Journal of Tropical Forest Science 35(1): 1–9 (2023)
https://doi.org/10.26525/jtfs2023.35.1.1
ISSN: 0128-1283, eISSN: 2521-9847

DISEASE RESISTANCE OF EUCALYPT CLONES TO *CERATOCYSTIS MANGINECANS*

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Submitted July 2021; accepted May 2022

Ceratocystis manginecans is causing serious damage to *Eucalyptus* plantations in Vietnam. When the stems of 8-month-old ramets of two widely-planted *Eucalyptus* clones were inoculated with nine *C. manginecans* isolates in a nursery trial, all caused disease. Using the same methodology, the two most pathogenic isolates were used to rank the resistance of 18 *Eucalyptus* clones. Significant ($p < 0.001$) differences among clones were observed for both lesion length and percentage mortality, 90 days after inoculation. Three *Eucalyptus* hybrid clones and seven *E. urophylla* clones were highly resistant, with mean lesion length from 0.99 to 2.75 cm and mortality ranging from 0 to 5.0%. The remaining eight clones, including the two clones used to screen the *C. manginecans* isolates, displayed low to moderate resistance with mean lesion length exceeding 5 cm and mortality ranging from 15.0 to 41.7%. This suggests that there are opportunities to deploy *Eucalyptus* hybrid and *E. urophylla* clones, resistant to *C. manginecans,* to enhance disease resistance through crossing among resistant genotypes.

Keywords**:** *Ceratocystis manginecans*, clone, *Eucalyptus*, inoculation, disease resistance

INTRODUCTION

Ceratocystis species are primary pathogens of *Eucalyptus* that have caused wilt disease in plantations of *Eucalyptus* hybrid in Congo; *E. grandis* in South Africa, Uruguay and South Africa, and together with related hybrids in Brazil and China (Roux et al. 2000, Roux et al. 2004, Van-Wyk et al. 2010, Ferreira et al. 2013, De Beer et al. 2014, Li et al. 2014). Wilt symptoms in *Eucalyptus* have been associated with attack by many *Ceratocystis* species including *C. eucalypti*, *C. pirilliformis*, *C. atrox*, *C. sublaevis*, *C. chinaeucensis*, *C. cercfabiensis* and *C. manginecans* (Kile et al. 1996, Barnes et al. 2003, Van-Wyk et al. 2007a, Van-Wyk et al. 2011, Chen et al. 2013, Liu et al. 2015). They have been found to be a causal agent of fungal wilt disease in *Eucalyptus* plantations in Vietnam (Thu et al. 2021, Trang et al. 2022).

Ceratocystis manginecans has been identified as a serious pathogen of many tree species in several plant families (Van-Wyk et al. 2007b, Al-Adawi et al. 2013, Pornsuriya & Sunpapao 2015, Thu et al. 2021). *Ceratocystis manginecans* was first reported causing wilt disease in *Mangifera indica* in Oman and Pakistan (Van-Wyk et al. 2007b). It has also been recorded as a wilt pathogen on *Prosopis cineraria* in Oman, *Dalbergia sissoo* in Pakistan, and *Mimusops elengi* in Thailand (Al-Adawi et al. 2013, Pornsuriya & Sunpapao 2015). It is commonly recorded in *Acacia* plantations in Indonesia and Vietnam, and also in *Dalbergia* and *Chukrasia* plantations in Vietnam (Tarigan et al. 2011, Chi et al. 2019b, Chi et al. 2021, Thu et al. 2021, Trang et al. 2022).

Acacia dominates timber production in Vietnam, with over 2 million hectares of plantations, however, several thousand hectares of *Acacia* plantations have been severely affected by wilt disease caused by *C. manginecans* and subsequently replaced by *Eucalyptus* (Bon et al. 2020, Chi et al. 2020, Thu et al. 2021, Chi 2022a). The area of *Eucalyptus* plantations in Vietnam has increased from 200,000 ha in 2013 to about 400,000 ha by 2020 (Harwood & Nambiar 2014, Thu et al. 2021).

In Brazil, variation in disease resistance to *Ceratocystis* species has been demonstrated within *E. grandis*, *E. saligna* and *E. urophylla*, and among clones of *E. grandis* × *urophylla* hybrid (Zauza et al. 2004, Rosado et al. 2009, Firmino et al. 2013). This suggests the potential to select and breed varieties with sufficient resistance to manage wilt disease in the future.

Since 1990, more than 60 high-yielding *Eucalyptus* seed sources and clones have been selected in Vietnam (Anh 2020, Hai & Dao 2013). However, no reports evaluating the resistance of these selected *Eucalyptus* varieties to *Ceratocystis* have been published to date. As a first step towards understanding and ranking the vulnerability of selected *Eucalyptus* varieties in Vietnam to *C. manginecans* wilt disease, the study investigated the pathogenicity of nine *C. manginecans* isolates from *Eucalyptus* plantations in Vietnam by applying them in the nursery to young plants of two commercial eucalypt clones. The two most virulent of these isolates were then used to rank eighteen selected *Eucalyptus* clones including the two commercial clones, for their resistance to *C. manginecans*.

MATERIALS AND METHODS

Fungal material

Nine *C. manginecans* isolates, six from *E. urophylla* plantations in five provinces in Vietnam and three from *E. camaldulensis* plantations in two provinces, were ranked for pathogenicity in the first part of the study (Table 1) (Chi & Thu 2016, Trang et al. 2022).

Plant material

Two of the most widely planted commercial eucalypt clones (*Eucalyptus* hybrid clone U6 and *E. urophylla* clone PN14) were used for the ranking of the pathogen isolates. These two clones, and an additional 16 eucalypt clones (Table 2) were evaluated for their resistance to the two most virulent isolates. Origins of the clones and their productivity in clonal field trials are presented in Table 2. More than 60 varieties of *Eucalyptus* (52 clones and 12 seed sources) are recognised as national or technically advanced varieties in Vietnam (Hai & Dao 2013, MARD 2017). However, many of them are difficult to clone. The clones selected for this study are all easy to propagate via stem cuttings (Anh 2020).

Pathogenicity testing of isolates

The pathogenic potential of nine isolates was evaluated by the under-bark inoculation method described by O'Gara et al. (1997) using eight-month-old ramets of clones U6 and PN14.

The clonal ramets were set out in a randomised complete block design with five blocks, each located in a separate plastic misthouse, 2.0 m in height. Each block comprised a total of 120 trees (10 rows x 12 trees), with the nine *Ceratocystis* isolates and control allocated at random to each row, which had 6-tree row plots of both clones. Ramets were planted in sun-pasteurised soil at a distance of 20 cm within rows and 40 cm between rows. Inorganic fertiliser (NPK 20-20-20 + micronutrients) dissolved in water (30 mg L^{-1}) was applied to the soil every 10 days. At the time

Table 1 Pathogen background of the *Ceratocystis manginecans* isolates (Chi & Thu 2016, Trang et al. 2022)

Isolate	Eucalyptus host	Location (district/province)	GenBank number
E101	*Eucalptus urophylla*	Phuc Yen, Vinh Phuc	MW386081
E103	*Eucalptus camaldulensis*	Lap Thach, Vinh Phuc	MW386082
E104	*Eucalptus camaldulensis*	Lap Thach, Vinh Phuc	MW386083
E106	*Eucalptus urophylla*	Son Duong, Tuyen Quang	MW386084
E244	*Eucalptus urophylla*	Dai Tu, Thai Nguyen	MW386085
E253	*Eucalptus urophylla*	Dai Tu, Thai Nguyen	MW386086
E256	*Eucalptus urophylla*	Phu Ninh, Phu Tho	MW386087
E264	*Eucalptus urophylla*	Cam Lo, Quang Tri	MW386088
E273	*Eucalptus camaldulensis*	Nghia Dan, Nghe An	MW386089

Table 2 Genetic background of the selected *Eucalyptus* clones and their productivities in clonal field trials

Taxon	Clone	Origin of clone*	Classified variety**
Eucalptus urophylla × *Eucalptus grandis*	DH32-29	China	Technically advanced clone[8]
Eucalptus urophylla × *Eucalptus pellita*	PNCT3	China	Technically advanced clone[6]
Eucalptus urophylla × *Eucalptus pellita*	$PNCT_{IV}$	China	Technically advanced clone[6]
Eucalptus urophylla × *Eucalptus camadulensis*	U6	China	Technically advanced clone[2]
Eucalptus urophylla	E15	Vietnam	Technically advanced clone[9]
Eucalptus urophylla	E28	Vietnam	Technically advanced clone[9]
Eucalptus urophylla	PN3d	Vietnam	National advanced clone[5]
Eucalptus urophylla	PN10	Vietnam	Technically advanced clone[4]
Eucalptus urophylla	PN21	Vietnam	Technically advanced clone[5]
Eucalptus urophylla	PN24	Vietnam	Technically advanced clone[5]
Eucalptus urophylla	PN46	Vietnam	Technically advanced clone[3]
Eucalptus urophylla	PN47	Vietnam	Technically advanced clone[3]
Eucalptus urophylla	PN54	Vietnam	National advanced clone[7]
Eucalptus urophylla	PN108	Vietnam	National advanced clone[7]
Eucalptus urophylla	PN116	Vietnam	Technically advanced clone[4]
Eucalptus urophylla	NC3	Vietnam	New clone
Eucalptus urophylla	QY23	Vietnam	New clone
Eucalptus urophylla	PN14	Vietnam	Technically advanced clone[1]

* China = clone imported from China, Vietnam = from family trial in Vietnam (Arnold et al. 2020, Hai & Dao 2013, MARD 2017); ** advanced clones were recognised by Vietnam's Ministry of Agriculture and Rural Development, Decision No: [1]3645/QĐ/BNN-KHCN dated 28/12/1998, [2]2159/QĐ-BNN-KHCN dated 15/6/1999, [3]2722/QĐ/BNN-KHCN dated 7/9/2004, [4]1773/QĐ-BNN-TCLN dated 19/7/2005, [5]1686/QĐ/BNN- KHCN dated 9/6/2006, [6]388/QĐ-BNN-TCLN dated 7/3/2014, [7]3893/QĐ/BNN-TCLN dated 20/9/2016, [8]4572/QĐ-BNN-TCLN dated 8/11/2017 and [9]1734/QĐ-BNN-TCLN dated 23/4/2021

of inoculation, plants had attained heights of over 70 cm and stem diameters of 0.7–0.8 cm, 30 cm above ground. A 4 mm diameter potato dextrose agar (PDA) plug with 14-day-old mycelia was placed under the bark of each stem with the mycelia towards the wood, ca. 10 cm above the ground, covered with moist, sterile cotton wool and sealed with paraffin tape. The PDA without mycelium was used as control treatment.

Lesion length (L) on each plant and percentage of dead plants (D) were recorded 30, 60 and 90 days after inoculation; by day 90 about half of the total number of plants developed symptoms of infection. For each of the nine isolates, a single wood sample was taken from one randomly selected infected plant. These samples were collected from the lesion-affected stem, distant from the inoculation wound. The pathogen was re-isolated from each sample and sequenced. The assessment was carried out according to the method described by Trang et al. (2022), using two β-tubulin primers: βT1a (TTCCCCCGTCTCCACTTCTTCATG), and βT1b (GACGAGATCGTTCATGTTGAACTC).

Based on mean lesion length at day 90, pathogenicity was assigned to one of five categories: L = 0 cm (nil), L ≤ 3 cm (weak), 3 cm < L ≤ 6 cm (average), 6 cm < L ≤ 9 cm (strong) and L > 9 cm (very strong).

Screening eucalypt clones for disease resistance

Screening of the eighteen *Eucalyptus* hybrid and *E. urophylla* clones for disease resistance was evaluated using the same under-bark inoculation method. Two *C. manginecans* isolates (E256 and E273) which were strongly pathogenic in the pathogenicity test, and a control treatment using PDA without mycelium, were used. The plants were set out in a randomised complete block design with five blocks, each block being

located in a separate plastic mist house. Each block had 18-tree clonal line plots with six plants allocated to each of the two isolates and the control treatment. Plant spacing, age and size at inoculation, and the timing and method of assessment for L and D were the same as for the pathogenicity test described above.

In addition, disease resistance (T) of each individual plant at day 90 was classed at one of the 5 levels: 5 = very strong, no lesions on stem, leaves healthy and green; 4 = strong leaves healthy and green; 3 = moderate, some leaf yellowing; 2 = weak, all leaves yellow and 1 = nil, leaves wilted, dried, fallen and/or dead (Figure 1).

Data analysis

First, plot mean values of all response variates were calculated. The development of symptoms over time in the first experiment (pathenogenicity testing of isolates) was examined by graphing mean L and D for each isolate at days 30, 60 and 90. Univariate analysis of variance (ANOVA) was then conducted on plot means for L and D (both experiments) and T (screening of *Eucalyptus* clones) using Release 12.1 of the GenStat software package. For both experiments, analysis of variance used replicate as the block factor and isolate × clone in factorial combination as the treatment structure. The analyses tested the significance of the main treatment effects (isolate and clone) and their interaction. In both experiments, the control PDA plug without pathogen inoculum did not result in any disease lesions or mortality, thus data from the control treatment was omitted from the statistical analyses. Plots of residual versus fitted values were examined to check that residuals were normally distributed. Duncan's Multiple Range Test was used for comparisons among treatments (*Ceratocystis* isolates in the pathogenicity test and clones in the clone screening test).

RESULTS

Pathogenicity of *Ceratocyctis manginecans* isolates

All nine *C. manginecans* isolates formed lesions on both *Eucalyptus* hybrid (U6) and *E. urophylla* (PN14) ramets. The nine re-isolated pathogen samples were all identified as *C. manginecans*.

At day 30, isolates differed significantly ($p < 0.001$) for L, but not for D. At both day 60 and day 90, isolates differed significantly for both L and D ($p < 0.001$). Averaged across the nine isolates, mean L was 1.42 cm at day 30, 3.49 cm at day 60 and 7.45 cm at day 90, while the corresponding means for D were 0.7, 7.0 and 23.9%, respectively. Clearly, day 90 was a more appropriate timing for assessment than days 30 or 60 as damage symptoms developed progressively to time. The development of L and D over time in the pathogenicity testing of the nine pathogen isolates is presented in Figure 2.

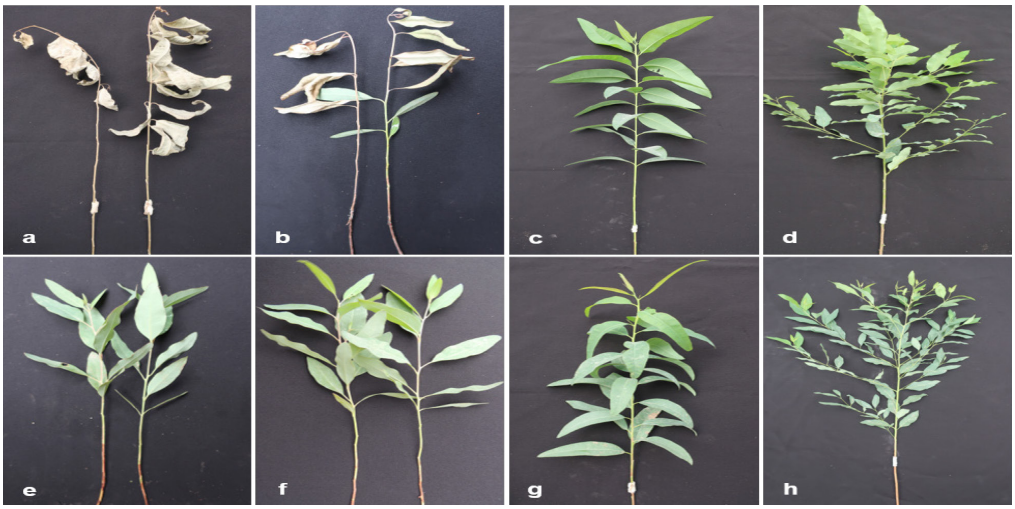

Figure 1 Responses at day 90 in the clone screening experiment to under-bark inoculation with mycelium of *Ceratocystis manginecans* isolate E256: a = susceptible clone PN14, resistance score 1 for both ramets, b = susceptible clone PN21, resistance scores of ramets 1 and 2, c & d = resistant clones PN108 and PNCT3, resistance scores 5

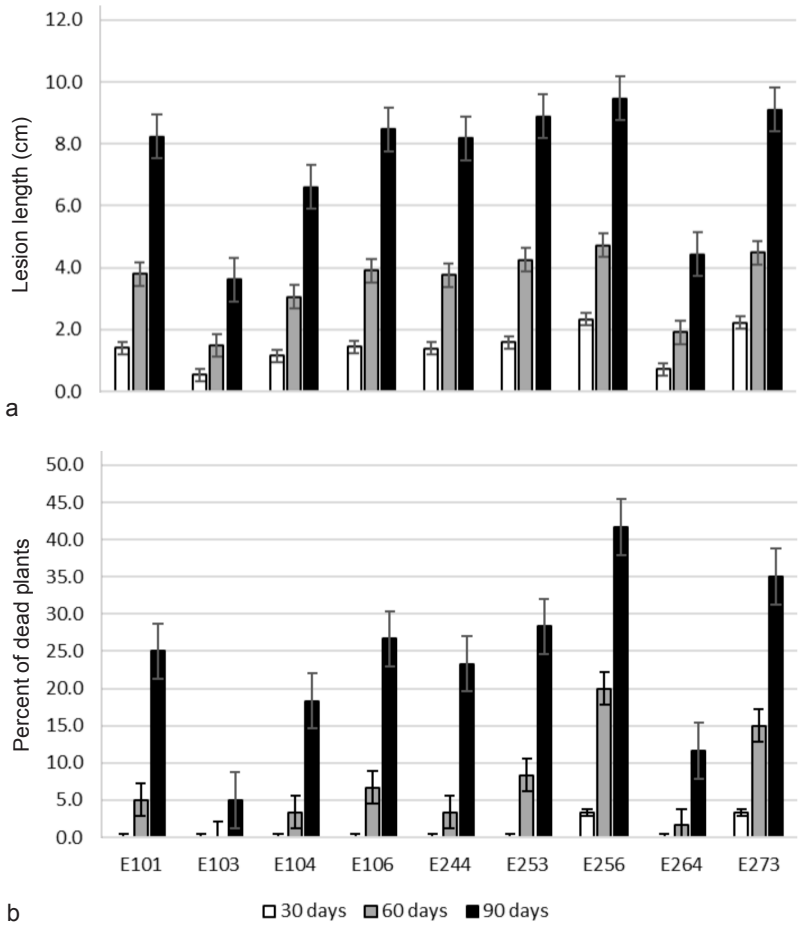

Figure 2 Pathogenicity of *Ceratocystis manginecans* isolates 30, 60 and 90 days after inoculation (mean of clones PN14 and U6): a = lesion length, b = percent of dead plants, error bars are critical difference

The effects of clone, and isolate × clone, were non-significant ($p > 0.05$) in the analysis of variance for L, D and T. Disease symptoms for the two *Eucalyptus* clones were very similar for all nine isolates. Figure 2 and Table 3 therefore present mean L and D for each isolate averaged for the two clones.

Based on the intervals used to classify L after 90 days of inoculation, the nine isolates fell into three groups: very strong (isolates E256 and E273), strong (E101, E104, E106, E244 and E253), and average (E103, E264). Duncan multiple range tests indicated that isolates E256 and E273 both were significantly ($p < 0.05$) higher for L than some, but not all of the five isolates ranked as strong, while isolate E256, but not E273, had significantly higher mortality than all five strong isolates. As isolates E256 and E273 ranked highest for both L and D, they were selected for the screening experiment.

Screening of *Eucalyptus* clones

By day 90, all 18 clones developed visible lesions from artificial inoculation with the selected isolates. There were significant ($p < 0.001$) differences among the clones for L, D and T. The two pathogen isolates did not differ significantly ($p > 0.05$) in their effects on L, D or T, nor was the interaction between isolate and clone significant for any of the response variates. Table 4 therefore presents the mean lesion length, mortality and resistance score for each clone, averaged across the two isolates. Clone mean values of L ranged from 0.99 to 9.55 cm, means for D ranged from 0 to 41.7%, and means for T ranged from 1.25 to 4.58 (Table 4).

Based on mean lesion length, survival and resistance, clones were grouped into four overall resistance classes: strong (10 clones), moderate (1 clone), weak (3 clones) and nil

Table 3 Pathogenicity of *Ceratocystis manginecans* isolates 90 days after inoculation of two hosts, clones PN14 and U6

Isolate	L (cm)	D (%)	Pathogenicity
E256	9.47 ± 0.82^{d}	41.7^{f}	Very strong
E273	9.11 ± 0.91^{cd}	35.0^{ef}	Very strong
E253	8.89 ± 1.45^{cd}	28.3^{de}	Strong
E106	8.47 ± 0.62^{c}	26.7^{d}	Strong
E101	8.24 ± 0.63^{c}	25.0^{cd}	Strong
E244	8.18 ± 0.81^{c}	23.3^{cd}	Strong
E104	6.61 ± 0.86^{b}	18.3^{bc}	Strong
E264	4.43 ± 0.50^{a}	11.7^{ab}	Average
E103	3.62 ± 1.20^{a}	5.0^{a}	Average
Control (PDA)	0.00	0.0	Nil
Mean of isolates	7.45	23.89	
LSD	0.92	7.29	
p	< 0.001	< 0.001	

The values are the means for the two clones; values followed by the same letters in a column are not different among isolates at p = 0.05 according to Duncan's multiple range test; L (cm) = lesion length, D (%) = percent of dead plants, Un = inoculated control treatment excluded from statistical analysis

Table 4 Mean lesion length (L), percent of dead plants (D), and disease resistance score (T) of 18 *Eucalyptus* clones after inoculation with two *Ceratocystis manginecans* isolates, E256 and E273

Clone	L (cm)	D (%)	T	Overall resistance class
E28	0.99^{a}	3.3^{a}	4.58^{d}	Strong
DH32-29	1.40^{a}	5.0^{a}	4.33^{d}	Strong
PN108	1.17^{a}	0^{a}	4.42^{d}	Strong
PN54	1.11^{a}	1.7^{a}	4.42^{d}	Strong
PN3d	1.51^{a}	0^{a}	4.17^{d}	Strong
PNCT3	2.63^{b}	5.0^{a}	4.05^{d}	Strong
E15	2.51^{b}	3.3^{a}	4.04^{d}	Strong
$PNCT_{IV}$	2.73^{b}	3.3^{a}	4.03^{d}	Strong
PN10	2.55^{b}	5.0^{a}	4.03^{d}	Strong
PN46	2.75^{b}	5.0^{a}	4.01^{d}	Strong
PN116	5.12^{c}	15.0^{b}	3.04^{c}	Moderate
QY23	8.08^{d}	28.3^{cde}	2.08^{b}	Weak
NC3	8.58^{de}	25.0^{c}	2.02^{b}	Weak
PN47	7.88^{d}	25.0^{cd}	2.03^{b}	Weak
U6	9.19^{ef}	36.7^{ef}	1.38^{a}	Nil
PN21	9.20^{ef}	35.0^{ef}	1.29^{a}	Nil
PN24	9.33^{ef}	33.3^{def}	1.27^{a}	Nil
PN14	9.55^{f}	41.7^{f}	1.25^{a}	Nil
Mean	4.77	15.09	3.14	
LSD	0.82	7.89	0.59	
p	< 0.001	< 0.001	< 0.001	

The values are the means for the two isolates; values followed by the same letters in a column are not different among isolates at p = 0.05 according to Duncan's multiple range test; resistance score was based on leaf appearance

(4 clones, including clones U6 and PN14 (Figure 1a). Three *Eucalyptus* hybrid clones (DH32-29, PNCT3 and $PNCT_{IV}$) and seven *E. urophylla* clones (PN3d, PN10, PN46, PN54, PN108, E15, E28) displayed strong and significantly greater resistance than the other clones; L ranged from 0.99 to 2.75 cm, D from 0 to 5.0%, and T from 4.01 to 4.58 (Figure 1c, d). Clone PN14 had the highest mortality, though this was not significantly different to that in clones U6, PN21 and PN 24. Duncan multiple range tests showed that the ten strongly resistant clones could be separated into two groups based on a significant difference in L, but they did not differ significantly in D.

DISCUSSION

This study identified productive *Eucalyptus* clones that displayed high resistance in the nursery against two highly virulent isolates of *C. manginecans*. Use of such resistant clones and avoidance of susceptible clones should enable growers to reduce damage from this disease in their plantations.

In these trials, the incubation and lethality period for nine *C. manginecans* isolates was about 60 days. The lesion length after 30 days was very small and only a few plants died when inoculated with isolates E253, E256 and E273. After that, the lesion length and mortality increased significantly at 60 and 90 days (Figue 2). Most isolates of *C. manginecans* have also been reported to take about 50–60 days to cause plant death when inoculated on *E. camaldulensis* cuttings and *Chukrasia tabularis* seedlings, while plant death occurred about 20 days after inoculation for *A. mangium* and *A.* hybrid and *D. tonkinensis* (Chi et al. 2019b, Chi et al. 2020, Chi et al. 2021, Trang et al. 2022).

Lesion length is a simple parameter for evaluating the pathogenicity of *C. manginecans* because it is straight forward to observe and measure. However, mortality is the ultimate expression of damage caused by disease. Therefore L and D should be both considered in the assessment of pathogenicity and screening of resistant varieties, as has been done for *Acacia* spp. and *C. tabularis* in Vietnam (Chi et al. 2021, Chi 2022b). In the current study, mean values of L and D for the eighteen *Eucalyptus* clones were very closely correlated, enabling consistent rankings of the clones for their overall resistance. The T of clones based on their foliage appearance also aligned cloesely with L and D (Table 4).

This study examined the pathogenicity of *Ceratocystis* isolates when applied under-bark to 8-month-old clonal plants growing in soil inside plastic misthouses. The levels of damage and death arising from natural infection in the field are likely to be affected by plantation age and environmental conditions. Heavily infected two-year-old plantations of eucalypt clones, U6 and PN14, had average mortality levels of 16.8 and 18.1%, respectively (Chi & Thu 2016, Thu 2016). These field levels of mortality were lower than the mortalities of 36.7 and 41.7% for the same clones observed in the nursery trial following inoculation with two highly pathogenic isolates of *C. manginecans* (Table 4).

Artificial inoculation by *C. manginecans* in the field has not yet been undertaken to minimise the risk of disease spread in *Eucalyptus* and *Acacia* plantations in Vietnam. However, artificial wounding of trees in a trial of 45 *A. auriculiformis* clones allowed *C. manginecans* to infect stems naturally, enabling the identification of seven clones with very strong disease resistance (Chi et al. 2019a). More field research is required to understand the range of damage, death and reduction in timber yield in *Eucalyptus* plantations caused by *Ceratocystis* infection.

The results of *Eucalyptus* screening in this study have important implications for commercial afforestation. Three clones of *Eucalyptus* hybrid (DH32-29, PNCT3 and $PNCT_{IV}$) and seven clones of *E. urophylla* (PN3d, PN10, PN46, PN54, PN108, E15 and E28), recognised as national or technically advanced varieties (Table 2), showed strong resistance to *C. manginecans* in the nursery. *Eucalyptus* is being considered to replace *Acacia* plantations that have been severely damaged by *Ceratocystis* wilt disease in Indonesia, Malaysia and Vietnam (Lee 2018, Chi et al. 2020). *Eucalyptus* hybrid clones, DH32-29, PNCT3 and $PNCT_{IV}$, have been planted widely in Vietnam since 2018 (Anh 2020). However, clone DH32-29 was recorded as susceptible to the stem-boring beetle *Batocera lineolata* (Quang et al. 2022). The remaining nine clones, with strong resistance are therefore recommended as suitable replacements for the two susceptible clones, U6 and PN14. One noteworthy feature of the results is the absence of a significant interaction between *Ceratocystis* isolates and *Eucalyptus* clones, in both pathogenicity testing of isolates and screening

of *Eucalyptus* clones. This lack of interaction, if found to be more general, would simplify expanded screening programs, as clone could be screened using a small number of virulent isolates.

Similar genetic variation in resistance to *Ceratocystis* has been reported in other studies on *Eucalyptus* and other tree genera. From 18 commercial clones of the *Eucalyptus* hybrid (*E. grandis* × *E. urophylla*) in Brazil, four clones were tolerant to *Ceratocystis* species (Zauza et al. 2004). An inoculation experiment on *E. grandis* and *E. urophylla* showed twelve genotypes resistant and nine susceptible to *C. fimbriata* (Rosado et al. 2009). Screening of 20 *Eucalyptus* clones in Brazil found five clones tolerant to *C. fimbriata*, including two clones of *E. urophylla*, one of *E. grandis* and two of *E. saligna*, while most *E. grandis* genotypes were more susceptible (Firmino et al. 2013). Screening for resistance to wilt disease has also been undertaken for *Acacia* species and *Chukrasia tabularis* in Vietnam (Brawner et al. 2020, Chi et al. 2021).

To date, eucalypt breeders in Vietnam have selected improved eucalypt varieties (clones or seedlots) mainly for growth, stem form and resistance to leaf diseases such as *Cryptosporiopsis eucalypti* (Hai & Dao 2013). The demonstration of strong genetic differences in resistance to *Ceratocystis* wilt disease among the clones tested in this study suggests that the other advanced *Eucalyptus* varieties recognised by the Ministry of Agriculture and Rural Development of Vietnam should be also evaluated for *Ceratocystis* resistance. Screening for wilt disease should also be undertaken for new *Eucalyptus* clones as part of their development, prior to commercialisation in Vietnam.

CONCLUSION

Ceratocystis manginecans can damage *Eucalyptus*, one of the main genera for plantations in Vietnam. There was significant variation in disease resistance among *Eucalyptus* clones. Ten of 18 clones tested displayed strong resistance to the disease. These clones could be potential sources of disease resistance for use in plantations and future breeding. Other selected *Eucalyptus* clones and varieties developed in Vietnam should be evaluated for their resistance to *C. manginecans*.

ACKNOWLEDGMENT

The authors would like to thank Harwood C for helpful advice and suggestions towards the paper.

REFERENCES

Al-Adawi AO, Barnes I, Khan IA et al. 2013. *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. *Australasian Plant Pathology* 42: 179–193.

Anh NT. 2020. *Preserve and Preserve Genetic Resources of Pulpwood Species*. Vietnam Paper Corporation, Phu Tho.

Arnold RJ, Xie YJ, Luo JZ, Wang HR & Midgley SJ. 2020. A tale of two genera: exotic *Eucalyptus* and *Acacia* species in China. 2. Plantation resource development. *International Forestry Review* 22: 153–168.

Barnes I, Roux J, Wingfield BD, Dudzinski MJ, Old KM & Wingfield MJ. 2003. *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. *Mycologia* 95: 865–871.

Bon PV, Harwood CE, Chi NQ, Thinh HH & Kien ND. 2020. Comparing wood density, heartwood proportion and bark thickness of diploid and triploid *Acacia* hybrid clones in Vietnam. *Journal of Tropical Forest Science* 32: 206–216.

Brawner J, Chi NM, Chi N, Glen M, Mohammed C, Thu PQ & Kien ND. 2020. Tolerance of *Acacia* populations following inoculation with the *Ceratocystis canker* and wilt pathogen in Vietnam. *Tree Genetics & Genomes* 16: 77.

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

Chi NM. 2022a. *Ceratocystis manginecans* wilt disease in forest trees and its management strategy in Vietnam. *Journal of Forestry Science and Technology* 1: 46–52.

Chi NM. 2022b. Pathogenicity of *Ceratocystis manginecans* in inoculated *Acacia* roots. *Indian Phytopathology* 75: 231–237.

Chi NM & Thu PQ. 2016. *Ceratocystis* wilt disease of *Eucalyptus* in Vietnam. *Science and Technology Journal of Agriculture and Rural Development* 6: 119–123.

Chi NM, Thu PQ & Mohammed C. 2019a. Screening disease resistance of *Acacia auriculiformis* clones against *Ceratocystis manginecans* by artificial and natural inoculation methods. *Australasian Plant Pathology* 48: 617–624.

Chi NM, Thu PQ, Huy PD & Anh NT. 2020. *Ceratocystis* wilt disease of *Acacia* plantations in Vietnam Paper Corporation. *Vietnam Journal of Forest Science* 2: 91–100.

Chi NM, Nhung NP, Trang TT et al. 2019b. First report of wilt disease in *Dalbergia tonkinensis* caused by *Ceratocystis manginecans*. *Australasian Plant Pathology* 48: 439–445.

Chi NM, Trang TT, Nhung NP et al. 2021. Ceratocystis wilt in *Chukrasia tabularis* in Vietnam: identification, pathogenicity and host tolerance. *Australasian Plant Pathology* 50: 17–27.

De Beer ZW, Duong TA, Barnes I, Wingfield BD & Wingfield MJ. 2014. Redefining *Ceratocystis* and allied genera. *Studies in Mycology* 79: 187–219.

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 Pathology* 43: 153–164.

Firmino AC, Junior HJT, Gava De Souza IC & Furtado EL. 2013. Resistance of *Eucalyptus* genotypes to *Ceratocystis* sp. *Scientia Forestalis* 41: 165–173.

Hai VD & Dao DN. 2013. *Some Forest Plant Varieties have been Recognized as Technically Advanced Varieties and National Varieties*. Vietnam Administration of Forestry, Hanoi.

Harwood CE & Nambiar EKS. 2014. Productivity of acacia and eucalypt plantations in Southeast Asia. 2. Trends and variations. *International Forestry Review* 16: 249–260.

Kile GA, Harrington TC, Yuan ZQ, Dudzinski MJ & Old KM. 1996. *Ceratocystis eucalypti* sp. nov., a vascular stain fungus from eucalypts in Australia. *Mycological Research* 100: 571–579.

Lee SS. 2018. Observations on the successes and failures of *Acacia* plantations in Sabah and Sarawak and the way forward. *Journal of Tropical Forest Science* 30: 468–475.

Li J, Zhang Y, Xu KC, Yang JY, Han YH, Sun YX & Huang Q. 2014. First report of wilt of *Eucalyptus* caused by *Ceratocystis fimbriata* in China. *Plant Disease* 98: 1744–1744.

Liu F, Mbenoun M, Barnes I et al. 2015. New *Ceratocystis* species from *Eucalyptus* and *Cunninghamia* in South China. *Antonie Van Leeuwenhoek* 107: 1451–1473.

MARD (Ministry of Agriculture and Rural Development). 2017. *Decision No. 4572/QD-BNN-TCLN dated 8/11/2014 of the Ministry of Agriculture and Rural Development of Vietnam on Recognizing Forest Plant Varieties as Technically Advanced*. Ministry of Agriculture and Rural Development, Hanoi.

O'gara E, Mccomb JA, Colquhoun IL & Hardy GESJ. 1997. The infection of non-wounded and wounded periderm tissue at the lower stem of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi*, in a rehabilitated bauxite mine. *Australasian Plant Pathology* 26: 135–141.

Pornsuriya C & Sunpapao A. 2015. A new sudden decline disease of bullet wood in Thailand is associated with *Ceratocystis manginecans*. *Australasian Plant Disease Notes* 10: 26–31.

Quang DN, Chi NM, Thao DV et al. 2022. Damage caused by *Batocera lineolata* Chevrolat (Coleoptera: Cerambycidae) in *Eucalyptus* and its management in Vietnam. *International Journal of Tropical Insect Science* 42: 1389–1399.

Rosado CCG, Guimarães LMDS, Titon M, Lau D, Rosse L, De Resende MDV & Alfenas AC. 2009. Resistance to *Ceratocystis* wilt (*Ceratocystis fimbriata*) in parents and progenies of *Eucalyptus grandis* x *E. urophylla*. *Silvae Genetica* 59: 99–106.

Roux J, Van-Wyk M, Hatting H & Wingfield MJ. 2004. *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. *Plant Pathology* 53: 414–421.

Roux J, Wingfield MJ, Bouillet JP, Wingfield BD & Alfenas AC. 2000. A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *Forest Pathology* 30: 175–184.

Tarigan M, Roux J, Van-Wyk M, Tjahjono B & Wingfield MJ. 2011. A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *South African Journal of Botany* 77: 292–304.

Thu PQ. 2016. Results of a survey of insect pests and diseases of the main forest plantation species in Vietnam. *Vietnam Journal of Forest Science* 1: 4257–4264.

Thu PQ, Quang DN, Chi NM, Hung TX, Binh LV & Dell B. 2021. New and emerging insect pest and disease threats to forest plantations in Vietnam. *Forests* 12: 1301.

Trang TT, Thu PQ, Khai PQ et al. 2022. First report of canker and wilt disease in eucalypt caused by *Ceratocystis manginecans* in Vietnam. *Indian Phytopathology* 75: 287–291.

Van-Wyk M, Wingfield BD & Wingfield MJ. 2011. Four new *Ceratocystis* spp. associated with wounds on *Eucalyptus*, *Schizolobium* and *Terminalia* trees in Ecuador. *Fungal Diversity* 46: 111–131.

Van-Wyk M, Pegg G, Lawson S & Wingfield MJ. 2007a. *Ceratocystis atrox* sp. nov. associated with *Phoracantha acanthocera* infestations on *Eucalyptus grandis* in Australia. *Australasian Plant Pathology* 36: 407–414.

Van-Wyk M, Heath RN, Tarigan M, Vermeulen M & Wingfield MJ. 2010. Comparison of procedures to evaluate the pathogenicity of *Ceratocystis fimbriata sensu lato* isolates from *Eucalyptus* in South Africa. *Southern Forests: a Journal of Forest Science* 72: 57–62.

Van-Wyk M, Al Adawi AO, Khan IA et al. 2007b. *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Diversity* 27: 213–230.

Zauza EAV, Alfenas AC, Harrington TC, Mizubuti ES & Silvai JF. 2004. Resistance of *Eucalyptus* clones to *Ceratocystis fimbriata*. *Plant Disease* 88: 758–760.