



Recognising ceratocystis disease symptoms in a *Eucalyptus pellita* nursery

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Abstract

Early recognition of symptoms of infection is essential to prevent diseases from spreading through nurseries and into planted systems. In plantations, symptoms of *Ceratocystis manginecans* infection are typically wilt and canker but symptoms may differ at the nursery stage and as yet have not been described for *Eucalyptus pellita*. In this study, disease symptoms were recorded over a three-month period in an *E. pellita* nursery and isolates trapped. Then, to clearly identify those relating to ceratocystis disease and any related mortality, two *C. manginecans* isolates were re-inoculated by either spraying or dipping of healthy plantlets raised by tissue culture and mini-cuttings. Many disease symptoms were observed in the nursery but only perithecia on the leaves and stems was a clear indication of the presence of a *Ceratocystis* pathogen. Nine pathogenic fungal species, including *C. manginecans*, were isolated and identified by DNA analysis. Koch's postulates indicated that leaf blight associated with *C. manginecans* was black-brown in colour, irregular, with uneven edges and surrounded by a yellow margin or halo on the upper leaf surface. Mortality following inoculation of plantlets by spraying immediately after planting was > 70%; by contrast, mortality of mini-cuttings was < 50% if spraying was done just before transplanting but < 20% if done 7 or 14 days after transplanting. Isolate EP313C caused significantly greater mortality than isolate EP106C. The research showed that *C. manginecans* and other pathogens were present in the nursery environment and must be identified and managed as part of good nursery practice.

Keywords *Ceratocystis manginecans* · *Ceratocystis fimbriata*

Introduction

Eucalyptus pellita is propagated through seed, tissue-culture plantlets, and mini-cuttings. In the nursery, eucalypt planting stock is susceptible to pathogens that cause damping-off, root and stem rot, wilting, and leaf blight or spots (Brown and Ferreira 2000). Symptoms typical of some of these pathogens have been described (Brown and Ferreira

2000; Alfenas et al. 2009), but those that are associated with ceratocystis disease symptoms on *Eucalyptus* spp. are yet to be examined in a systematic way. The aim of this study was to develop a system for recognising symptoms associated with ceratocystis disease in a nursery that produces *E. pellita* planting stock for commercial plantations.

A proper and precise identification of pathogens is crucial for the successful control of diseases. In eucalypt nurseries, fungal pathogen infection is often characterized by water-soaked lesions on the root or stem, and necrotic, discoloured, dry, brown, black or sunken areas on the stem (Alfenas et al. 2009; Viljoen et al. 1992). Pathogens from fungal groups such as *Rhizoctonia solani* Kühn., *Fusarium* spp., *Pythium* spp., *Phytophthora cinnamomi* Rands, *Colletotrichum gloeosporoides* (Penz.) Penz. & Sacc., *Botrytis cinerea* Pers, and *Calonectria* spp. are commonly associated with raising eucalypt planting stock (Alfenas et al. 2009; Brown and Ferreira 2000; Viljoen et al. 1992; Crous et al. 2019), as is *Ralstonia solanacearum*, a bacterium that

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causes wilting and mortality (Mafia et al. 2012). Abiotic factors, manifested as sun scald, leaf scorch, poor nutrition, and water stress, can also lead to disease development in *Eucalyptus* spp. (Brown and Ferreira 2000).

Determining the causal pathogen by visual inspection of symptoms that appear on the leaves and stems can be challenging, as they are often common to many diseases, as well as other factors. For example, *Calonectria* spp. produce light purple to dark brown spots, angular to circular, or irregular, depending on the species, host species, and age (Brown and Ferreira 2000; Crous et al. 2019; Wang and Chen 2020); *Teratosphaeria* spp. produce pale green leaf dots on the leaves that gradually become yellow, the underside of the leaf may be covered in brown-black spores, and other characteristics may discriminate the different species (Carnegie et al. 2008; Crous et al. 2019; Andjic et al. 2019); *Colletotrichum* spp. cause discrete light brown lesions with a red-purple border (Viljoen et al. 1992; Mangwende et al. 2021); *Coniella* spp. cause leaf blight with circular necrotic rings and brown pycnidia along the leaf margin (Park et al. 2000). Water-soaked lesions and interveinal necrosis are typical symptoms of the disease caused by the bacterium *Xanthomonas axonopodis* (Ferraz et al. 2018). As these diseases are among those that infect *Eucalyptus* species and the symptoms may overlap those caused by ceratocystis disease, microscopic examination is an essential requirement; DNA analysis may also be necessary to validate a pathogen's identity.

Ceratocystis disease, like many other diseases (Brown and Ferreira 2000; Alfenas et al. 2009), can spread in a variety of ways into nurseries. Ceratocystis spores can be disseminated through contaminated soil (Marin et al. 2003), human transfer from contaminated areas (Roux and Wingfield 2009; Engelbrecht et al. 2007; Tarigan et al. 2011; Ferreira et al. 2013), insect frass transported by wind and rain (Souza et al. 2013; Harrington 2007), and nursery practices (Ferreira et al. 2013). Water is also an efficient vehicle for transferring ceratocystis spores (Grosclaude et al. 1991; Vigouroux and Stojadinovic 1990). Consequently, if ceratocystis disease is considered a potential disease threat in the nursery, a reliable method is required to first identify its presence so that systems can be put in place to anticipate and mitigate subsequent disease attacks.

To better understand the expression of ceratocystis disease symptoms in *E. pellita* in a commercial nursery, this study established two separate experiments for recognising potential and actual disease symptoms: the first involved observation of disease symptoms in the nursery using microscopy, and trapping suspected pathogens for DNA identification; the second used *Ceratocystis manginecans* isolates for the testing of Koch's postulates and to clearly describe its disease symptoms and pathogenicity. The objectives were to: 1) identify the pathogens that were present

in a commercial nursery raising *E. pellita* planting stock; (2) confirm that *C. manginecans* was among the pathogens present; and (3) demonstrate the nature of *C. manginecans* disease symptoms on *E. pellita* in the absence of other fungal pathogens by satisfying Koch's postulates. The hypothesis tested was that inoculation with *C. manginecans* will cause mortality in *E. pellita* mini-cuttings and plantlets raised by tissue culture.

Materials and methods

Symptom observations

The development of ceratocystis disease symptoms in mini-clonal-cuttings of *Eucalyptus pellita* was observed in a nursery belonging to PT Arara Abadi, Sinarmas Forestry (SMF) in Riau Province, Sumatera, Indonesia. As part of standard nursery practice, cuttings are exposed to three contrasting environments: a shaded greenhouse (GH) for 0–21 day-old cuttings, relative humidity (RH) 81–82%, maximum incident light 4300 lx, watering 6.7 ml/m²/min; a shaded outdoor area (SA) for 22–28 day-old cuttings, mean RH 73–74%, maximum incident light 34,700 lx, watering 200 ml/m²/min; an open area (OA) for cuttings > 29 days old, ambient humidity and incident light, watering 1360 ml/m²/min. The cuttings in GH were planted in a 75 cm³ tube that was arranged on a tray with capacity for 96 tubes. Just before the cuttings were moved to SA, their heights were graded, dead material and debris were removed, and the spacing doubled (48 cuttings per tray). In OA, the spacing was further increased (33 cuttings per tray). The observations were carried out on the then two most commonly-grown clones, SMAA7700EP (hereafter referred to as clone 77) and SMWK3610EP (clone 361).

In each age class, leaves were inspected for dark blight, stems for black lesions, and both for the presence of perithecia. The inspection and tissue sampling were performed on sixty-one days over a three-month period. The leaf and stem samples were selected randomly from symptomatic plants at each nursery stage, and an area between healthy and discoloured tissue was chosen for isolation. To test for the presence of and to trap the *Ceratocystis* pathogen, each portion of sample was placed onto a wound created on a healthy phyllode of *A. mangium* following its surface sterilisation with 70% alcohol (Indrayadi et al., 2023). Four separate samples were applied to each phyllode then incubated at 28 °C for 7 days (Indrayadi et al., 2023). A total of 65 phyllodes were used through the inspection period. Mycelial morphology and microscopic identification of the spores were used to identify and record the fungal growth on the phyllode. Each discovered fungus from the phyllode was

cultured on Potato Sucrose Agar (PSA) for 5 days and those with similar culture appearance were grouped. The identify of each group of fungi was validated through DNA analysis. The identified isolates have been deposited in the Indonesian Centre for Biodiversity and Biotechnology (ICBB), Bogor except for *Ceratocystis manginecans*.

DNA analysis

Fungal DNA was extracted using the method developed by Izumitsu et al. (2012) and rDNA ITS amplified by PCR using the ITS1 and ITS4 primers (White et al. 1990). For *Ceratocystis* species, RPB11 and FG1093 gene regions were also amplified (Fourie et al. 2015). The PCR products were submitted to 1st BASE (Indonesia) for sequencing (forward and reverse); to facilitate identification, the NCBI nucleotide database was searched using the DNA sequences.

Inoculum preparation

Ceratocystis isolates EP106C, isolated from a plantation of clone 77, and isolate EP313C that was isolated from the nursery producing this clone, were grown on PSA in Petri dishes. The PSA was prepared by boiling 200 g of potato to produce 1 L of broth, adding 20 g agar and 20 g sucrose, and then autoclaving for 20 min at 121° C. The spores of *C. manginecans* were collected by scraping 14-day-old cultures with a spatula then adding 10 mL sterile water and 0.2 ml Tween 80 to make a spore suspension. Total spore concentration of ascospores, aleurioconidia, and doliform and cylindrical conidia, was determined using a Neubauer Haemocytometer. The inoculum was diluted to 1×10^2 spores/ μ l. For the other fungal isolates, mycelial inoculum was prepared from 10-day-old cultures using a 3-mm diameter cork borer.

Re-inoculation

Fungi isolated from diseased plants in the nursery were re-inoculated into mini-cuttings of *E. pellita* clone 77. Spore suspensions of *Ceratocystis* isolates were sprayed onto seven-day-old cuttings. Five isolates of the other pathogens were re-inoculated onto the leaves of one-month-old cuttings of the same material by first making a wound with a sterile needle, placing agar plug inoculum onto the wound, and then sealing it with tape. After one week, disease symptoms were documented and the pathogen re-isolated to confirm Koch's postulates.

Media preparation

For each mini-cutting and rooted plantlet, cocopeat and rice husk mixed in a 2:1 ratio was filled into a 75 cm³ polytube; a planting hole for the mini-cuttings was created in the centre of the tube. Before transplanting, fungicide containing the active ingredient Propineb® was applied to the media at a concentration of 0.5 g per L of water. 3 g granular Agrimore® (29-10-10 N:P:K) dissolved in 1 L water was applied twice weekly.

Plant materials for re-inoculation

Two types of material were used, rooted plantlets of clone 77 from tissue culture and clonal cuttings harvested from mother plants of clones 77 and 361. For the latter, a cutting that consisted of four pairs of leaves was excised from the mother plant; the 4th pair was removed entirely as was 2/3 of the 3rd and 2nd pair using sharp scissors before planting. This is standard nursery practice and is done to reduce water loss from the cuttings (Saya et al. 2008).

Inoculation

Experiment 1 The rooted plantlets were gently removed from the agar using tap water before inoculating and planting in a polytube. The first treatment involved inoculating a 1 ml spore suspension from a *C. manginecans* isolate into the planting hole and also dipping the plant into the liquid inoculum before planting. For the second treatment, the inoculum was sprayed directly onto the plantlet after planting; the control treatment was sprayed with sterilised water. There were ten plants with six replications in each treatment.

Experiment 2 Mini-cuttings of clones 77 and 361 were used. For the first treatment, a spore suspension of *C. manginecans* was sprayed onto the cuttings just before planting (S-0D); for the second and third treatment it was sprayed at seven (S-7D) and 14 (S-14D) days after planting, respectively; a control received no inoculum. There were eight cuttings with six replications in each treatment.

Plants in both experiments were monitored for 12 weeks. The analysis of variance of plant mortality was tested using PROC GLM of the SAS statistical software (SAS Institute, North Carolina) and the overall F-test significant at the $\alpha=0.05$ was examined using Fisher's LSD.



Fig. 1 Symptoms appearing on plants in the nursery sampled for fungal isolation: (a) dark blight originating at a node and developing along the internode – red arrow; (b) perithecia developing on the node; (c) irregular blight symptom starting from the cut edge of leaves; (d) irregular

brown-black blight on upper side of emerging leaves; (e) perithecia neck (yellow arrow) developing on edge of infected leaf, (f) symptoms on old leaves in the nursery open area (OA).

Table 1 Fungal isolates obtained from plants with suspected ceratocystis disease symptoms in the *E. pellita* nursery

Fungi	Clone		Sample		Nursery area		
	SMAA7700EP	SMWK3610EP	Leaf	Stem	GH	SA	OA
<i>C. manginecans</i>	14	4	15	3	6	4	8
Other fungi	147	101	194	54	97	70	81

Results

Potential ceratocystis disease symptoms in the nursery

Disease symptoms on the *E. pellita* mini-cuttings were hard to distinguish in the nursery; the following were considered indicative of ceratocystis disease. A common symptom was the appearance of black lesions (Fig. 1a) and clusters of perithecia (Fig. 1b) along the internode (Fig. 1a) or at the leaf base (Fig. 1b). A second symptom was an irregular black-brown blight that developed from the cut surface where 2/3 of the leaf had been removed (Fig. 1c) and on

the upper side of emerging leaves (Fig. 1d). The most crucial indicator was perithecia on the edge of a leaf (Fig. 1e). Irregular black-brown blight on old leaves of the OA mini-cuttings was also considered a potential ceratocystis disease symptom (Fig. 1f).

The potential presence of *Ceratocystis manginecans* based on morphological and microscopic identification was observed on 12 of the 61 days the cuttings were inspected. Fungal isolations were attempted on those 12 days and 266 isolates obtained (Table 1). Isolates were grouped into 14 morphological groups and the rDNA ITS sequenced from a representative for each group to assist in identification (Table 2). *Ceratocystis manginecans* was confirmed by

Table 2 Identification, based on morphological characters and rDNA ITS sequence data, of fungal isolates from nursery plants showing symptoms of suspected ceratocystis infection

Isolate code/ ICBB code	Morphological ID	BLAST search result	Consensus ID	GenBank accession
EP328C/ ICBB10015	<i>Cylindrocyclidium</i> sp.	Over 99% similarity to a variety of <i>Calonectria</i> species, including type isolates of <i>C. papillata</i> (KY653267.1), <i>C. sulawesiensis</i> , <i>C. humicola</i> and <i>C. cerciana</i> .	<i>Calonectria</i> sp.	OR553243
EP340C/ ICBB10016	<i>Fusarium</i> sp.	Sequence noisy but highest match (87%) to <i>Fusarium oxysporum</i> MW800341.1 and 85–87% similarity to type isolates from a range of other <i>Fusarium</i> species	<i>Fusarium</i> sp.	Not submitted
EP353C/ ICBB10017	<i>Colletotrichum</i> sp.	Over 99% sequence similarity to type isolates of many species of <i>Colletotrichum</i> , including <i>C. siamense</i> (JX010171.1), <i>C. ti</i> , <i>C. clidemiae</i> and <i>C. aeschynomenes</i> .	<i>Colletotrichum</i> sp.	OR553247
EP344C/ ICBB10018	Unidentified	Sequence similarity of 99% to an unidentified <i>Diaporthe</i> sp. (KY790595) and 95–97% similarity to the type strains of multiple <i>Diaporthe</i> spp., including <i>D. sackstonii</i> , <i>D. miriciae</i> , <i>D. novem</i> , <i>D. tulliensis</i> .	<i>Diaporthe</i> sp.	OR553248
EP341C/ ICBB10019	<i>Coniella</i> sp.	Noisy sequence but highest match (91%) is to <i>Coniella eucalyptigena</i> , (NR_137983.1) with 85–90% similarity to other <i>Coniella</i> spp.	<i>Coniella</i> sp.	Not submitted
EP350C/ ICBB10020	<i>Fusarium</i> sp.	Sequence similarity of 99% to type isolates of a range of <i>Fusarium</i> species, including <i>F. venetii</i> (MH855494.1), <i>F. regulare</i> , <i>F. neerlandicum</i> , and <i>F. liriodendri</i> , with up to 98% sequence identity with many other <i>Fusarium</i> species.	<i>Fusarium</i> sp.	OR553244
EP349C/ ICBB 10021	Unidentified	Over 99% sequence similarity to several <i>Chaetomella</i> species and 95% similarity to type isolate of <i>C. zambiensis</i> (NR_156280).	<i>Chaetomella</i> sp.	OR553245
EP347C/ ICBB10022	<i>Pestalotiopsis</i> sp.	Sequence identical to type isolate of <i>Pseudopestalotiopsis avicenniae</i> (NR_147541.1) and over 99% similarity to type isolates of five other <i>Pestalotiopsis</i> species.	<i>Pseudopestalotiopsis aff. avicenniae</i>	OR553251
EP351C/ ICBB10023	<i>Penicillium</i> sp.	Sequence similarity of 99.3% to type isolate of <i>Talaromyces aurantiacus</i> (MH857871.1) and 98.7% to type isolate of <i>T. fusiformis</i> .	<i>Talaromyces aff. aurantiacus</i>	OR553253
EP348C/ ICBB10024	<i>Penicillium</i> sp.	Sequence similarity of 98–99% to type isolates from many species of <i>Talaromyces</i> , including <i>T. beijingensis</i> , (NR_172251).	<i>Talaromyces</i> sp.	OR553252
EP357C/ Not submitted	<i>Aspergillus</i> sp.	Sequence identical to type isolates of <i>Aspergillus oryzae</i> and <i>A. flavus</i> and 99% similar to many other <i>Aspergillus</i> spp.	<i>Aspergillus aff. oryzae</i>	OR553249
EP345C/ ICBB10025	<i>Penicillium</i> sp.	Sequence similarity of 99.5% to type strains of <i>Penicillium mariae-crucis</i> (MH861584.1, MH861585.1), 95–99% similarity to type strains of multiple other <i>Penicillium</i> species	<i>Penicillium</i> sp.	OR553246
EP352C/ IC10026BB	<i>Gliocladium</i> sp.	Over 99% similarity to type isolates from <i>Clonostachys rosea f. catenulata</i> (NR_165993.1), <i>C. solani f. nigrovirens</i> and <i>Hypomyces corticiicola</i> . Over 90% similarity to type isolates of multiple <i>Clonostachys</i> spp.	<i>Clonostachys</i> sp.	OR553250
EP106C/ ICBB9999 & EP313C	<i>Ceratocystis</i> sp.	RPBII and <i>tefl</i> (Ep106C only) sequences identical to ex-type isolate of <i>C. manginecans</i> (KJ601620.1, EF433317.1.), ITS sequence identical to ex-type isolate of <i>C. acaciivora</i> , syn. <i>C. manginecans</i> (EU588656.1), ribosomal protein coding region FG1093 in Ep106C identical to that from ex-type isolate of <i>C. manginecans</i> (KJ601698), while Ep313C differed by a single nucleotide transition from T to C.	<i>Ceratocystis manginecans</i>	RPBII - ON023587, ON023588 <i>tefl</i> - OM779105 ITS - OM780184, OM780185 FG1093 - OM779110, OM779111

sequencing of the rDNA ITS, RPBII and FG1093 regions. It was present in all the nursery stages, and more common in leaf than stem samples and in clone 77 than clone 361 (Table 2).

Sequences of the rDNA ITS had over 99% similarity to named isolates listed in NCBI for 12 of the morphologically delineated groups, with 88–90% matches for the remaining two groups (Table 2). A *Diaporthe* sp. and *Chaetomella raphigera* were the two linked only through molecular identification. Although the morphological identification

was otherwise similar to that based on sequence similarity, the latter was more specific to species level and provided a more up-to-date taxonomy. Besides *C. manginecans*, eight pathogenic fungal species were identified. One species of *Clonostachys* was also isolated and the other fungi were from the ubiquitous genera *Penicillium*, *Aspergillus*, and *Talaromyces*.

Symptoms following re-inoculation

The six pathogenic fungi examined were associated with different forms of blight on the leaves of *E. pellita* (Fig. 2). The blight caused by *C. manginecans* was characterised by an irregular shape with uneven edges and a black-brown colour, surrounded by a yellow margin or halo on the upper leaf surface (Fig. 2b) and a watery patch on the lower leaf surface (Fig. 2c). Clusters of perithecia with their ascospores (Fig. 2d, yellow arrow) were mainly on the edge of the leaves and this was the main feature that distinguished ceratocystis disease symptoms from those of the other re-inoculated fungi. Stem rot on the node or leaf base was also produced by *C. manginecans*. (Fig. 2e). Leaf infections spread rapidly to the stem, resulting in death of the entire plant. The symptoms expressed by the other fungi were a light-dark brown irregular or concentric blight without the presence of a halo and wilting of the plant.

DNA analysis

DNA sequencing confirmed the identity of isolates EP106C (isolated from plantation tree and used in pathogenicity tests) and EP313C (isolated from nursery plantlets and also used

in pathogenicity tests) as *C. manginecans*. The sequenced gene regions, RPBII and FG1093, of both isolates were identical to those of an ex-type isolate of *C. manginecans*, CMW13851. The ITS sequences were identical to the ex-type isolate of *C. acaciivora*, subsequently synonymised with *C. manginecans* (Fourie et al. 2015).

Dipping vs. spraying inoculum

The percentage of rooted *E. pellita* plantlets that survived after being inoculated with *Ceratocystis* isolates through dipping was not significantly different from the control. However, plant mortality following spraying the inoculum was significantly greater than from dipping (Fig. 3). Isolate EP313C appeared to be more aggressive than isolate EP106C, though the differences in both treatments were not statistically significant. Mortalities occurred only in the first month after inoculation, and the remaining plants remained healthy until age 12 weeks.

The survival rate of *E. pellita* mini-cuttings sprayed with inoculum before planting was significantly lower than when inoculum was applied 7 or 14 days later. (Fig. 4A). Isolate EP313C caused significantly greater mortality than isolate EP106C (Fig. 4B). The mortality of clone 77 was



Fig. 2 Symptoms produced following re-inoculation with *C. manginecans* and five of the eight other pathogens isolated from the nursery samples: (a–e) *C. manginecans* disease symptoms on leaves and stem;

and leaf diseases caused by: (f) *Colletotrichum siamense*; (g) *Fusarium* sp.; (h) *Diaporthe* sp.; (i), *Coniella eucalyptigena*; (j) *Calonectria cerciana*

Fig. 3 Plant survival after inoculating *E. pellita* plantlets using two methods of application and two isolates of *C. manginecans*

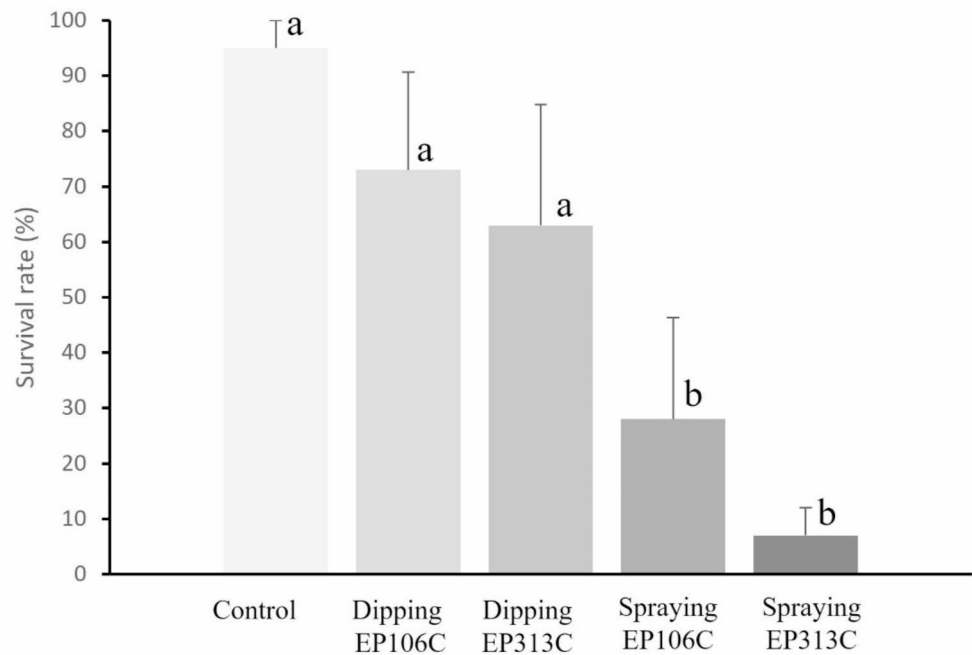
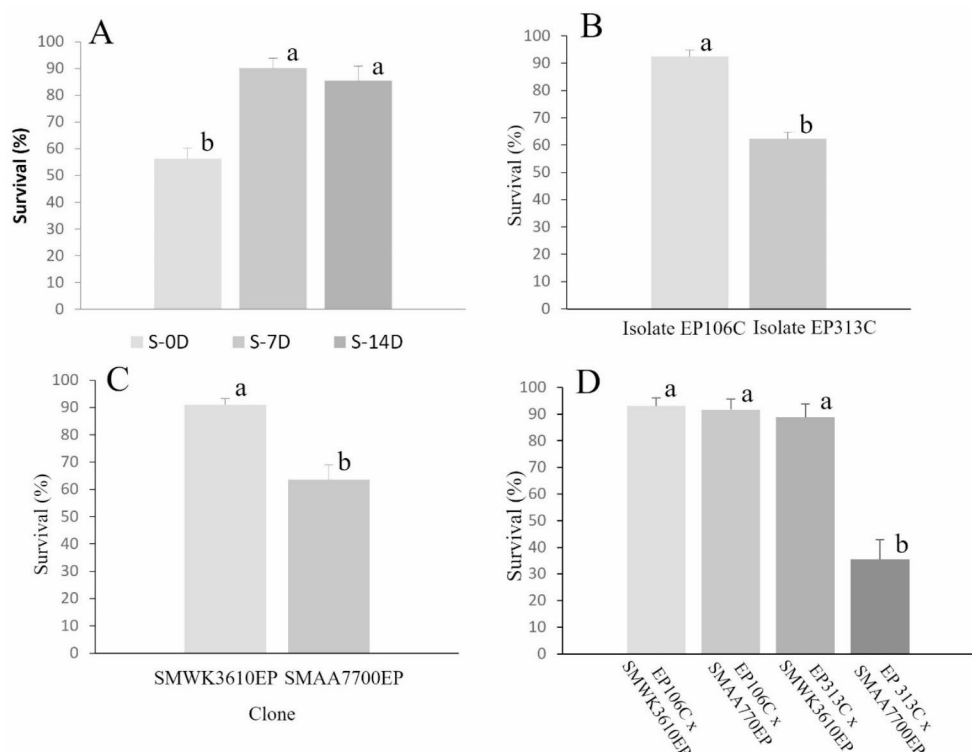


Fig. 4 The survival rate of *E. pellita* mini-cuttings eight weeks after spraying with *C. manginecans* inoculum; (A) Effect of inoculation timing, (B) Comparison of two ceratocystis isolates, (C) Comparison of two eucalyptus clones, (D) Clone by isolate interaction. Note: control without spraying pathogen inoculum excluded from the analysis because of 100% survival



significantly greater than that of clone 361 (Fig. 4C), and the mortality of clone 77 inoculated with isolate EP313C was greater than for the other three isolate $\times$ clone combinations (Fig. 4D). Eight weeks after spraying, no new necrotic symptoms developed.

Perithecia of *C. manginecans* developed on the plants after the spraying treatment in both experiments. Perithecia of *C. manginecans* developed on the decaying stems of the

rooted plantlets (Fig. 5a - red arrow) and decaying leaves of the mini-cuttings (Fig. 5b - red arrow) and produced ascospores on the cut edges of the leaves (Fig. 5b - green arrow). Recognisable symptoms developed on the leaves: an irregular and elliptical blight with uneven edges, and a black-brown centre surrounded by a yellow margin or halo (Fig. 5c, d). There were no clear differences between the abaxial and adaxial leaf surfaces at early stages of infection.



Fig. 5 Symptoms caused by *C. manginecans* in the reinoculation experiments: perithecia on decaying plantlets (a) and leaves (b) (red arrows); sticky ascospore mass emerging from perithecium on the

edge of cut leaf (green arrow) and perithecia (red arrows) (b); irregular black-brown blight with surrounding halo (c); blight symptoms on plants in tray (d); symptomless controls (e)

Discussion

Nine pathogenic fungal species, including *Ceratocystis manginecans*, were isolated from the *E. pellita* nursery, and symptoms that appeared associated with ceratocystis disease were identified. We focussed on *C. manginecans* as this disease is increasingly impacting eucalypt plantations in Indonesia (Indrayadi et al., 2023) as well as elsewhere (Trang et al. 2022; Ferreira et al. 2013; Roux et al. 2020).

The most reliable symptom of *C. manginecans* was the emergence on both leaves and stems of perithecia, a characteristic often associated with advanced development of Ascomycota fungi (Trail 2007). One of the eight other pathogens found, *Coniella eucalyptigena* is another ascomycete that produces perithecia and causes concentric ring leaf blight in eucalypts (Silva et al. 2020b; Alvarez et al. 2016). It was more challenging to identify the causal agent from the other symptoms of the different diseases present, particularly the leaf blight, based solely on a visual inspection. For example, even though the Koch's postulates' findings indicated that an irregular black-brown blight with a surrounding halo is a symptom of ceratocystis disease, it was first necessary to isolate from the perithecia to make an accurate diagnosis. Black-brown blight caused by other ceratocystis diseases can also be seen on other species, for example those on the genus *Syngonium* caused by *Ceratocystis fimbriata* (Dhakal 2018). It is also possible for other pathogenic fungi such as some of those identified in the nursery, to randomly develop halos (Alfenas et al. 2009). Thus, when the typical symptoms of *C. manginecans* are

observed that include the presence of perithecia, this should be taken as a sign that control measures are immediately required, and if there is any uncertainty, molecular identification should be sought.

Of the total number of isolations made from suspected ceratocystis disease symptoms in the nursery, less than 10% were *C. manginecans*. This comparatively low level indicates a low inoculum load entering and/or its low establishment in this *E. pellita* nursery. Alternatively, the leaf blight symptoms associated with the other pathogens may have masked signs of ceratocystis infection. For example, symptoms of *Fusarium*, *Colletotrichum* and *Calonectria* are more pronounced on the leaves of many plant species (Mangwende et al. 2021; Okungbowa and Shittu 2012; Rodrigues et al. 2014; Li et al. 2017). *Chaetomella raphigera* as well as *C. eucalyptigena* cause leaf spot on *Eucalyptus* species (Crous et al. 2019; Cao et al. 2021). *Colletotrichum siemense* can attack the leaves of *Capsicum annuum* (Silva et al. 2019); and *Pseudopestalotiopsis theae* those of *Camellia sinensis* and *Phoenix dactylifera* (date palm) (Maharachchikumbura et al. 2014; Tao et al. 2020), plant species that are grown in areas surrounding the nursery. Although these fungal species are not known to attack mature *Eucalyptus* species, these diseases may have entered the nursery due to the proximity of these other hosts. As these other pathogens were associated with mortality of the *E. pellita* cuttings, it is necessary to investigate not only the origin of the *C. manginecans* inoculum but also that of other potential pathogens as part of good nursery practice.

Based on the low number of ceratocystis isolations made, the level of infection was similar in each of the three nursery environments. The very high humidity levels and very low incident light are expected to be the main factors supporting the emergence of ceratocystis disease in the greenhouse environment (Stahr and Quesada-Ocampo 2020). The low rate of infection following transfer to the shaded outdoor environment would have been assisted by the removal of dead plants and other dead and decaying material prior to transfer (Galanti and Lutgen 2021; Ferreira et al. 2013), as spores released from perithecia are easily spread by routine activities in nurseries (Ferreira et al. 2013). In addition, the increased spacing between plants and the lower humidities and higher light levels would also decrease the risk of infection. Full exposure to the natural environment and a further increase in spacing may have further restricted disease development in the open environment. Such an interpretation still requires some qualification: the low numbers in the greenhouse and shaded outdoor environments may also have been linked to a failure to recognise cryptic symptoms of ceratocystis disease such as internal stem lesions. The relative contributions of contamination from the surrounding environment and nursery management remain unknown. More than 500 different species of pathogenic fungi commonly infect leaves of *Eucalyptus* spp. (Crous et al. 2019). This study has shown that several fungal species present in Indonesia can infect *E. pellita* mini-cuttings, pointing to a requirement for continual disease monitoring and control as part of nursery practice.

A mycoparasitic fungus was identified among the isolates, indicating a potential role of biological control in the nursery. *Clonostachys rosea* is available as a commercial product for controlling diseases like damping-off and stem rot though it has not been tested against *C. manginecans* (Thambugala et al. 2020). *Diaporthe* spp. have been shown to produce antagonistic antibacterial compounds (Tanapi-chatsakul et al. 2018), however it appears that endophytic *Diaporthe* spp. pose more of a risk of becoming diseases in woody plants (Gomes et al. 2013; Crous and Wingfield 2018). Hence, it is necessary to further investigate the potential pathogenicity of these antagonists before considering their use as biological control agents in reducing the threat of *C. manginecans* in *E. pellita* nurseries.

Inoculation of tissue-cultured *E. pellita* plantlets by spraying with a spore suspension of *C. manginecans* caused significant levels of mortality (> 70%) in plantlets, but inoculation by dipping plantlets into the spore suspension was less effective. Tissue culture is frequently utilised for large-scale propagation of mother plants (Hussain et al. 2012); however, because plantlets are relatively small and succulent, and need to acclimatise to nursery conditions, they are highly susceptible to diseases (Dale et al. 2008). The greater

risk of mortality from spraying plantlets compared to dipping highlights the problem of above-ground contamination when managing nurseries for *C. manginecans*. Nevertheless, in spite of the application of Propineb® to the media at transplanting, *C. manginecans* still caused unacceptable levels of mortality. The lack of mortality one month after inoculation may relate to the pathogen's inability to adapt and infect more mature, intact plant tissues as well as the activation of the plant defence system as the plantlets grow (Lapin and Van den Ackerveken 2013).

Similarly, reinoculation by spraying caused significant though lower (< 50%) levels of mortality in mini-cuttings but only if the treatment was applied just before planting. Fungal infections caused by *Ceratocystis* spp. mainly occur through wounds (Harrington 2013), and those created by cutting the leaves and that at the base of the cutting may have acted as portals for disease entry. The substantial reduction in mortality if the reinoculation was delayed for 7 days may be linked to healing of these wounded tissues. *Ceratocystis* pathogens are hemibiotrophs that require living plant tissue for their early establishment (Sun et al. 2020), and like the majority of diseases that are usually found in the early stages of growth in eucalypt nurseries, can induce damping off disease (Alfenas et al. 2009). Given that mortality of eucalypts during their propagation in nurseries is a major cause of economic loss (Brown and Ferreira 2000; Alfenas et al. 2009), more attention needs to be given to managing the consequences of creating artificially wounded tissue.

Within a species, the level of aggressiveness of pathogen isolates may be influenced by their origin (Harrington et al. 2011). Although originating from the same *Eucalyptus* clone, 77, isolate EP313C was isolated from plants in the nursery whereas isolate EP106C was obtained from an infected plantation tree. The higher aggressiveness of isolate EP313C suggests that it was better adapted to the nursery environment than isolate EP106C because this was its source of isolation (Engelbrecht et al. 2007; Valdetaro et al. 2019). This finding implies that the origin, habitat, and the clone of the host from which an isolate was obtained might all influence the aggressiveness of a *C. manginecans* isolate.

Clone 361 was consistently more tolerant to *C. manginecans* than clone 77. In commercial plantations established to clone 361 from the same nursery stock there have been no reports to date of ceratocystis disease symptoms and *C. manginecans* has not been isolated from natural wounding (Sagitarianto F. personal comm.). *Eucalyptus* spp. express contrasting levels of tolerance to ceratocystis disease infection (Silva et al. 2020a), and *E. pellita* is considered one of the more disease-tolerant (Guimarães et al. 2010). However, within a eucalypt species, different levels of tolerance are found (Zauza and Alfenas 2004). This experiment has shown this to be the case for *E. pellita* and that inoculation

by spraying shoots may offer a reliable method of screening for disease tolerance to *C. manginecans*, similar to the use of phyllode inoculation as a rapid, preliminary test for tolerance of *A. mangium* to ceratocystis (Nasution et al. 2022).

This study has shown that inoculation of nursery plants of *E. pellita* with *C. manginecans* results in mortality, clearly demonstrating that this disease must be managed in a nursery setting. Several pathogens, including *C. manginecans* were isolated from the nursery, but common and overlapping symptoms meant that DNA analysis was required for their correct identification. Re-inoculation experiments indicated that airborne sources of inoculum may lead to greater mortality than soil-borne sources, particularly if fungicides are used in potting media. While mini-cuttings can quickly become less vulnerable to disease entry, managing artificial wounding to reduce disease susceptibility must be addressed. In addition, the mix of pathogens and the complexity of the factors that drive disease development, show that comprehensive control for all diseases which embraces sanitation, fungicides and antagonists, is potentially required in nurseries raising *E. pellita*.

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Declarations

Conflict of interest The authors have no conflict of interest to declare that is relevant to this article.

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References

Alfenas AC, Zauza EAV, Maria RG, Assis TF (2009) Clonagem e doenças do eucalipto. Editora UFV, 2 edn. Minas Gerais, Brazil

- Alvarez LV, Groenewald JZ, Crous PW (2016) Revising the Schizoparmaceae: Coniella and its synonyms Pilidiella and Schizoparme. *Stud Mycol* 85:1–34. <https://doi.org/10.1016/j.simyco.2016.09.001>
- Andjic V, Carnegie AJ, Pegg GS, Hardy GESJ, Maxwell A, Crous PW, Pérez C, Wingfield MJ, Burgess TI (2019) 23 years of research on Teratosphaeria leaf blight of *Eucalyptus*. *For Ecol Manag* 443:19–27. <https://doi.org/10.1016/j.foreco.2019.04.013>
- Brown BN, Ferreira F (2000) Disease during propagation of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds) Disease and pathogens of eucalypts. CSIRO Publishing, Collingwood Vic, Australia
- Cao X-X, Wei J-G, Hou J-G, Fan C-L, Luo J, Wu Y-J, Yang X-H, Luo J-T, Yang X-B (2021) First report of *Chaetomella raphigera* causing brown spot on *Eucalyptus urophylla* × *E. grandis* in China. *J Plant Pathol* 103(1):403–403. <https://doi.org/10.1007/s42161-020-00737-6>
- Carnegie A, Lawson S, Smith T, Pegg G, Stone C, McDonald J (2008) Healthy hardwoods: a field guide to pests, diseases and nutritional disorders in subtropical hardwoods. Forest and Wood Products Australia, Victoria
- Crous PW, Wingfield MJ (2018) Fungi infecting woody plants: emerging frontiers. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 40(1):1–3. <https://doi.org/10.3767/persoonia.2018.40.00>
- Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, Summerell BA, Edwards J, Taylor PWJ, Groenewald JZ (2019) Foliar pathogens of eucalypts. *Stud Mycol* 94:125–298. <https://doi.org/10.1016/j.simyco.2019.08.001>
- Dale A, Hughes BR, Donnelly D (2008) The role of micropropagation in producing specific pathogen-tested plants. *HortScience* 43(1):74–77
- De Silva DD, Groenewald JZ, Crous PW, Ades PK, Nasruddin A, Mongkolporn O, Taylor PWJ (2019) Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. *IMA Fungus* 10(1):1–32. <https://doi.org/10.1186/s43008-019-0001-y>
- Dhakal U (2018) Survey for *Ceratocystis Fimbriata* on *Syngonium* species. Host range test and molecular characterization of the isolates collected. University of Hawai'i at Manoa, Hawai'i, United States
- Engelbrecht CJ, Harrington TC, Alfenas AC (2007) Ceratocystis wilt of *Cacao*—A disease of increasing importance. *Phytopathology* 97(12):1648–1649
- Ferraz HGM, Badel JL, da Silva Guimaraes LM, Reis BP, Totola MR, Goncalves RC, Alfenas AC (2018) *Xanthomonas axonopodis* pv. *Eucalyptorum* pv. nov. causing bacterial leaf blight on *Eucalypt* in Brazil. *Plant Pathol J* 34(4):269–285. <https://doi.org/10.5423/PPJ.OA.01.2018.0014>
- Ferreira MA, Harrington TC, Gongora-Canul CC, Mafia RG, Zauza EAV, Alfenas AC (2013) Spatial-temporal patterns of ceratocystis wilt in *Eucalyptus* plantations in Brazil. *Forest Pathol* 43(2):153–164. <https://doi.org/10.1111/efp.12013>
- Fourie A, Wingfield MJ, Wingfield BD, Barnes I (2015) Molecular markers delimit cryptic species in *Ceratocystis sensu stricto*. *Mycological Progress* 11:1020. <https://doi.org/10.1007/s11557-014-1020-0>
- Galanti R, Lutgen H (2021) Greenhouse and Nursery Sanitation Tools, Equipment, Workers, and Visitors. The College of Tropical Agriculture and Human Resources (CTAHR) <https://www.ctahr.hawaii.edu/site/Info.aspx>. Accessed 18 June 2022
- Gomes RR, Gliencie C, Videira SI, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* 31:1–41. <https://doi.org/10.3767/003158513X666844>

- Grosclaude C, Olivier R, Pizzuto JC, Romiti C (1991) Dissemination of *Ceratocystis fimbriata* f. *platani* inoculum by river water. Eur J for Pathol 21(3):168–171
- Guimarães LMS, Titon M, Lau D, Rosse LN, Oliveira LSS, Rosado CCG, Christo GGO, Alfenas AC (2010) *Eucalyptus pellita* as a source of resistance to rust, ceratocystis wilt and leaf blight. Cropp Breed Appl Biotechnol 10(2):124–131. <https://doi.org/10.12702/1984-7033.v10n02a04>
- Harrington TC (2013) Ceratocystis diseases. In: P.Gonthier GN (ed) Infectious forest diseases. CABI, Wellesford, UK, pp 230–255
- Harrington TC (2007) The genus *Ceratocystis*: where does the oak wilt fungus fit? In: Billings RF, Appel DN (eds) National Oak Wilt Symposium, Austin, Texas, June 4–7 2007. pp 27–43
- Harrington TC, Thorpe DJ, Alfenas AC (2011) Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of *Ceratocystis fimbriata*. Am Phytopathological Society 101(5):555–566
- Hussain A, Ahmed I, Nazir H, Ullah I (2012) Plant tissue culture: current Status and Opportunities. In: Leva A, Rinaldi LMR (eds) Recent advances in plant in vitro culture. IntechOpen, London, pp 1–28
- Indrayadi H, Glen M, Siregar BA, Ratkowsky D, Rimbawanto A, Tjahjono B, Mohammed C (2023) Cross-inoculation of commercial forestry, amenity and horticulture tree species with *Ceratocystis* isolates collected from different host species. Plant Disease, in press
- Izumitsu K, Hatoh K, Sumita T, Kitade Y, Morita A, Tanaka C, Gafur A, Ohta A, Kawai M, Yamanaka T, Neda H, Ota Y (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. Mycoscience 53(5):396–401. <https://doi.org/10.1007/s10267-012-0182-3>
- Lapin D, Van den Ackerveken G (2013) Susceptibility to plant disease: more than a failure of host immunity. Trends Plant Sci 18(10):546–554. <https://doi.org/10.1016/j.tplants.2013.05.005>
- Li J, Wingfield MJ, Liu Q, Barnes I, Roux J, Lombard L, Crous PW, Chen S (2017) *Calonectria* species isolated from *Eucalyptus* plantations and nurseries in South China. IMA Fungus 8(2):259–286. <https://doi.org/10.5598/ima fungus.2017.08.02.04>
- Mafia RG, Alfenas AC, Penchel Filho RM, Ferreira MA, Alfenas RF (2012) Bacterial wilt: pathogen spread and disease effects on eucalyptus cloning. Revista Árvore 36(4):593–602. <https://doi.org/10.1590/s0100-67622012000400002>
- Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014) *Pestalotiopsis* revisited. Stud Mycol 79(1):121–186. <https://doi.org/10.1016/j.simyco.2014.09.005>
- Mangwende E, Truter M, Aveling TAS, Chirwa PW (2021) Anthracnose leaf spot pathogens, *Colletotrichum fructicola* and *Colletotrichum cigarro*, associated with *Eucalyptus* seed produced in South Africa. Australas Plant Pathol 50(5):533–543. <https://doi.org/10.1007/s13313-021-00807-y>
- Marin M, Castro B, Gaitan A, Preisig O, Wingfield BD, Wingfield MJ (2003) Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. J Phytopathol 151(7–8):395–405. <https://doi.org/10.1046/j.1439-0434.2003.00738.x>
- Nasution A, Indrayadi H, Glen M, Evans K, Ratkowsky D, Brawner J, Gafur A, Mohammed C (2022) Phyllode inoculation provides a rapid protocol for preliminary screening of *Acacia* species for tolerance to Ceratocystis wilt and canker disease. Eur J Plant Pathol 163(2):321–339. <https://doi.org/10.1007/s10658-022-02479-w>
- Okungbowa FI, Shittu HO (2012) Fusarium wilt: an overview. Environ Res J 6(2):83–102
- Park RF, Keane PJ, Wingfield MJ, Crous PW (2000) Fungal disease of Eucalypt foliage. In: Keane PJ, Kile GA, Podger FD, Brown AV (eds) Eucalypts. CSIRO Publishing, Collingwood Vic, Australia
- Rodrigues AL, Pinho DB, Lisboa DO, Nascimento RJ, Pereira OL, Alfenas AC, Furtado GQ (2014) *Colletotrichum theobromicola* causes defoliation, stem girdling and death of mini-cuttings of eucalyptus in Brazil. Trop Plant Pathol 39(4):326–330
- Roux J, Wingfield M (2009) *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. South Forests: J for Sci 71(2):115–120. <https://doi.org/10.2989/sf.2009.71.2.5.820>
- Roux J, Wingfield M, Fourie A, Noeth K, Barnes I (2020) Ceratocystis wilt on *Eucalyptus*: first record from South Africa. South Forests: J for Sci 82(1):24–31. <https://doi.org/10.2989/20702620.2019.1686687>
- Saya RA, Mankessi F, Toto M, Marien J-N, Monteuis O (2008) Advances in mass clonal propagation of *Eucalyptus urophylla* x *E. grandis* in Congo. BOIS ET FORÊTS DES TROPIQUES 297(3):16–25
- Silva AC, Betancourth BML, Ferreira DC, Elerati TL, Rodrigues FÁ, Alfenas AC (2020a) Responses of resistant and susceptible hybrid clones of *Eucalyptus urophylla* x *Eucalyptus grandis* to infection by *Ceratocystis fimbriata*. Ann for Sci 77(45):1–19. <https://doi.org/10.1007/s13595-020-00932-6>
- Silva X, Roux J, Asiegbu FO (2020b) Diseases of Eucalypts in Paraguay and First Report of *Teratosphaeria zuluensis* from South America. Forests 11(10):1035. <https://doi.org/10.3390/f11101035>
- Souza AGC, Maffia LA, Murta HM, Alves YH, Pereira RM, Picanco MC (2013) First report on the association between *Ceratocystis fimbriata*, an agent of mango wilt, *Xyleborus affinis*, and the sawdust produced during beetle colonization in Brazil. Plant Dis 97(8):1116–1116. <https://doi.org/10.1094/pdis-12-12-1204-pdn>
- Stahr M, Quesada-Ocampo LM (2020) Assessing the role of temperature, inoculum density, and wounding on disease progression of the fungal pathogen *Ceratocystis fimbriata* causing black rot in sweetpotato. Plant Dis 104(3):930–937. <https://doi.org/10.1094/PDIS-12-18-2224-RE>
- Sun Y, Li M, Wang Y, Li L, Wang M, Li X, Xu M, Loake GJ, Guo M, Jiang J (2020) *Ceratocystis fimbriata* employs a unique infection strategy targeting peltate glandular Trichomes of Sweetpotato (*Ipomoea batatas*) plants. Phytopathology 110(12):1923–1933. <https://doi.org/10.1094/PHYTO-05-20-0165-R>
- Tanapichatsakul C, Monggoot S, Gentekaki E, Pripdeevech P (2018) Antibacterial and antioxidant metabolites of *Diaporthe* spp. isolated from flowers of *Melodorum fruticosum*. Curr Microbiol 75(4):476–483. <https://doi.org/10.1007/s00284-017-1405-9>
- Tao Y, Quan X, Khokhar I, Anjum T, Song H, Mukhtar I (2020) First Report of *Pseudopestalotiopsis theae* causing leaf spot of date palm (*Phoenix dactylifera*) in China. Plant Dis 105(2):508. <https://doi.org/10.1094/PDIS-06-20-1356-PDN>
- Tarigan M, Wingfield MJ, van Wyk M, Tjahjono B, Roux J (2011) Pruning quality affects infection of *Acacia mangium* and *A. crassiparpa* by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*. South Forests: J for Sci 73(3–4):187–191. <https://doi.org/10.2989/20702620.2011.639498>
- Thambugala KM, Daranagama DA, Phillips AJL, Kannangara SD, Promptutha I (2020) Fungi vs. fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. Front Cell Infect Microbiol 10:604923. <https://doi.org/10.3389/fcimb.2020.604923>
- Trail F (2007) Fungal cannons: explosive spore discharge in the Ascomycota. FEMS Microbiol Lett 276(1):12–18. <https://doi.org/10.1111/j.1574-6968.2007.00900.x>
- Trang T, Thu P, Khai T, Tuan T, Hinh T, Nam N, Thuy P, Chi N (2022) First report of canker and wilt disease in eucalypt caused by *Ceratocystis manginecans* in Vietnam. Indian Phytopathol 75(1):287–291
- Valdetaro D, Harrington TC, Oliveira LSS, Guimarães LMS, McNew DL, Pimenta LVA, Gonçalves RC, Schurt DA, Alfenas AC (2019)

- A host specialized form of *Ceratocystis fimbriata* causes seed and seedling blight on native *Carapa guianensis* (andiroba) in amazonian rainforests. Fungal Biol 123(2):170–182. <https://doi.org/10.1016/j.funbio.2018.12.001>
- Vigouroux A, Stojadinovic B (1990) Infection of plane tree by *Ceratocystis fimbriata* f. *platani* after contamination on water as growing substrate of wounded roots. Eur J for Pathol 20(2):118–121
- Viljoen A, Wingfield MJ, Crous PW (1992) Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: a review. S Afr for J 161(1):45–51. <https://doi.org/10.1080/00382167.1992.9630424>
- Wang Q, Chen S (2020) *Calonectria pentaseptata* causes severe leaf disease of cultivated *Eucalyptus* on the Leizhou Peninsula of Southern China. Plant Dis 104(2):493–509. <https://doi.org/10.1094/PDIS-05-19-1009-RE>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18(1):315–322
- Zauza EAV, Alfenas AC (2004) Resistance of *Eucalyptus* clones to *Ceratocystis fimbriata*. Plant Dis 88(7):758–760

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