Comparison of Hyphal Fragments and Spores to Evaluate the Pathogenicity of the *Eucalyptus* Leaf and Shoot Pathogen *Calonectria pseudoreteaudii*

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Abstract: The genus *Calonectria* includes many aggressive plant pathogenic species with a worldwide distribution. Calonectria leaf blight is one of the most prominent diseases of *Eucalyptus* trees in Southeast Asian and South American plantations. Inoculation trials to evaluate pathogenicity of *Calonectria* species typically use conidial suspensions but this is not possible for species that do not sporulate sufficiently in culture. *Calonectria pseudoreteaudii* is one of the most aggressive species to *Eucalyptus* in China, but most isolates fail to produce conidia in culture, requiring an alternative procedure for artificial inoculation. This study compared inoculations utilizing conidial and hyphal fragment suspensions. Two *Eucalyptus* genotypes were used, and these were inoculated with different concentrations of hyphal fragments or conidia of three *C. pseudoreteaudii* isolates. Three days after inoculation, the treated *Eucalyptus* plants displayed similar disease symptoms, irrespective of whether they had been inoculated with conidia or hyphal fragments. This was consistent for all of *C. pseudoreteaudii* isolates and also the different *Eucalyptus* genotypes. The results demonstrate that hyphal fragment suspensions can be used to provide a reliable indication of *C. pseudoreteaudii* isolate pathogenicity when conidia are not available for inoculation studies.

Keywords: Calonectria leaf blight; Forest disease; Fungal pathogen; Inoculation method.

1. INTRODUCTION

Calonectria species are widely distributed in tropical and sub-tropical regions of the world (Crous 2002; Lombard et al. 2010). Many species in this genus are important plant pathogens, and cause diseases on more than 335 plant species residing in over 100 families. Susceptible species include forest trees, agricultural crops, and horticultural plants (Crous 2002; Guan et al. 2010; Ivors et al. 2012). *Calonectria* species infect roots, stems, branches, and leaves of their hosts, causing symptoms such as cutting rot, root disease, stem cankers, damping-off, as well as leaf and shoot blight. In the case of severe infections, entire plants can die (Crous 2002; Lombard et al. 2009, 2010; Taniguchi et al. 2008).

Inoculation using conidial suspension is a common method to evaluate the pathogenicity of *Calonectria* species. Such inoculations have been used to determine the pathogenicity of *C. pseudonaviculata* and *C. henricotiae* causing boxwood blight (Gehesquière et al. 2016; Richardson et al. 2020; Sacher et al. 2020) as well as other *Calonectria* species on a wide range of plants including examples from Brazil (Alfenas et al. 2013a, 2013c, 2016), Tunisia (Lombard et al. 2011) and China (Chen et al. 2011; Wang and Chen 2020; Wu and Chen 2021). These have included studies comparing the pathogenicity of isolates (Alfenas et al. 2013a, 2013c; Chen et al. 2011; Lombard et al. 2011; Wang and Chen 2020; Wu and Chen 2021) as well as those considering the relative susceptibility of various hosts (Alfenas et al. 2013a, 2013c, 2016; Chen et al. 2011; Guo et al. 2016; Richardson et al. 2020; Wang and Chen 2020; Wu and Chen 2021).

Inducing *Calonectria* species to produce sufficient numbers of conidia for research can be challenging (Alfenas et al. 2013b; Wang and Chen 2020; Wu and Chen 2021). For this reason, the pathogenicity of many *Calonectria* species has not been determined (Liu et al. 2020). Different isolates of the same *Calonectria* sp. can vary widely in their ability to produce conidia, frustrating attempts to understand isolate-specific pathogenicity and diversity (Wu and Chen 2021). As an alternative, hyphal fragments could be used for pathogenicity tests. These have also previously been utilized to evaluate the pathogenicity of other fungal pathogens that exhibit low or variable conidial production. For example, Li et al. (2007) and Meng et al. (2014) evaluated the pathogenicity of *Sclerotinia sclerotiorum*, the causal agent of Sclerotinia stem rot of oilseed rape and sunflower,

using hyphal fragment suspensions sprayed onto plants. Likewise, Park et al. (2008) used hyphal fragment suspensions to determine the pathogenicity of *Rhizoctonia solani*, the cause of rice sheath blight. Recently, Pham et al. (2021) used the same approach to prove the pathogenicity of *Elsinoe necatrix*, a pathogen that causes *Eucalyptus* scab and shoot malformation. In the case of *Calonectria* species, Guo et al. (2016) compared conidial and hyphal fragment suspensions in pathogenicity tests with *C. pseudonaviculata*, and showed that boxwood plants inoculated with hyphal fragments resulted in the same symptoms as those treated with conidia.

Infection by *Calonectria* spp. is usually initiated with the germination of conidia, microsclerotia or ascospores (Crous 2002). The conidia of *C. pteridis*, *C. pseudoreteaudii* on *Eucalyptus* leaves, and conidia of *C. pseudonaviculata* on boxwood leaves have been shown to produce germ tubes giving rise to mycelium that penetrates leaf tissue directly through the stomata (Graca et al. 2009; Guo et al. 2020; Ye et al. 2017), after which they colonize the tissues to eventually cause leaf blight (Alfenas et al. 2016; Graça et al. 2009; Ye et al. 2017). Johnston and Beute (1975) showed that infection of peanut roots occurs via mycelium developing directly from microsclerotia. These studies suggest that *Calonectria* spp. have no specialized infection structures and that the mycelium causes infections.

On *Eucalyptus*, *Calonectria* species initially infect the leaves causing a blight disease. This disease has caused substantial damage to *Eucalyptus* trees in Asian and South American plantations (Aiello et al. 2020; Bose et al. 2021; Rodas et al. 2005). In China, Calonectria leaf blight has emerged as a serious disease of *Eucalyptus* trees in Fujian, Guangdong, Guangxi, Hainan, and Yunnan Provinces resulting in substantial economic losses to the local forestry industry (Deng et al. 1997; Wang and Chen 2020; Wu and Chen 2021; Zhou and Wingfield 2011; Zhu et al. 2002). For this reason, screening *Eucalyptus* genotypes for tolerance to infection, as well as studies on their biology is a priority for forestry companies.

Calonectria pseudoreteaudii is considered the most important species of *Calonectria* in *Eucalyptus* growing areas of China (Wang and Chen 2020; Wu and Chen 2021). Most *Calonectria* isolates including those of *C. pseudoreteaudii* fail to produce abundant conidia in culture, requiring an alternative procedure for artificial inoculation. The aim of this study was consequently to critically compare inoculation protocols using conidial and hyphal fragment suspensions of this pathogen.

2. MATERIALS AND METHODS

2.1. *Calonectria* isolates and plants for inoculation

Three previously studied *C. pseudoreteaudii* isolates (CSF13317, CSF13636, and CSF16056; Wang and Chen 2020; Wu and Chen 2021), two (CSF13317, CSF13636) of which were used in a study by Wang and Chen (2020) and identified as *C. pentaseptata*, prior to the taxonomic revision of Liu et al (2020) were used in the present study. Previous pathogenicity tests indicated that all three isolates were highly pathogenic on the tested *Eucalyptus* genotypes, and these three isolates all produced abundant conidia when the tests were conducted (Wang and Chen 2020; Wu and Chen 2021). The identification of these three isolates was based on their morphological characteristics as well as phylogenetic analyses using DNA sequences of multiple gene regions (Liu et al. 2020; Wang and Chen 2020; Wu and Chen 2021). In the latter studies, the pathogenicity of these isolates was confirmed by inoculating *Eucalyptus* plants with conidia. The isolates have been preserved in the culture collection of the Research Institute of Fast-growing Trees/China Eucalypt Research Centre of the Chinese Academy of Forestry (CAF) in Zhanjiang, Guangdong Province, China.

Two *Eucalyptus* genotypes, the *E. urophylla* × *E. tereticornis* genotype CEPT1845 and the *E. urophylla* × *E. grandi* genotype CEPT1846, which are widely planted in southern China, were used for inoculations. Plants were obtained in June 2020 from tissue culture and cultivated under greenhouse conditions (20–25 °C and natural photoperiods). The *Eucalyptus* plants were three-months-old and approximately 40 cm in height at the time of inoculation.

2.2. Preparation of inoculum

To prepare conidial suspensions, isolates were cultured on 2% malt extract agar (MEA; 20 g malt extract and 20 g agar per liter of water) in Petri dishes at 25 °C for seven days. Sterile water was then added to the Petri dishes, and a sterilized, soft-bristle paint brush was used to dislodge the mycelium from the agar surface. The water was then drained and the dishes placed upside down in an incubator for 3–4 days at 25 °C. This resulted in large number of conidia being produced on the surface of the cultures as shown for *Calonectria pteridis* by Graça et al. (2009) and *C. pseudoreteaudii* (Wang and Chen 2020; Wu and Chen 2021). Sterile water was then added to the surface of the cultures and conidia were dislodged with sterile soft brush. The retrieved conidial

suspension was transferred to a sterilized beaker, and the concentration of the conidial suspension was measured using a hemocytometer. To prepare a series of conidial concentrations, sterile water was added, and adjusted to four final concentrations of 1×10^5, 5×10^4, 1×10^4, and 1×10^3 conidia/mL. To test for conidial germination, 10 μL of the conidial suspension for each isolate was transferred to fresh MEA medium. After 4 hours, 100 conidia on the MEA medium were randomly selected, and the germinated conidia were counted.

To produce hyphal fragment suspensions, each isolate was cultivated on 2% MEA in a Petri dish at 25 °C for seven days. A sterilized bamboo stick was used to scrape the mycelium from the agar surface and transferred to 2% malt broth medium. After incubation at 25 °C with shaking (200 rpm) for three hours, the mycelial suspension was examined under a microscope to ensure that only mycelial fragments were present. After incubation for additional two days, the mycelial cultures were homogenized at the maximum allowable speed using a homogenizer (VRera, Jiangsu, China), and appropriate time of homogenization was set to derive mycelial fragments of approximately the same length range as those of the conidia (Wang and Chen 2020; Wu and Chen 2021). The fragments from the hyphal suspension were mounted in a drop of sterile water on glass slides and examined under an Axio Imager A1 microscope (Carl Zeiss, Munich, Germany) and an AxioCam ERc 5S digital camera with Zeiss Axio Vision 4.8 software (Carl Zeiss). For each isolate, the lengths of 100 hyphal fragments were measured. Minimum, maximum, and average (mean) values were determined, and the results were presented as (minimum−) (average − standard deviation) − (average + standard deviation) (−maximum).

A spectrophotometer was used to measure the absorbance of the hyphal fragment suspension at a wavelength of 600 nm (ABS_{600}), and the ABS_{600} values were adjusted to 1.0, 0.5, 0.25, 0.125, 0.06, 0.03, and 0.015. The concentration of the hyphal fragment suspension for each of the three *Calonectria* isolates at different absorbance levels was measured using a hemocytometer and the same method as that used to measure the conidial suspensions. To test for growth rate of the hyphal fragments, 10 μL of each suspension was transferred to fresh MEA medium. After 4 hours, 100 hyphal fragments were randomly selected for observation and assessment.

2.3. Inoculation procedures

Inoculation trials were conducted using three *C. pseudoreteaudii* isolates (CSF13317, CSF13636,

and CSF16056) on each of the two *Eucalyptus* genotypes (CEPT1845 and CEPT1846). These plants were inoculated with conidial or hyphal fragments for each of the three isolates at different concentrations. The hyphal fragment concentrations were ABS_{600} = 1.0, 0.5, 0.25, 0.125, 0.06, 0.03, and 0.015, and the concentrations of the conidia were 1×10^5, 5×10^4, 1×10^4, and 1×10^3 conidia/mL. After inoculation, re-isolations were conducted for all treatments and the entire experiment was repeated once.

For inoculations, plants were treated by spraying with either conidia or hyphal fragments. Prior to inoculation, plants were placed in plastic chambers in a greenhouse for 24 hours where the temperature was maintained at 25–27 °C and the humidity at 70%–80%. Plants were then sprayed with conidial or hyphal fragments including the adaxial and abaxial surfaces of the leaves until run-off. For the controls, sterile water was sprayed onto plants in the same manner as for the inoculum. Six plants of each *Eucalyptus* genotype were inoculated for each isolate at each concentration of conidia or hyphal fragments and the same number of plants were included as controls for each of the two experiments. After inoculation, all plants were maintained at 25–27 °C and at 70%–80% humidity throughout the experiment.

For both conidial and hyphal fragment inoculations, a disease index (DI = ∑ (representative rating scale × number of diseased leaves / maximum rating scale (5) × total number of leaves examined) was developed to evaluate the results following the approach of Mishra et al. (2009). The percentage of leaf area with lesions was evaluated for all inoculated leaves using the "Leaf Doctor" software developed by Pethybridge and Nelson (2015). A 0–5 rating scale was thus established where 0 = no lesions, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% leaf area with lesions.

Re-isolations were conducted after lesions were evaluated, for both conidial and hyphal fragment inoculations. For re-isolations, small pieces of discolored leaves (approximately 0.04 cm^2) from the edges of the resultant lesions were cut and placed on 2% MEA at room temperature. Re-isolations were conducted for randomly selected leaves from four randomly selected plants of each *Eucalyptus* genotype across all treatments and controls. Colonies appeared one day after re-isolation and the resulting isolates were identified based on morphological characteristics.

196 **2.4. Data analyses**

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198 The results of the inoculations were assessed after 72 hours and plant susceptibility was compared
199 at different hyphal fragment and conidial concentrations. To test whether the resulting disease index
200 data were normally distributed, a Kolmogorov–Smirnov normality test was conducted using SPSS
201 Statistics v. 22.0. software (IBM Corp., Armonk, NY, USA). Disease indices across treatments
202 were transformed using Rank Transformation in the SPSS Statistics v. 22.0 package, if the resulting
203 disease index data were not normally distributed based on the test. SPSS Statistics v. 22.0. software
204 was used to perform analysis of variance (ANOVA). A first step was to test for differences between
205 the results for the repeated experiments where conidia or hyphal fragments were used. Based on the
206 disease indices resulting from inoculations with the three isolates at all concentrations of hyphal
207 fragments or conidia, on both *Eucalyptus* genotypes, we analyzed the differences in pathogenicity
208 between inoculations and an ANOVA test was conducted.

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210 **3. RESULTS**

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212 **3.1. Viable inoculum properties**

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214 The mycelial cultures were homogenized at 20,000 rpm for two minutes to produce more than 80%
215 of the hyphal fragment with lengths comparable to those of the conidia (69−116 μm) and following
216 the approaches of Wang and Chen (2020) and Wu and Chen (2021). Average hyphal fragment
217 lengths for isolates CSF13317, CSF13636, and CSF16056 were consequently128 μm, 119 μm and
218 79 μm, respectively (Table 1). The germination/growth assay showed that the conidia and hyphal
219 fragments for the three studied isolates were viable (Fig. 1), with germination percentages being
220 97%, 98%, and 98% for the conidia, and growth rates being 89%, 88%, and 95% for the hyphal
221 fragments of isolates CSF13317, CSF13636, and CSF16056 respectively. The average
222 concentrations of the hyphal fragments for the three *Calonectria* isolates at ABS_{600} 1.0, 0.5, 0.25,
223 0.125, 0.06, 0.03, and 0.015 were 1.5×10^5, 6.9×10^4, 4.2×10^4, 2.5×10^4, 9.7×10^3, 6.1×10^3, and
224 2.8×10^3 hyphal fragments/mL, respectively (Table 2).

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226 **3.2. Similarity between the inoculation methods**

The two *Eucalyptus* genotypes inoculated with either the hyphal fragments or conidia displayed symptoms 24 hours after inoculation. All inoculated *Eucalyptus* plants displayed similar symptoms including leaf spots, and leaf blight, while the control plants showed no disease symptoms. The leaves were assessed 72 hours after inoculation and the disease indices were determined (Fig. 2 and Fig. 3). Both the hyphal fragment and conidial inoculum gave rise to lesions and symptoms were consistently more severe at higher concentrations of inoculum (Fig. 4). *Calonectria pseudoreteaudii* was re-isolated from the inoculated leaves, irrespective of whether they had been treated with conidia or hyphal fragments and the pathogen was never isolated from the controls.

The disease index data were not normally distributed based on a Kolmogorov–Smirnov normality test ($P < 0.05$). Thus, all the data for the disease indices were transformed (Kolmogorov–Smirnov normality test, $P = 0.2$) by conducting a Rank transformation using the SPSS Statistics v. 22.0.

The ANOVA results showed that the results of the replicated pathogenicity tests with hyphal fragments or conidial suspensions were not significantly different ($P > 0.05$). Thus, data for the two experiments with either the hyphal fragments or conidia were pooled for subsequent analyses.

3.3. Isolate and *Eucalyptus* genotypic effect on inoculation and resultant blight symptoms

Results of each of the conidial or hyphal fragment inoculation with each of the three isolates on each of two *Eucalyptus* genotypes, showed that isolate CSF13636 was more pathogenic than isolates CSF13317 and CSF16056, with the exception of the inoculation on CEPT1846 using 1×10^3 conidia/mL (Fig. 5). Similarly, at each concentration of conidia or hyphal fragments, isolates CSF13317, CSF13636, and CSF16056, resulted in consistently larger lesions and greater lesion numbers on *Eucalyptus* genotype CEPT1845 than those of *Eucalyptus* genotype CEPT1846 (Fig. 5).

3.4. Effect of inoculum type concentrations on blight infection

The statistical results showed that the pathogenicity of the three isolates was different and the two *Eucalyptus* genotypes differed in their tolerance to infection. Furthermore, the three isolates all

showed a decreasing trend in levels of infection with decreasing concentrations of conidia or hyphal fragments (Fig. 5). For each *Eucalyptus* genotype inoculated with each isolate, disease indices associated with hyphal fragment inoculation at a concentration of $ABS_{600} = 1.0$ was not significantly different from that associated with inoculation using conidia at a concentration of 5×10^4 conidia/mL ($P > 0.05$). This was with the exception of isolates CSF13317 and CSF16056 inoculated on CEPT1846. Disease indices for hyphal fragment inoculations at a concentration of $ABS_{600} = 0.25$ were not significantly different to those for the conidial inoculum at a concentration of 1×10^4 conidia/mL ($P > 0.05$). In this case, the exception was for isolate CSF13636 inoculated on CEPT1845. Disease indices for inoculations with the hyphal fragment suspensions at a concentration of $ABS_{600} = 0.015$ were not significantly different to those for the conidial suspension at the concentration of 1×10^3 conidia/mL ($P > 0.05$) (Fig. 5).

4. DISCUSSION

In this study, a detailed comparison of inoculations with the important *Eucalyptus* pathogen *C. pseudoreteaudii* using either conidia or hyphal fragments showed that the two techniques provide similar results. This was confirmed using three different isolates of the pathogen on two different *Eucalyptu*s genotypes. The results provide evidence that *C. psuedoreteaudii* inoculations can be achieved relying on hyphal fragments when isolates do not produce conidia in culture. This will be valuable in future studies where it is necessary to assess *C. psuedoreteaudii* relative pathogenicity on *Eucalyptus* genotypes important to *Eucalyptus* forestry.

The *Eucalyptus* genotypes inoculated in this study with either hyphal fragments or conidia showed symptoms, such as leaf blight, similar to those of Calonectria leaf blight on *Eucalyptus* in plantations. A previous study by Guo et al. (2016) also successfully used inoculations with *C. pseudonaviculata* hyphal fragments, making it possible to distinguish resistant genotypes of boxwood. The present study supports Guo et al. (2016) finding and provides critical quantitative data.

An important consideration when using hyphal fragments for inoculation is to effectively quantify the concentration of these fragments to ensure consistency and repeatability of the method. Two methods have commonly been used to achieve this goal. These include using a spectrophotometer

to measure the absorbance value of the suspension (Granade and Artis 1980; Guidry and Trelles 1962; Pinzari et al. 2017; Zang et al. 2010) or by using a hemocytometer to count propagules (Li et al. 2007; Markantonatou et al. 2020; Park et al. 2008; Pham et al. 2021). Hyphal fragment suspensions prepared by any of these methods produced symptoms on the inoculated plants (Guo et al. 2016; Markantonatou et al. 2020; Park et al. 2008; Pham et al. 2021; Zang et al. 2010). Steps such as breaking hypha into lengths similar to size of the conidia and using a spectrophotometer to quantify the concentration of the hyphal fragment suspension, which was done in the present study, should ensure consistency and reproducibility of these assays.

A reliable inoculation technique requires confidence that the conidia and hyphal fragments are equally viable. Therefore, it is necessary to test for germination and hyphal fragment growth in order to utilize the hyphal fragment inoculation method. Measuring the viability of the hyphal fragments and conidia of *C. pseudoreteaudii* in the present study showed that these structures maintained their activity after homogenization. Importantly, the hyphal fragments were used shortly after homogenization. It is also important to consider the germination of conidia is different for different species of *Calonectria* (Daughtrey 2019; Liu et al. 2021; Sharma and Mohanan 1990) and can be influenced by environmental factors (Crous 2002; Daughtrey 2019; Miller et al. 2018). Careful attention must consequently be given to factors that have been shown to negatively impact inoculum viability when conducting inoculation studies either with conidia or hyphal fragments.

Results of this study demonstrate that suspensions of hyphal fragments can be used in lieu of conidial suspensions to evaluate the pathogenicity of *C. pseudoreteaudii* on *Eucalyptus* genotypes. In previous tests to evaluate the pathogenicity of *Calonectria* species, conidial suspension concentrations of 5×10^4 conidia/mL (Van Laere et al. 2019; Wu and Chen. 2021) or 1×10^4 conidia/mL (Freitas et al. 2019) have been used. For the *C. pseudoreteaudii* pathogenicity assay conducted on *Eucalyptus* in the present study, a hyphal fragment suspension concentration of ABS600 = 1.0 could be used in lieu of a conidial suspension with a concentration of 5×10^4 conidia/mL to evaluate pathogenicity via inoculation. These results make it possible to undertake studies related to the infection biology of *Calonectria* species including those that could lead to the identification of disease-tolerant planting stock.

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Figure Legends

Figure 1. *Calonectria pseudoreteaudii* conidia and hyphal fragments used in this study. **a–d**, Germinated macroconidia. **e–h**, Hyphal fragments after homogenization. **i–l**, Elongated hyphae. Scale bar: **a–l** = 20 μm. Red arrows indicate the conidial germination and hyphal fragment growth. **a–d, and k:** isolate CSF13317; **e–j, and l:** isolate CSF16056.

Figure 2. The rating scales for non-infected and infected leaves of *Eucalyptus* genotype CEPT1845. **a–b**, Scale 0, no lesions. **c, e, g, i, and k**, Scales 1, 2, 3, 4, and 5, respectively. **d, f, h, j, and l**, Leaf Doctor (Pethybridge and Nelson 2015) scanning images of **c, e, g, i, and k,** respectively.

Figure 3. The rating scales for non-infected and infected leaves of *Eucalyptus* genotype CEPT1846. **a–b:** scale 0, no lesions. **c, e, g, i, and k:** Scales 1, 2, 3, 4, and 5, respectively**. d, f, h, j, and l:** Leaf Doctor (Pethybridge and Nelson 2015) scanning images of **c, e, g, i, and k,** respectively.

Figure 4. Symptoms on leaves of *Eucalyptus* genotype CEPT1846 after inoculation with sterile water, conidia suspensions, and hyphal fragment suspensions. **a–b**, Scale 0, no lesions, after being spay-inoculated with the sterile water**. c, e, g, i, and k**, representative symptoms of scales 1, 2, 3, 4, and 5 leaves, respectively, after being spay-inoculated with the conidial suspensions. **d, f, h, j, and l**, Representative symptoms of scales 1, 2, 3, 4, and 5 leaves, respectively, after being spay-inoculated with the hyphal fragment suspensions.

Figure 5. Disease index on *Eucalyptus* genotype CEPT1845 and CEPT1846 resulting from inoculation with different concentrations of hyphal fragment suspension and conidial suspension with each isolate of CSF13317, CSF13636, and CSF16056 (*Calonectria pseudoreteaudii*), and the controls. Error bars represent standard error of the means. Bars topped with different numbers indicate treatment means that are significantly different ($P < 0.05$). "*" represents no lesions produced by the negative controls.

Table 1. Mycelial and conidial lengths of the three *Calonectria pseudoreteaudii* isolates used in this study.

Isolate	Hyphal fragments or conidia	Length[a]	Average of length	Reference
CSF13317	Hyphal fragments	(64–) 94.5–162 (–200) µm	128 µm	This study
CSF13636	Hyphal fragments	(49–) 84–153.5 (–197.5) µm	119 µm	This study
CSF16056	Hyphal fragments	(32–) 42.5–99 (–184) µm	79 µm	This study
CSF13317	conidia	(83–) 94–107 (–116) µm	100.5 µm	Wang and Chen 2020
CSF13636	conidia	(69–) 77.5–90.5 (–101) µm	84 µm	Wang and Chen 2020
CSF16056	conidia	(77.5–) 87–104.5 (–112.5) µm	96 µm	Wu and Chen 2021

[a] Measurements are presented in the format [(minimum–) (average – standard deviation) – (average + standard deviation) (–maximum)].

Table 2. The mycelial fragments concentrations of three *Calonectria pseudoreteaudii* isolates at different ABS_{600}.

ABS_{600}	Isolate			Average
	CSF13317[a]	CSF13636	CSF16056	
1	1.2×10^5	1.5×10^5	1.6×10^5	1.5×10^5
0.5	4.9×10^4	7.6×10^4	8.1×10^4	6.9×10^4
0.25	3×10^4	4.5×10^4	5.2×10^4	4.2×10^4
0.125	1.8×10^4	3×10^4	2.8×10^4	2.5×10^4
0.06	7.8×10^3	1.3×10^4	8.8×10^3	9.7×10^3
0.03	5.4×10^3	7.8×10^3	5×10^3	6.1×10^3
0.015	2.8×10^3	3.6×10^3	2×10^3	2.8×10^3

[a] Measurements: mycelial fragments/mL.

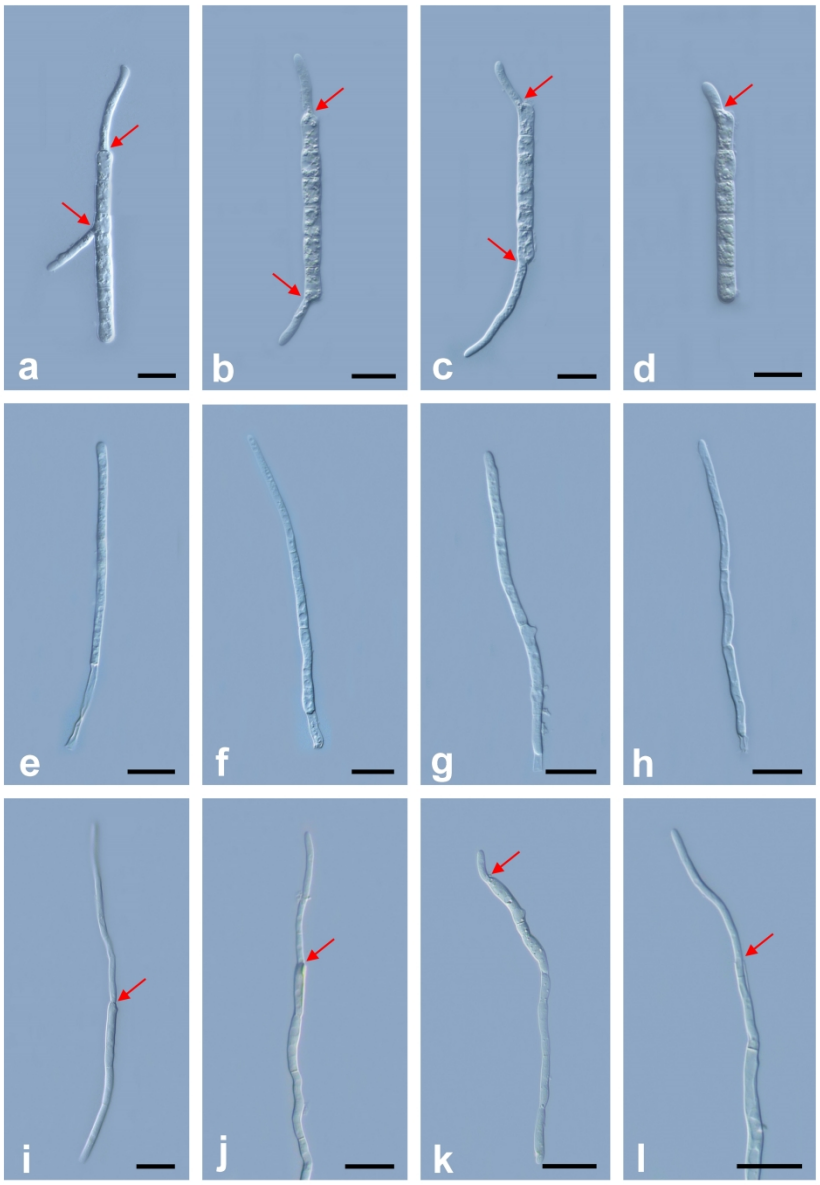

Figure 1. Calonectria pseudoreteaudii conidia and hyphal fragments used in this study. a–d, Germinated macroconidia. e–h, Hyphal fragments after homogenization. i–l, Elongated hyphae. Scale bar: a–l = 20 µm. Red arrows indicate the conidial germination and hyphal fragment growth. a–d, and k: isolate CSF13317; e–j, and l: isolate CSF16056.

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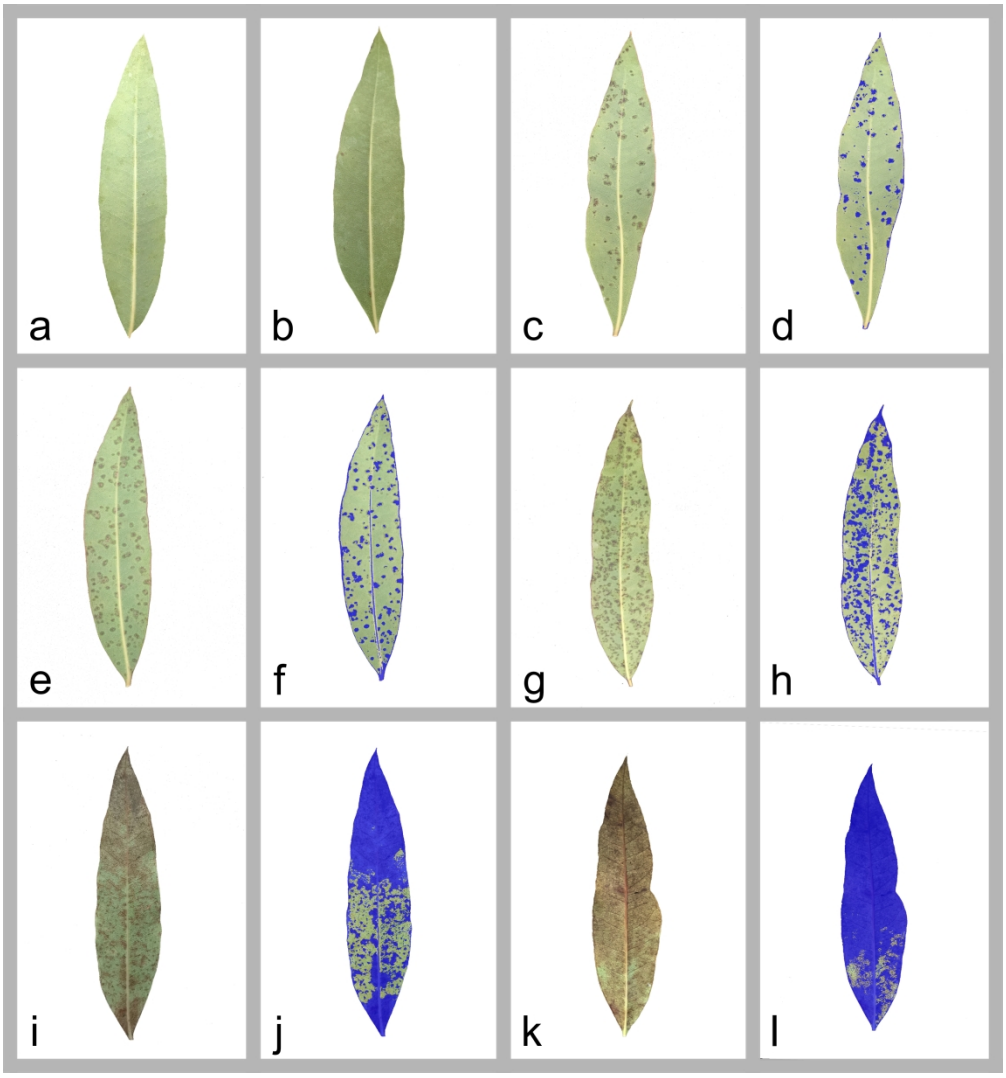

Figure 2. The rating scales for non-infected and infected leaves of Eucalyptus genotype CEPT1845. a–b, Scale 0, no lesions. c, e, g, i, and k, Scales 1, 2, 3, 4, and 5, respectively. d, f, h, j, and l, Leaf Doctor (Pethybridge and Nelson 2015) scanning images of c, e, g, i, and k, respectively.

178x190mm (600 x 600 DPI)

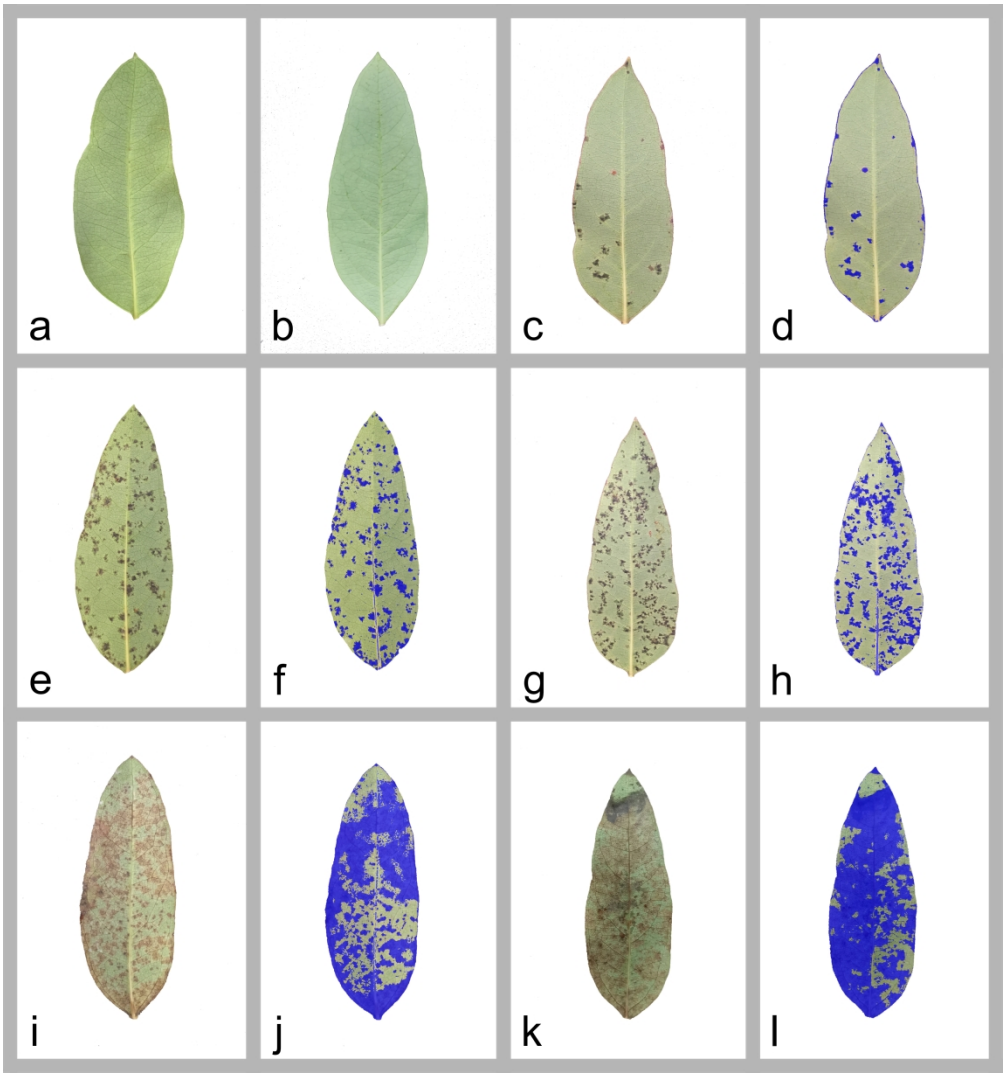

Figure 3. The rating scales for non-infected and infected leaves of Eucalyptus genotype CEPT1846. a–b: scale 0, no lesions. c, e, g, i, and k: Scales 1, 2, 3, 4, and 5, respectively. d, f, h, j, and l: Leaf Doctor (Pethybridge and Nelson 2015) scanning images of c, e, g, i, and k, respectively.

178x190mm (600 x 600 DPI)

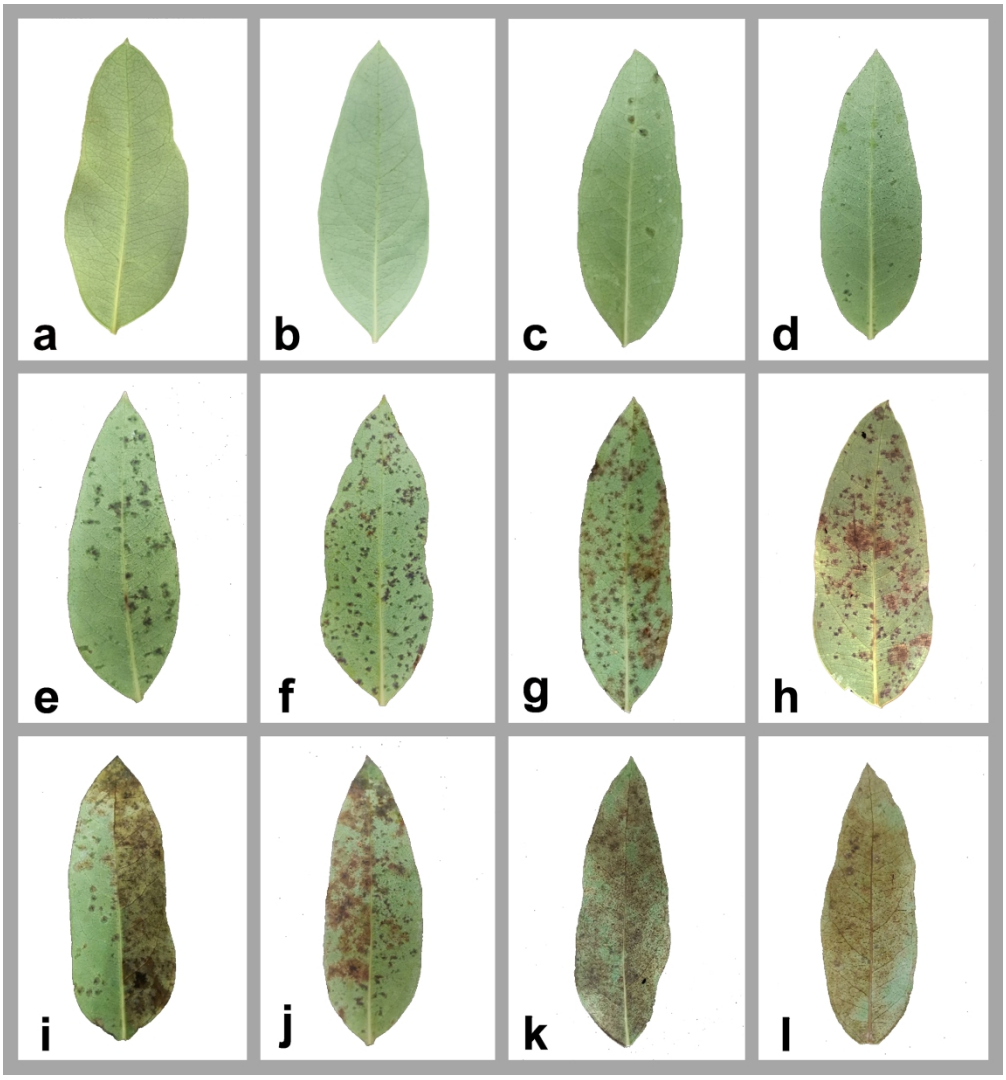

Figure 4. Symptoms on leaves of Eucalyptus genotype CEPT1846 after inoculation with sterile water, conidia suspensions, and hyphal fragment suspensions. a–b, Scale 0, no lesions, after being spay-inoculated with the sterile water. c, e, g, i, and k, representative symptoms of scales 1, 2, 3, 4, and 5 leaves, respectively, after being spay-inoculated with the conidial suspensions. d, f, h, j, and l, Representative symptoms of scales 1, 2, 3, 4, and 5 leaves, respectively, after being spay-inoculated with the hyphal fragment suspensions.

178x190mm (600 x 600 DPI)

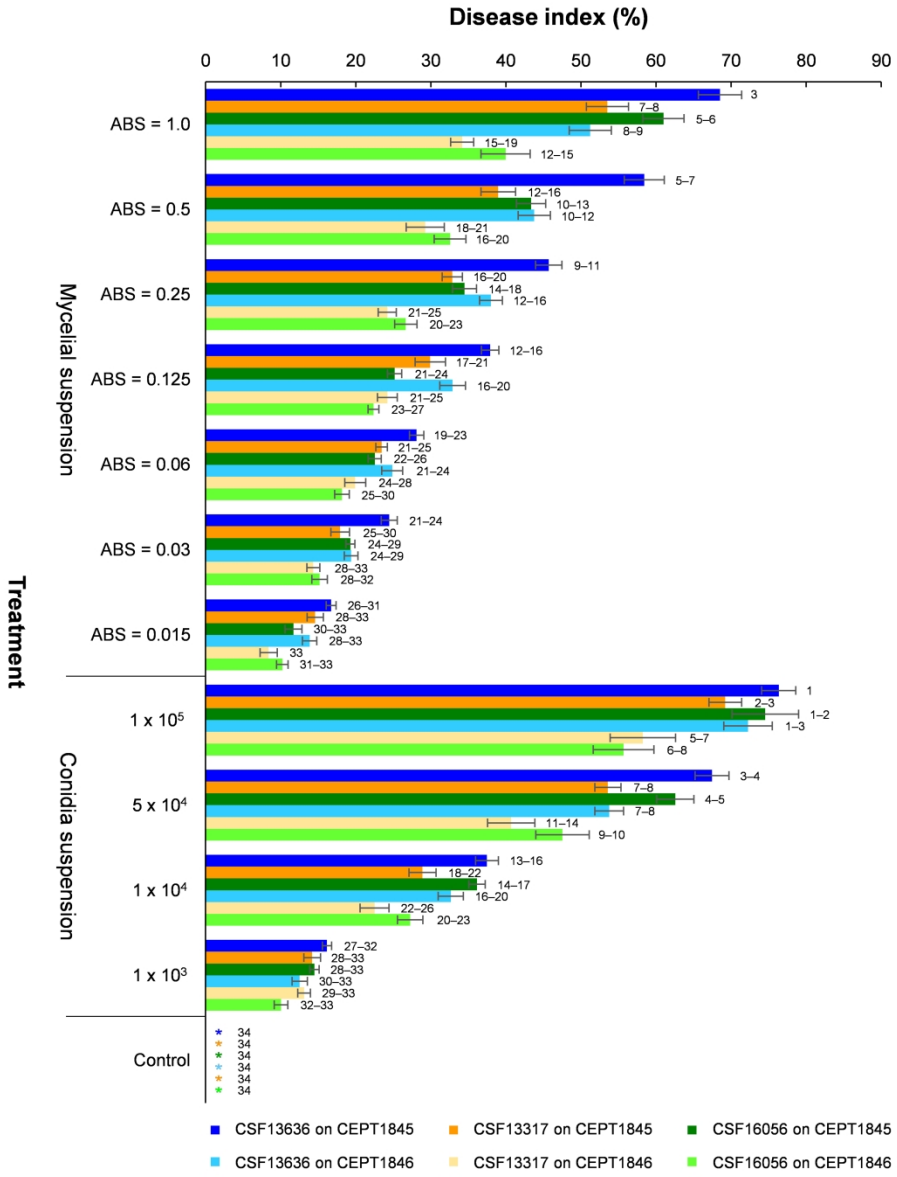

Figure 5. Disease index on Eucalyptus genotype CEPT1845 and CEPT1846 resulting from inoculation with different concentrations of hyphal fragment suspension and conidial suspension with each isolate of CSF13317, CSF13636, and CSF16056 (Calonectria pseudoreteaudii), and the controls. Error bars represent standard error of the means. Bars topped with different numbers indicate treatment means that are significantly different ($P < 0.05$). "*" represents no lesions produced by the negative controls.

178x230mm (600 x 600 DPI)