting a tangential longitudinal section (approximately 50 mm) of the newly infected xylem with the stain. The duku plants which were collected as samples were around 10 to 100 years old, and are therefore prone to infection in the plantation. Symptoms of wilt disease were evaluated as follows, the extent of lesion progression from discoloration of bark and wood, presence of sap flow from the surface of the lesion, the extent of leaf wilting or shedding, and death of the tree. The wood samples were stored in plastic bags and refrigerated before isolation.

Isolation of *Ceratocystis* was carried out based on carrot bait method (Moller and De Vay, 1968). Discolored wood was placed between two carrot slices that were first treated with streptomycin sulfate (100 mg/l) and incubated at room temperature to induce fungal sporulation on the slices. Wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 min, and rinsed with distilled water. Afterward, there were dried in laminar airflow planted directly on malt extract agar (MEA) media at room temperature (25°C) for 7-10 days to induce direct sporulation in MEA.

Masses of single ascospores which developed at the tips of ascomata on wood slices planted directly on MEA or infected carrots were transferred to 2% MEA (20 g/l malts, 20 g/l agar) (Biolab, Midrand, South Africa) in a new Petri dish, after which these cultures were incubated at 25°C.

Morphological characterization. The morphological characteristics of the observed fungi were represented by isolates originating from eight regions that were severely affected by *Ceratocystis*, namely Ogan Komering Ulu (Kepayang; CAL32194), East Ogan Komering Ulu (Bantan Pelita; CAL32367), South Ogan Komering Ulu (Simpang; CAL32164), Ogan Komering Ilir (Pairing; CAL30673), Musi Banyuasin (Sanga Desa; CAL32156), Musi Rawas (Tuah Negri; CAL31663), North Musi Rawas (Lawang Agung; CAL31654), and Muara Enim (Ujan Mas; CAL31351). Morphological observations of *Ceratocystis* isolate used the structure of the fungus which was cultured on 2% MEA media and incubated for 10 days at 25°C. Samples were prepared by placing fungal structures on glass slides in lactic acid and observing these structures under a light microscope. For each isolate, 100 replicate were established for the measurements of length and width of the base, ascomata neck, ascospores, bacilliform conidia, barrel-shaped conidia, and chlamydospores (Al Adawi et al., 2013).

Growth in culture. To determine the growth rate in culture, 4 mm mycelium-covered agar plugs were taken from the outer edge of 10-days-old cultures and placed face down in the center of a 90 mm Petri dish containing 2% MEA. Furthermore, a total of eight isolates were selected which represent the most severely affected areas from each region, namely CAL32194, CAL32156, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673, and CAL31351. Each isolate was replicated four times and planted in an incubator at a temperature of 10-30°C with an interval of 5°C. Also, the diameter of the colony was measured every 2 days for 14 days and the average was calculated.

DNA extraction, amplification, sequencing, and phylogenetic analyses. The pure cultures used for the DNA extraction were 14 isolates that represent each affected area, namely Ogan Komering Ulu (CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, and CAL32192), East Ogan Komering Ulu (CAL32367), South Ogan Komering Ulu (CAL32164), Ogan Komering Ilir (CAL30673), Musi Banyuasin (CAL32156 and CAL32157), Musi Rawas (CAL31663), North Musi Rawas (CAL31654), and Muara Enim (CAL31351). These isolates were grown in potato dextrose broth (PDB) for DNA extraction at 25°C for 10 days. Mycelium from PDB cultures was filtered, dried, and grounded into a fine powder using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration, as well as purity, were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA).

Amplification and polymerase chain reaction (PCR) sequencing were obtained from two gene regions, namely beta-tubulin which include βT1a (TTCCCCCGTCTCCACTTCTTCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Glass and Donaldson, 1995) as well as internal transcribed spacer (ITS) which include; ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Furthermore, the amplification was performed in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq-DNA-polymerase, 0.2 mM of each dNTP), 1 µl of each forward and reverse primer, 1 µl DNA template, and 27 µl sterile water. Also, PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were initial denaturation for 3 min at 94°C, 30 cycles for 30 seconds at 94°C for 30 s, for 30 s at 52°C, and 1 min at 72°C for. Amplification was completed at 72°C for 10 min and the PCR prod-

uct was stored at 10°C. The PCR amplicon was sequenced at 1st BASE (Malaysia), while the DNA sequences were compared with the GenBank database through a nucleotide BLAST search located at the National Center for Biotechnology Information (NCBI), Bethesda, MD, USA. The relevant sequences were transferred and then processed using the BioEdit software (Hall, 1999).

Trees were visualized and edited in MEGA v. 7 with maximum parsimony (MP) analysis and bootstrap of 1,000 replicates (Kumar et al., 2016). Branch support for nodes was obtained by performing 1,000 bootstrap replicates of the aligned sequences. For MP analysis, the metrics calculated included tree length, retention index, and consistency index. Also, *C. virescens* was used as the out-group taxon and the in-group was considered to be monophyletic.

Inoculation trials. These studies were conducted using ten isolates of *C. fimbriata.* The isolates were selected from the most severely affected area namely Ogan Komering Ulu and Musi Banyuasin (Table 1) and representing from two different type of haplotype ITS5 and ITS7b. Inoculation was designed using two studies to evaluate the pathogenicity of the isolates. First inoculation was tested their pathogenicity on *L. domesticum*. Two-year-old *L. domesticum* plants were collected from local seedlings with a stem diameter of 2-3 cm and a height of 50-60 cm and were put into a 15 cm diameter pot containing peat soil used for the experiment. All the plants were kept in the experimental house and watered twice a day.

The second inoculation test was performed to determine the specificity of the host range in *A. mangium*, *A. carsicarpa*, *E. urophylla*, *D. costulata*, *H. brasiliensis*, *A. scholaris*, and *M. cajuputi*. The age of the plant used for inoculation was four months with a stem diameter of 2-3 cm and a height of 70-80 cm, which was collected from a forest plant nursery in South Sumatra, planted in the same pot media and maintained as described for the first experiment.

Inoculation was performed using the isolates grown in MEA for 2 weeks. The plants were injured with a sterile scalpel by making an L-shaped (10 mm long) incision on the seedling stem, approximately 10 cm above the soil surface, and inserting agar mycelium (4 mm diam.) into each wound site. Ten host plants were inoculated with each *Ceratocystis* isolate and the same number of seedlings was inoculated with sterile MEA as a control. The plants were arranged in a randomized block design, and all inoculated wounds were covered with moistened sterile cotton and parafilm.

The inoculated plants were kept in the experimental house and watered twice a day. After 45 days, the peel tissue from the seedlings was incised at the top and bottom of the site and the length of the lesion was measured. The length of lesions in inoculated plants was measured after 45 days. To re-isolate the inoculated pathogens, wood samples were collected from the edges of the lesions and grown on

Table 1. Incidence of *Ceratocystis* wilt in duku orchards of South Sumatra

Location (tree/location)	Incidence (%)		
	May 2019	June 2020	February 2021
Ogan Komering Ulu			
Kartamulya (*n* = 89)	53.9	64	85.4
Saleman (*n* = 74)	41.9	58.1	95.9
Singapura (*n* = 83)	56.6	70.4	73.5
Pengaringan (116)	84.5	95.7	100
Reksa Jiwa (*n* = 91)	59.3	72.5	84.6
Tebat Agung (*n* = 67)	10.5	16.4	31.3
Padang Bindu (*n* = 71)	5.6	15.5	19.7
Kepayang (*n* = 103)	86.4	100	100
East Ogan Komering Ulu			
Bantan Pelita	-	7.7	20.5
South Ogan Komering Ulu			
Simpang	-	3.3	26.7
Tanjung Sari	-	1.8	8.9
Tanjung Beringin	-	5.2	11.1
Kisau	-	3.8	15.2
Ogan Komering Ilir			
Penyandingan	-	6.9	27.6
Ulak Kemang	-	2.7	19.2
Tanjung Lubuk	-	2.6	17.4
Musi Banyuasin			
Kasmaran	-	7.1	15.5
Babat Toman	3.8	14.1	29.5
Beruge	3.7	16.1	30.8
Sereka	6.8	20.5	47.9
Sanga Desa	85.7	100	100
Tanjung Raya	58.4	75.3	100
Musi Rawas			
Tuah Negri	-	-	40.2
Mambang	-	-	40.1
Lubuk Tuo	-	-	10.2
North Musi Rawas			
Beringin Jaya	-	-	56.1
Lawang Agung	-	-	43.6
Karang Waru	-	-	22.7
Rantau Kadam	-	-	8.2
Lesung Batu	-	-	5.8
Muara Enim			
Ujan mas	-	-	11.5

MEA plates or placed between two carrot slices.

Pathogenicity test data were analyzed using the SAS university edition software package (SAS Institute Inc., Cary, NC, USA). Furthermore, the analysis of variance (ANOVA) and Tukey's honestly significance difference (Tukey's honestly significant difference) test was used to determine the significant differences in the mean comparisons of the different treatments.

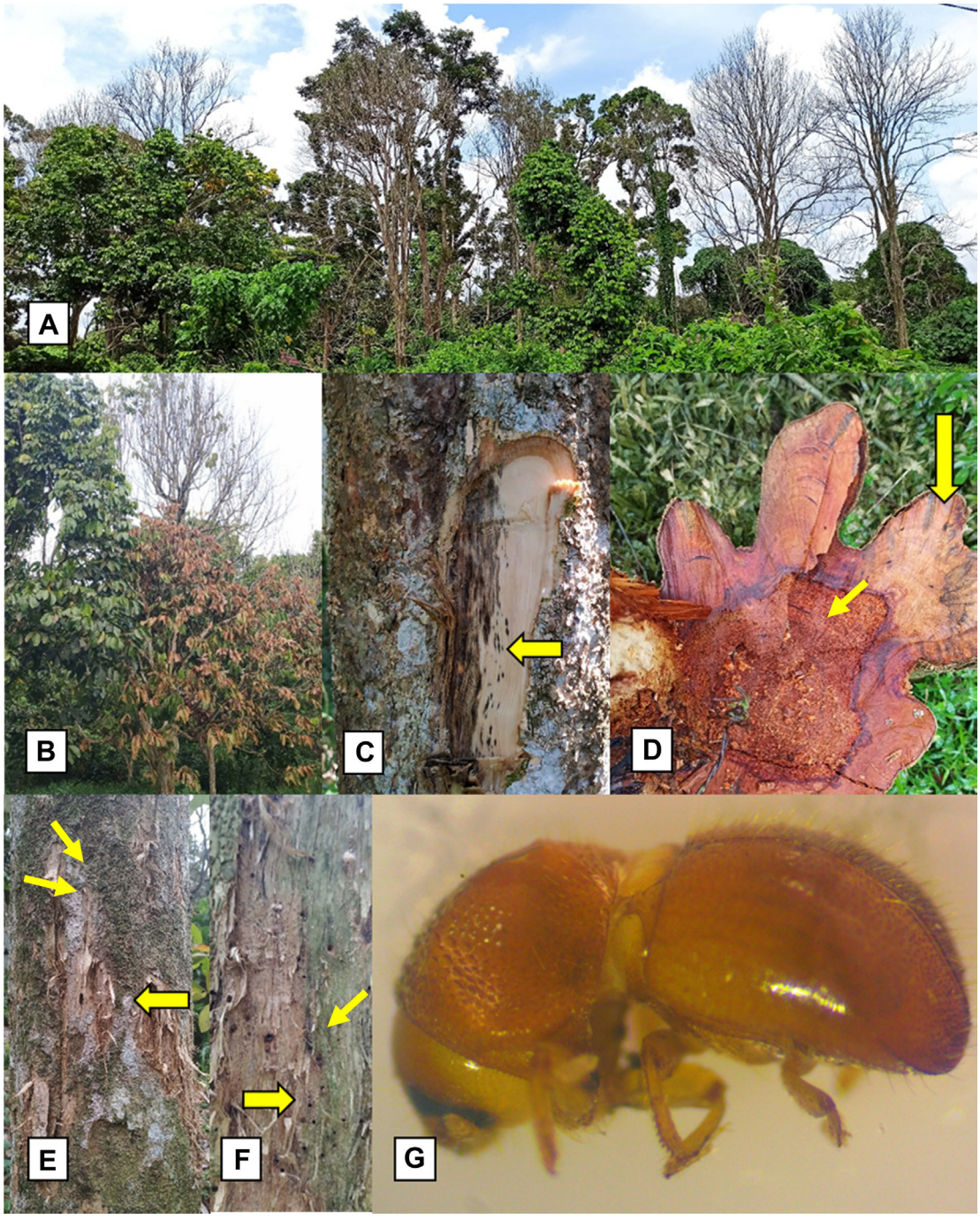

Fig. 2. Symptoms of wilt and die-back on *Lansium domesticum*. (A, B) Trees affected by *Ceratocystis fimbriata* experience rapid and simultaneous wilting of the leaves on the main branch or the entire canopy until it finally dies. (C, D) Dispersal pattern of discoloration in cross-section and the cambium area of wilted tree trunks (yellow arrows). (E) Squirrel bite caused peeled-off bark on diseased tree (yellow arrows). (F) A beetle hole on affected diseased wood (yellow arrow). (G) *Hypocryphalus mangiferae* as a vector for the spread of *Ceratocystis*.

Results

Diseases incidence, Sample collection, and fungal isolation. *Ceratocystis* wilt disease in duku was first reported in 2014 and was found only in 3 villages in Ogan Komering Ulu District, namely Belatung, Lubuk Batang Baru and Lubuk Batang Lama with an incidence of 100% (Suwandi et al., 2021). Currently, the attacked duku plantation has been destroyed and replaced with corn plants, the survey to observe this disease was continued considering the plant has high economic value and as the mascot of fruits in South Sumatra. Recent reports from 2019 to 2021 show that this disease has spread widely across various districts as centers of duku plantations in South Sumatra with varying levels of disease incidence (Table 1). It has spread widely in other plantations in the Ogan Komering Ulu District covering the Kartamulya, Saleman, Pengaringan, Mutual Jiwa, and Kepayang areas with the incidence of the disease reaching 100% in Pengaringan and Kepayang villages (Table 1). In the same year, it was also found that this disease attacks the duku trees sporadically in Musi Banyuasin District, within 271 km from the disease origin of Ogan Komering Ulu, and this has resulted in the death of all trees (100%) in the duku plantations in Sanga Desa and Tanjung Raya (Table 1).

Table 2. Recovery of *Ceratocystis fimbriata* from carrot baiting and direct isolation of wood onto the malt extract agar from samples collected from dying *Lansium domesticum* trees in Ogan Komering Ulu and Musi Banyuasin

District	Area	Year	Recovery of *C. fimbriata*, *n* (%)
Ogan Komering Ulu (26/40, 65%)	Kartamulya	2019	2/5 (40)
	Saleman	2019	5/5 (100)
	Singapura	2019	2/5 (40)
	Pengaringan	2020	5/5 (100)
	Reksa Jiwa	2020	2/5 (40)
	Tebat Agung	2020	3/5 (60)
	Padang Bindu	2020	2/5 (40)
	Kepayang	2020	5/5 (100)
East Ogan Komering Ulu (4/5, 80%)	Bantan Pelita	2021	4/5 (80)
South Ogan Komering Ulu (14/25, 56%)	Simpang	2021	4/5 (80)
	Tanjung Sari	2021	2/5 (40)
	Tanjung Beringin	2021	4/5 (80)
		2021	2/5 (40)
	Kisau	2021	2/5 (40)
Ogan Komering Ilir (8/15, 53.3%)	Penyandingan	2020	3/5 (60)
	Ulak Kemang	2020	3/5 (60)
	Tanjung Lubuk	2020	2/5 (40)
Musi Banyuasin (16/30, 53.3%)	Kasmaran	2021	1/5 (20)
	Babat Toman	2021	2/5 (40)
	Beruge	2021	1/5 (20)
	Sereka	2021	2/5 (40)
	Sanga Desa	2021	5/5 (100)
	Tanjung Raya	2021	5/5 (100)
Musi Rawas (12/15, 80%)	Tuah Negri	2021	4/5 (80)
	Mambang	2021	5/5 (100)
	Lubuk Tuo	2021	3/5 (60)
North Musi Rawas (16/25, 64%)	Beringin Jaya	2021	3/5 (60)
	Lawang Agung	2021	5/5 (100)
	Karang Waru	2021	3/5 (60)
	Rantau Kadam	2021	3/5 (60)
	Lesung Batu	2021	2/5 (40)
Muara Enim (3/5, 60%)	Ujan mas	2020	3/5 (60)

From 2020 to 2021, there were similar disease incidences on the duku plantations in Ogan Komering Ilir, within 158 km from the disease origin, and Muara Enim (within 152 km from the disease origin) with mild infestation with the incidence of less than 28% and 11.5%, respectively. In 2021, Musi Rawas (within 263 km from the disease origin), had a fairly incidence of 40.2%. In 2021, severe infestations were also detected in several villages of North Musi Rawas, within 345 km from the disease origin, especially Beringin Jaya and Lawang Agung with a percentage of 56.1% and 43.6%, respectively (Table 1). Due to the rapid development and spread of this disease in Ogan Komering Ulu and Musi Banyuasin in a short time, it is feared that this attack will kill duku plants in other districts in South Sumatra. Therefore, this disease destroys duku plant, which has high economic value and has become the mascot of the fruit flora of South Sumatra.

Infected duku tree is characterized by wilting leaves on certain twigs or branches. The leaves turn yellow, wilt, and dry, then it eventually dies due to a lack of nutrient supply to the plant. Although, it will take up to four to five months after the first symptoms for it to completely die. *Ceratocystis* disease attacks have resulted in the death of duku trees that are between 10 to 100 years old (Fig. 2A and B). Pathogen development on stems causes staining of vascular tissue and cankers on stems, and the initial symptoms shown are black streaks on the vascular tissue of the plant, as well as discoloration of the sapwood (Fig. 2C and D). There is a wound on the diseased tree caused by a squirrel scratch (Fig. 2E). In general, holes will appear on the

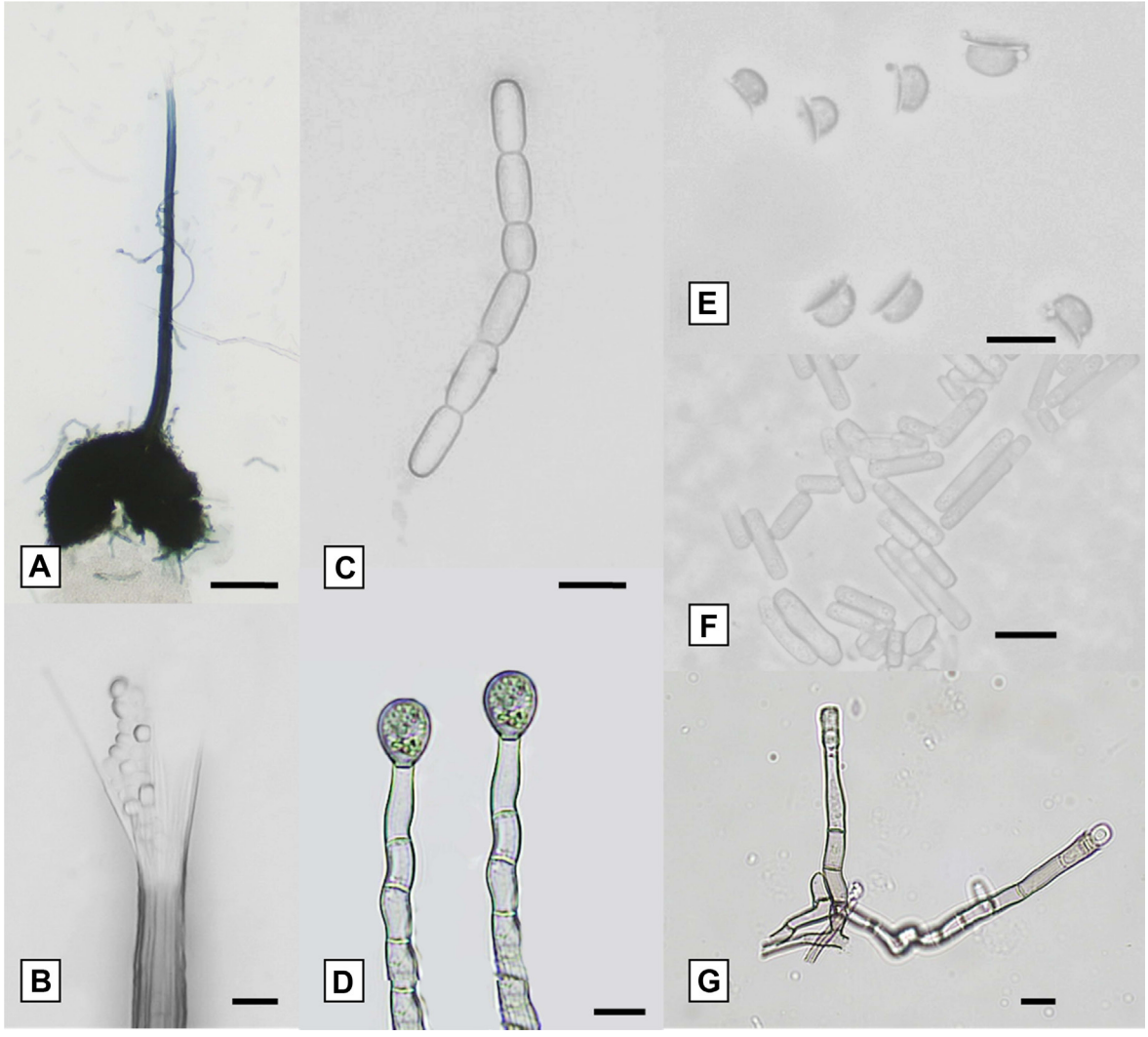

Fig. 3. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Lansium domesticum* stem lesion: (A) globose ascomata with a long neck, (B) divergent ostiolar hyphae, (C) barrel-shaped conidia, (D) chlamydospores, (E) hat-shaped ascospores, (F) cylindrical conidia, and (G) conidiophore/phialide. Scale bars: A = 100 µm, B-E, G = 10 µm, F = 5 µm.

infected duku stem caused by *Hypocryphalus mangiferae* (Fig. 2F) which is a vector insect for *Ceratocystis* (Fig. 2G).

Isolation of symptomatic xylem tissue in *L. domesticum* using carrot bait and direct planting into MEA media resulted in 16 isolates which represent Ogan Komering Ulu, East Ogan Komering Ulu, South Ogan Komering Ulu, Ogan Komering Ilir, Musi Banyuasin, Musi Rawas, North Musi Rawas, and Muara Enim areas which were severely affected by this disease. Meanwhile, the overall isolation percentage of *L. domesticum* samples from each region was 65%, 53.3%, 56%, 80%, 64%, 80%, 53.3%, and 60% for Ogan Komering Ulu, Musi Banyuasin, South Ogan Komering Ulu, East Ogan Komering Ulu, North Musi Rawas, Musi Rawas, Ogan Komering Ilir, and Muara Enim, respectively (Table 2).

Sixteen selected *Ceratocystis* isolates were collected from diseased duku plants, and there include CAL32194, CAL32191, CAL32196, CAL32195, and CAL32192 from Ogan Komering Ulu; CAL32159, CAL32156, CAL32157, and CAL32158 from Musi Banyuasin; CAL32164 from South Ogan Komering Ulu; CAL32367 from East Ogan Komering Ulu; CAL31654 from North Musi Rawas, CAL31663 from Musi Rawas; CAL30673 from Ogan Komering Ilir; and CAL31351 from Muara Enim. The isolate cultures obtained in this study were preserved in the

Table 3. Morphology of selected *Ceratocystis fimbriata* isolates from a different district in South Sumatra

Morphological characters[a]	Isolates							
	CAL32194	CAL32156	CAL32164	CAL32367	CAL31654	CAL31663	CAL30673	CAL31351
Ascomatal bases								
Shape	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
Ascomatal base (w)	134.3-312.4	122.9-291.4	135.7-325.2	141.3-317.1	137.9-321.1	132.1-334.9	137.9-346.1	122.1-316.9
Ascomatal base (l)	153.1-404.4	131-315.4	148.1-398.4	151.1-411.4	143.1-398.4	152.4-394.1	139.1-421.8	157.1-412.1
Ascomatal necks	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Neck (l)	415.4-768.4	354.9-677.7	413.7-798.8	439.9-736.4	475.8-813.6	484.6-790.9	463.8-723.6	484.6-780.9
Neck (w) top	11.5-26.8	7.06-18.4	11.3-21.9	11.1-25.4	10.1-17.9	11.3-21.7	11.1-22.9	11.3-21.7
Neck (w) bottom	24.8-47.9	20.3-39.7	23.6-42.6	22.6-51.2	23.7-43.8	22.67-42.9	23.7-43.6	22.67-44.8
Ostiolar hyphae								
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	32.2-43.5	30.4-40.1	32.7-44.7	32.7-42.2	33.5-43.9	33.7-44.8	33.5-42.9	31.7-44.8
Ascospores								
Hat-shaped ascospores (l)	3.4-5.7	3.3-5.2	3.2-5.4	3.4-4.9	3.2-4.4	3.1-5.1	3.1-4.3	3.3-4.9
Ascospores (w) without sheath	3.4-5.1	3.1-4.1	3.3-4.7	3.4-4.4	3.3-4.1	3.4-4.5	3.3-4.1	3.5-4.4
Ascospores (w) with sheath	5-6.8	4.1-6.1	5.1-6.7	5.3-6.4	5.2-6.5	5.5-6.7	5.2-6.3	5.4-6.6
Primary conidia (l)	12.1-27.5	10.6-18.9	13.8-23.8	12.2-29.3	13.2-25.7	14.9-24.8	12.5-21.6	13.7-24.6
Primary conidia (w)	3.5-7.4	3.2-4.3	3.1-5.1	3.4-4.1	3.2-5.1	3.4 -4.4	3.4-4.1	3.5-4.7
Secondary conidia (l)	6.3-11.6	5.7-10.1	6.6-11.8	7.9-11.8	6.7-11.9	6.8-11.5	6.5-11.5	6.2-11.3
Secondary conidia (w)	4.5-7.6	4.1-7.4	4.7-7.5	5.6-7.9	4.3-7.8	4.3-7.8	4.3-7.1	4.1-7.8
Chlamydospores								
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	10.7-15.1	8.7-15.1	11.3-15.6	9.7-17.8	10.7-15.4	10.1-16.5	10.3-14.6	10.4-14.5
Chlamydospores (w)	7.9-13.9	8.3-11.1	6.9-14.2	6.8-13.6	7.6-11.8	7.7-12.5	7.6-11.8	7.6-12.9
Culture growth rate[b]								
10°C	0	0	0	0	0	0	0	0
15°C	3.3-3.5	2.2-2.5	3.2-3.5	2.2-2.7	3.2-3.4	2.2-2.8	2.3-2.9	2.4-2.8
20°C	3.2-3.7	3.1-2.9	3.2-3.9	3.3-3.9	4.2-4.4	3.2-3.5	4.2-4.4	3.2-3.5
25°C	5.1-5.3	4.1-4.5	4.7-5.1	4.4-4.7	4.4-4.9	4.1-4.5	4.4-4.9	4.1-4.5
30°C	3.3-3.6	3.1-3.9	3.5-4.6	3.5-4.2	3.8-4.2	3.1-3.4	3.8-4.2	3.1-3.4

[a]All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm.
[b]Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after 14 days.

Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Morphological characterization and growth in culture. The isolates obtained had similar morphological characteristics when grown on MEA media. All isolates had light gray mycelia and dark gray to greenish colors, they also had black ascomata bases that were globose to subglobose (Fig. 3A) and produced an ascomata neck with divergent ostiolar hyphae at the ends (Fig. 3B). This fungus also produced chained barrel-shaped conidia (Fig. 3C), and chlamydospores (Fig. 3D), it also had hat-shaped ascospores (Fig. 3E). Cylindrical conidia (Fig. 3F) were generated from the primary phialidic conidiophore (Fig. 3G).

All morphological characteristics of the isolates studied were similar to the description of *C. fimbriata* which is isolated from *M. indica* (Van Wyk et al., 2007), *Prosopis cineraria* (Ghaf) in Oman, *Dalbergia sissoo* (Shisham) in Pakistan (Al Adawi et al., 2013), and the diseased *A. mangium* (Tarigan et al., 2011). However, there were no significant differences in the structural dimensions of all isolates for ascomata, ascospores, and chlamydospores (Table 3). All reported isolates were in the range of *C. fimbriata* and showed relatively similar growth responses. They did not grow at 10°C and optimal growth for all *Ceratocystis* isolates occurred between 25°C and 30°C (Table 3).

DNA extraction, amplification, sequencing, and phylogenetic analyses. For the ITS and β-tubulin gene regions, PCR amplification showed a fragment size of about 550 base pairs, and the product sequences were then stored in the GenBank database where it was compared with other *Ceratocystis* (Supplementary Table 1). A BLAST search using the β-tubulin gene in GenBank showed that isolates of the species *C. fimbriata sensu stricto* were grouped with 99% identical sequences (Fig. 4). Meanwhile, using ITS gene data, the isolates were dominated by the ITS5 which was 100% similar to that of WRC previously isolated from the duku plant where the disease originated, and a new ITS haplotype (ITS7b) of *C. fimbriata* (Fig. 5).

The phylogenetic relationships of these selected isolates with related taxa were analyzed using the MP method, and the result showed that isolates of *C. fimbriata* in *L. domesticum* were closely related to *C. fimbriata* in *Eucalyptus grandis* in Zimbabwe, *Camellia sinensis*, *Colocasia esculenta*, and *Punica granatum* in China, *Acacia* in Vietnam and Indonesia as well as *Mangifera indica* in Oman, Pakistan, and Indonesia. The phylogeny was assessed and analyzed using bootstrap analysis with 1,000 replications,

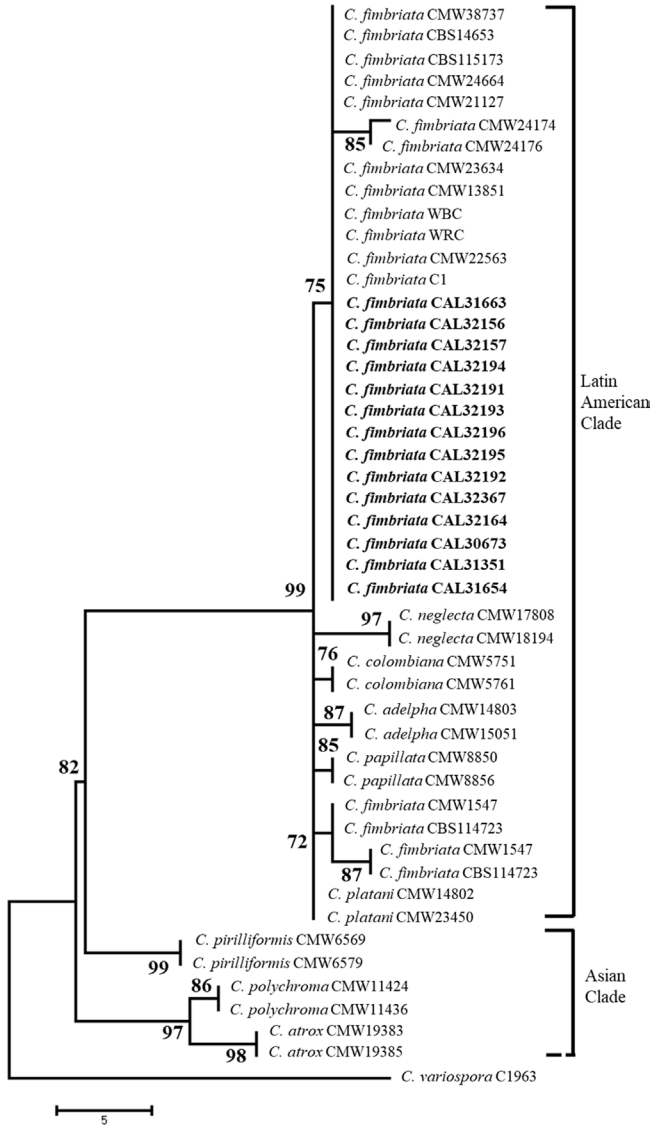

Fig. 4. The phylogenetic tree resulting from the maximum parsimony analysis of the β-tubulin sequence shows the relationship between *Ceratocystis fimbriata* from the *Lansium* tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. *C. variospora* is used as an outgroup.

as well as β-tubulin sequence respectively, and the result of the analysis showed that all isolates belonged to the Latin American Clade of *C. fimbriata sensu lato*. The similarity of this sequence to the previous case of *C. fimbriata* and the identification with phenotypic characteristics showed that the causative agent of sudden wilt disease in *L. domesticum* in Indonesia is classified as *C. fimbriata*.

Inoculation trials. *L. domesticum* seedlings inoculated in the first experiment showed discoloration in the bundle

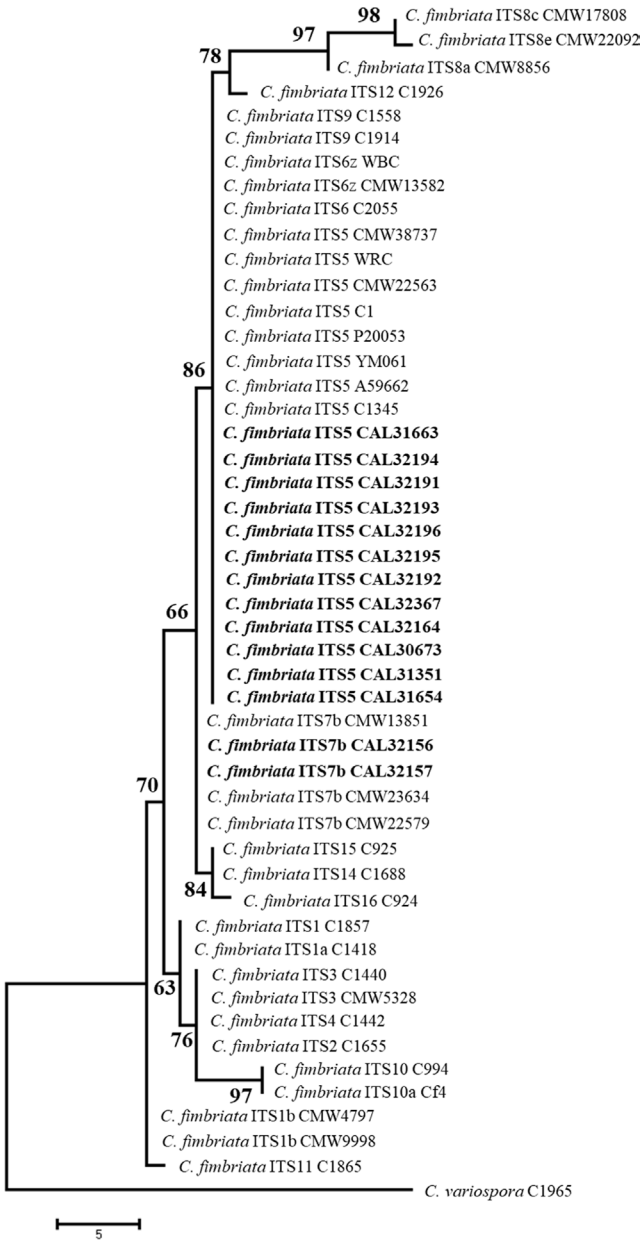

Fig. 5. The dendrogram formed from the maximum parsimony analysis shows the genetic linkage of the representative rDNA internal transcribed spacer (ITS) genotype in *Ceratocystis fimbriata sensu stricto*. Isolates from *Lansium domesticum* in Indonesia are marked in bold. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designation of Harrington et al. (2014). *C. variospora* is used as an outgroup taxon.

vessels, whereby 90% and 100% of it dies 45 days, as well as 70 days after pathogen inoculation respectively (Fig. 6A and B). ANOVA for lesion length in duku showed that there was no significant difference among all isolates inoculated to this host. All inoculated isolates resulted in lesion lengths of 6.86 to 19.81 cm in *L. domesticum* seedlings (Table 4). Statistical analysis showed a significant difference in lesion length between inoculated *L. domesticum* and control seedlings. Re-isolation of inoculated seedlings resulted in *C. fimbriata* and no fungus was found in the control nurseries.

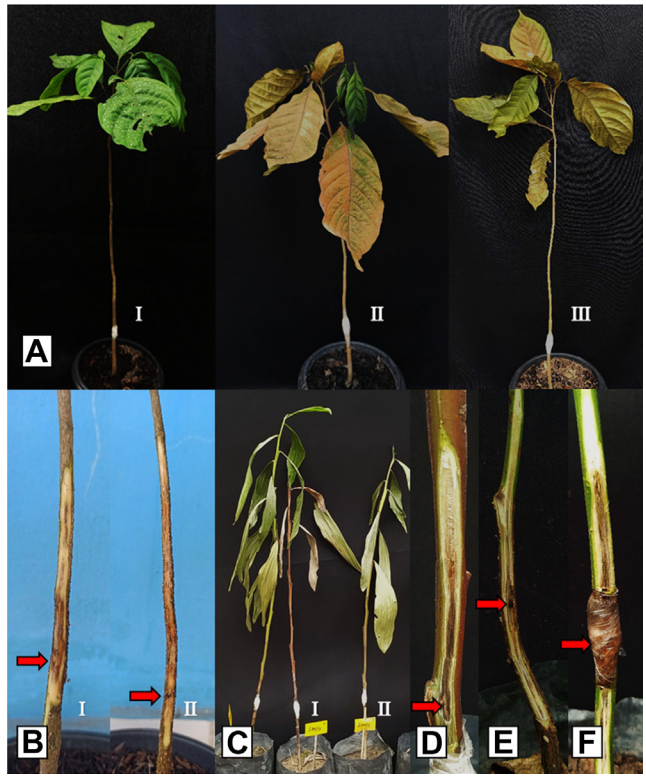

Fig. 6. Symptoms of mycelial plug inoculation with *Ceratocystis fimbriata* isolates (CAL32194 and CAL32159) from *Lansium domesticum* 45 days after inoculation. (A) Symptoms on 2-year-old duku seedlings (*L. domesticum*) inoculated with malt agar plug (control) (I), duku plants experienced complete wilting and finally died after being inoculated with CAL32194 (II) and CAL32159 (III). (B) The formation of an upward lesion from the inoculation site (red arrows) on duku plants after being inoculated by CAL32194 (I) and CAL32159 (II). (C, D) 4-month-old Acacia plants show symptoms of wilting and formation of upward lesions from the inoculation site (red arrow) after being inoculated by CAL32194 (I) and CAL32159 (II). (E) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Eucalyptus*, at 45 days of observation did not show any signs of wilting. (F) The formation of an upward lesion from the inoculation site (red arrow) on 4-month-old *Acacia crassicarpa*, at 45 days of observation did not show any signs of wilting.

The *A. mangium* seedlings inoculated with *C. fimbriata* showed typical symptoms of wilt disease, which include extensive vascular discoloration in all inoculated seedlings (Fig. 6C-F), and wilt was noted to reach 100% of all seedlings at day 70 after inoculation (Table 5). There was

Table 4. Pathogenicity of *Ceratocystis* isolates on *Lansium domesticum* under nursery condition

Isolates	Host test	*Lansium domesticum*		
		Lesion length[a] (cm)	Wilting and death at 45 days post inoculation	Wilting and death at 70 days post inoculation
CAL32156	10	16.35 f	7/10	10/10
CAL32157	10	15.49 ef	7/10	8/10
CAL32158	10	12.29 cd	5/10	5/10
CAL32159	10	11.02 c	2/10	5/10
CAL32191	10	11.73 cd	2/10	3/10
CAL32192	10	13.83 def	7/10	8/10
CAL32193	10	19.81 g	9/10	10/10
CAL32194	10	6.86 b	2/10	2/10
CAL32195	10	12.89 cde	5/10	6/10
CAL32196	10	11.19 cde	5/10	7/10
Control (MEA)	10	0.01 a	0/10	0/10
P-value		<0.001		

[a]Values followed by the same letters in a column are not different among isolates at $P = 0.05$ according to Tukey's honestly significant difference multiple range test.

no significant difference in the length of lesion produced by the *Ceratocystis* isolate used in the inoculation. The average length of lesions produced by all isolates of *C. fimbriata* inoculated to *A. mangium* seedlings was 9.94 to 20.93 cm (Table 5). Lesion and *Ceratocystis* fungus was not discovered in the control seedlings after re-isolation.

The isolates from *C. fimbriata* that were inoculated on other test seedlings, caused death and infection in plants which were characterized by the formation of significant lesions. In *A. crassicarpa*, *E. urophylla*, and *M. leucadendra* seedlings, all isolates caused moderately pathogenic symptoms with lesion lengths of 5.97-12.59 cm, 8.80-11.92 cm, and 1.94-5.17 cm, respectively. However, in *D. costulata*, *H. brasiliensis*, and *A. scholaris* plants, these isolates caused weakly symptoms with lesion lengths of 3.05-5.39 cm, 1.62-7.56 cm, and 3.36-6.51 cm, respectively, compared to controls with an average lesion length of 0.1 cm (the scar with a knife at the time of inoculation) (Table 5).

The members of the ITS5 and ITS7 haplotypes tested on all duku and other agroforestry plants showed approximately the same pathogenic ability to infect the tested plants. The re-isolation of the eight inoculated test plants resulted in a *C. fimbriata* culture, that confirmed Koch's postulate test. None of *Ceratocystis* isolates grew from control seedlings.

Discussion

Based on a survey conducted from 2019 to 2021, *Ceratocystis* has spread widely from its place of origin in the Ogan Komering Ulu District (Suwandi et al., 2021). Currently, the wilt disease has been found to affect the duku plants in other locations. *Ceratocystis* has been discovered to attack extensive areas with a radius of 345 km from its origin to South Ogan Komering Ulu, Musi Banyuasin, Ogan Komering Ilir, Muara Enim, Musi Rawas, and North Musi Rawas, with various severity levels, whereby it is very severe in Musi Banyuasin with a percentage of 100% the same as in Ogan Komering Ulu. Meanwhile, attacks in North Musi Rawas and other districts reached 56.1% and less than 30%, respectively.

The widespread of the disease in *L. domesticum* is closely related to the wood-boring insect *H. mangiferae* that comes from Southeast Asia, but it is well-known as a vector of *Ceratocystis* disease on mango plants in Oman and Pakistan (Al Adawi et al., 2006, 2013). *H. mangiferae* were seen in the field which has holes formed by this insect in *L. domesticum* plants, especially in the lesion area on wood. Squirrel rodents are also always seen on infected duku plants and cause the disease to spread widely by biting the infected stems and branches before moving to healthy plants (Suwandi et al., 2021). Additionally, the pruning of branches that have been infected with *Ceratocystis* through the use of agricultural tools without sterilization exacerbates the spread of this disease (Chi et al., 2019b) which is also caused by wind (Harrington, 2007). *Ceratocystis* is also transmitted from infected wild acacia around duku plantations or other plants that are hosts of this pathogen.

Field observations show that attacks from this disease occur from the trunk or branches at the top and go down to

Table 5. Host range test of *Ceratocystis* isolates on forest and agroforestry plants under nursery condition

Isolates	Host test	*Acacia mangium*			*Acacia carsicarpa*			*Eucalyptus urophylla*			*Dyera costulata*		
		Lesion length[a] (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length[a] (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length[a] (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi	Lesion length[a] (cm)	Wilting and death at 45 dpi	Wilting and death at 70 dpi
CAL32156	10	18.25 ef	10/10	10/10	9.86 de	0/10	1/10	11.32 b	0/10	1/10	4.25b	0/10	0/10
CAL32157	10	16.32 de	10/10	10/10	10.16 de	0/10	2/10	11.81 b	0/10	1/10	3.91b	0/10	0/10
CAL32158	10	14.49 cde	8/10	10/10	9.39 cd	0/10	1/10	9.33 b	0/10	0/10	3.63b	0/10	0/10
CAL32159	10	13.59 bcd	8/10	10/10	8.26 bcd	0/10	1/10	9.86 b	0/10	0/10	3.83b	0/10	0/10
CAL32191	10	11.73 bc	7/10	10/10	7.96 bcd	0/10	0/10	9.82 b	0/10	0/10	3.57b	0/10	0/10
CAL32192	10	15.54 cde	10/10	10/10	6.57 bc	0/10	0/10	10.59 b	0/10	0/10	5.15b	0/10	0/10
CAL32193	10	20.93 f	10/10	10/10	12.59 e	0/10	5/10	11.92 b	0/10	3/10	5.39b	0/10	0/10
CAL32194	10	9.943 b	5/10	10/10	5.97 b	0/10	0/10	8.80 b	0/10	0/10	3.05b	0/10	0/10
CAL32195	10	15.39 cde	9/10	10/10	7.82 bcd	0/10	2/10	11.20 b	0/10	2/10	4.02b	0/10	0/10
CAL32196	10	14.64 cde	8/10	10/10	8.64 bcd	0/10	1/10	11.15 b	0/10	1/10	3.60b	0/10	0/10
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01a	0/10	0/10
P-value		<0.001			<0.001			<0.001			<0.001		
		Hevea brasiliensis			*Alstonia scholaris*			*Melaleuca leucadendra*					
CAL32156	10	5.23 e	0/10	0/10	5.21 b	0/10	0/10	5.81 e	0/10	2/10			
CAL32157	10	4.05 de	0/10	0/10	4.75 b	0/10	0/10	5.17 de	0/10	2/10			
CAL32158	10	2.83 bcd	0/10	0/10	3.70 ab	0/10	0/10	3.15 bc	0/10	0/10			
CAL32159	10	2.58 bcd	0/10	0/10	3.50 ab	0/10	0/10	2.63 bc	0/10	0/10			
CAL32191	10	1.92 bc	0/10	0/10	3.43 ab	0/10	0/10	2.32 b	0/10	0/10			
CAL32192	10	3.87 de	0/10	0/10	3.98 ab	0/10	0/10	4.23 cde	0/10	1/10			
CAL32193	10	7.56 f	0/10	0/10	6.51 b	0/10	0/10	5.06 de	0/10	4/10			
CAL32194	10	1.62 ab	0/10	0/10	3.36 ab	0/10	0/10	1.94 b	0/10	0/10			
CAL32195	10	3.47 cde	0/10	0/10	3.86 ab	0/10	0/10	3.79 bcd	0/10	1/10			
CAL32196	10	3.19 bcd	0/10	0/10	3.83 ab	0/10	0/10	3.42 bcd	0/10	0/10			
Control (MEA)	10	0.01 a	0/10	0/10	0.01 a	0/10	0/10	0.01 a	0/10	0/10			
P-value		<0.001			<0.001			<0.001					

dpi, days post inoculation.

[a]Values followed by the same letters in a column are not different among isolates at $P = 0.05$ according to Tukey's honestly significant difference multiple range test.

the stem, which is spread by squirrels and insects. This disease also occur from the root and continues up to the base of the stem. The infection from these roots is caused by the spread of pathogenic inoculum through rainwater flow or splashes. In some locations in a district affected by the disease, the plants were able to grow healthy, while in other places the attacks were very severe. The variety of disease severity at each location and district is probably due to the various levels of resistance offered by the planted varieties of duku and the degree of soil fertility, which affects the growth and resistance of the plants. There was no correlation between the polyculture and monoculture systems of duku with the attack rate because *Ceratocystis* wilt disease was discovered in duku, which was grown in both polyculture and monoculture.

The identity of *C. fimbriata* as a pathogen associated with wilt disease in *L. domesticum* was determined based on morphological characteristics and a comparison of DNA sequences which include CAL32194, CAL32191, CAL32193, CAL32196, CAL32195, CAL32192, CAL32164, CAL32367, CAL31654, CAL31663, CAL30673 and CAL31351 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC while isolates CAL32156, CAL32157 with reference isolates CMW13851, CMW23634, CMW22579 were identified as belonging to *C. fimbriata* which was collected from *L. domesticum* in South Sumatra is part of *C. fimbriata* s.l. complex grouped into *C. fimbriata sensu stricto*. Comparison of ITS and β-tubulin gene sequences in each isolate obtained showed similarities to

C. fimbriata which was reported to attack duku (Suwandi et al., 2021), jackfruit (Pratama et al., 2021b), and bullet wood (Pratama et al., 2021a) plants.

In a previous study, there were two variations of the ITS rDNA sequence from two isolates, namely ITS5 and ITS6z haplotype of *C. fimbriata* (Suwandi et al., 2021). In this study, there were also two variations of the ITS rDNA sequence, namely the ITS5 and ITS7b haplotype. ITS5 haplotype was the most common genotype since it recovered from seven out of eight district in South Sumatra. ITS7b haplotype was the new genotype of *C. fimbriata* that affected *L. domesticum* in South Sumatra localized in Musi Banyuasin District. ITS6z was not isolated from this study. It might be due to the haplotype having a weak pathogenicity (Suwandi et al., 2021). From this and previous study, there are three the ITS haplotype *C. fimbriata* group isolated from *L. domesticum* (Meliaceae) including ITS5, ITS6z, and ITS7b that was the same as the haplotype *C. fimbriata* group from *acacia*, jackfruit, and bullet wood in Indonesia (Pratama et al., 2021a, 2021b; Tarigan et al., 2011). This shows that the genetic similarity of *Ceratocystis* in *L. domesticum* (Meliaceae) with *Ceratocystis* in *Acacia* is the result of crossing the ITS5, ITS6z, and ITS7b haplotypes. Therefore, it appears that the *Ceratocystis* pathogen that attacks *L. domesticum* (Meliaceae) in South Sumatra originates from *Acacia* which was first discovered in Riau.

This *Ceratocystis* wilt disease causes the death of duku plants in South Sumatra, and the symptoms include progressive loss of canopy which leads to the death of the tree, and the bark around the lesions and the wood turn dark blue to brown in the diseased trunk. In general, these symptoms are similar to those of *C. fimbriata* described in *Acacia* plants (Tarigan et al., 2010, 2011). *C. fimbriata* is a severe wilt pathogen that infects jackfruit (Pratama et al., 2021a) and causes a sudden decline in bullet wood disease (Pratama et al., 2021b), hence it has the potential to cause damage and destruction to duku in Indonesia.

C. fimbriata is best known for its severe damage inflicted on various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al., 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al., 2016); Araceae represented by *C. esculenta* (Oliveira et al., 2017); and Meliaceae represented by *L. domesticum* (Suwandi et al., 2021). However, recently it has been reported that *C. fimbriata* kills *A. heterophyllus,* Moraceae family in Indonesia (Pratama et al., 2021a). This supports the perspective that *C. fimbriata* has a wide host range, therefore having the potential of infecting other trees not previously mentioned.

Wilt disease of *L. domesticum* appears to be serious and it can devastate native trees like never before through host transfer (Roy, 2001; Wingfield et al., 2010). Pathogenicity test on duku showed that a very high attack intensity of 100% causes wilting and death of plants. Also, inoculation tests on various forest and agroforestry plant hosts showed that *C. fimbriata* derived from *L. domesticum* has a very aggressive on *A. mangium* (Suwandi et al., 2021), moderately pathogenic to *A. carsicarpa*, *E. urophylla*, and *M. cajuputi*, as well as weakly pathogenic to *D. costulata*, *A. scholaris*, and *H. brasiliensis*. This was shown by the formation of lesions on the stems which leads to the death of the inoculated seedlings.

The most pathogenic isolate from *L. domesticum* (CAL32193) resulted in the death of seedlings 25 days after inoculation. Furthermore, the death of acacia and eucalyptus plants showed similar symptoms, which include leaf wilting, and discoloration of the vascular tissue until the plant finally dies as found by Tarigan et al. (2011); and Roux et al. (2020). *Ceratocystis* is a very serious economical disease that has attacked *L. domesticum* in all duku production centers in South Sumatra hence it damages the income sources of farmers in this province. Also, the verification of *M. cajuputi* as an endogenous wetland plant that is infected and causes death, becomes a threat to the indigenous ones. Given the very wide host of *Ceratosystis*, the attack of this pathogen poses a serious threat to the biodiversity of Indonesia.

Sudden wilt disease on *L. domesticum* caused by *C. fimbriata* has spread widely to duku production centers in various districts of South Sumatra. Furthermore, the population consisted of individuals with uniform morphology dominated by ITS5 and ITS7b which were still localized in Musi Banyuasin, as well as being highly pathogenic in duku. *Ceratocystis* was also pathogenic to all forest test plants including wetland indigenous, posing a serious threat to the biodiversity of Indonesia.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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