

# GENETIC CONTROL ON HEARTROT DECAY OF ACACIA MANGIUM WILLD GENOTYPES

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*Acacia mangium* Willd. has been widely planted for wood production across Southeast Asia, where plantations have been frequently damaged by diseases such as heartrot. But little attention has been paid to creating disease-tolerant genotypes within the progeny tests of this species in these countries. This study evaluates disease status of *A. mangium* at 3 years of age in two progeny trials established in Tuyen Quang and Quang Tri provinces, Vietnam. Heartrot disease tolerance was inferred by quantifying damage incidence using acoustic tomography on standing trees and assessing lesion length after artificial inoculation with the fungal pathogen *Perenniporia tephropora* that is commonly associated with heartrot. Our results showed that the damage incidence and lesion length differed significantly among families. The heritability estimates for damage incidence ( $h^2 = 0.25$ ) and lesion length ( $h^2 = 0.38$ ) were moderate. Genotype by environment interactions did not impact damage incidence or lesion length. Our finding also indicates that the damage incidence measured by acoustic tomography on standing trees could be an indirect and highly reliable method for quantifying the genetic variation of this trait in *A. mangium*. Several *A. mangium* families with low incidence of heartrot and small lesion lengths following inoculation were identified for the continued development of *A. mangium* in Vietnam.

Keywords: *Acacia mangium*, disease tolerance, heartrot, heritability, *Perenniporia tephropora*

## INTRODUCTION

Tropical acacia plantations in Southeast Asia to provide locally wood products, furniture exports and support rural development have steadily increased (Nambiar et al. 2014). *Acacia mangium* Willd. has become an important tree species in forestry plantation programs across the tropics (Siddiq & Cao 2016). Recent studies on mechanical properties of solid timber indicated that the wood of *A. mangium* can be used for structural timber and meets requirements of construction uses (Firmanti & Kawai 2005, Hai et al 2015).

In Vietnam, *A. mangium* plantations established for solid-wood utilisation have been expanding as improved genetic material and silvicultural prescriptions have been developed. By 2020, the total area of acacia plantations

reached 2.35 million hectares (Phuc et al. 2021). As *A. mangium* is an exotic species that has been planted on a large scale, potential threats to plantations have been identified and studied intensely. Heartrot, rootrot, pink disease and wilt disease have been widely reported in acacia plantations (Old et al. 2000, Brawner et al 2015, Trang et al. 2017, Thu et al. 2021). Heartrot disease is a common disease in *A. mangium* species, particularly where the trees are grown for solid-wood products (Duong et al. 2022, Nguyen 2015, Ito 2002). Heartrot is caused by a saprotrophic fungal decay of heartwood, which reduces wood quality without killing the tree and there are no external symptoms on diseased trees (Caroline et al. 2006). When affected and unaffected stands are compared,

the productivity of *A. mangium* plantations with heartrot and wilt disease was less than 15m<sup>3</sup>/ha/year while the yield in unaffected stands was between 22 and 35m<sup>3</sup>/ha/year (Harwood & Nambiar 2014). The disease has been reported on trees as young as 2-years of age (Lee 2002). Various pathogens have been associated with heartrot, such as *Phellinus noxius*, *Rigidoporus hypobrunneus* and *Tinctoporellus epimiltinus* in Malaysia and Indonesia (Lee & Zakaria 1993), *P. kawakamii* in Hawaii (Larsen et al. 1985) and *Perenniporia tephropora* in Vietnam (Duong et al. 2022). Damage incidence is variable across stands with incidence ranged from low to high in Vietnam (Nguyen 2015, Duong et al. 2022), Malaysia (Sudin et al. 1993) and Bangladesh (Basak 1997). The incidence differed between regions, sites, ages (Barry et al. 2004), genetics (Old 2000) and silvicultural techniques, such as pruning (Prasad & Naik 2002, Barry et al. 2004).

*Acacia mangium* has become one of the main planted forest tree species in Vietnam, due to its wide adaptability, fast growth, straight stem and a wood quality that is suitable for lumber. A breeding program of 253 unique families within 16 original provenances of *A. mangium* in Vietnam has been developing since 1996 (Hai et al. 2015). In the first and second generation of breeding, intense focus was placed on the improvement of growth traits, stem quality and wood properties. This has led to the development of large timber plantations that are managed to provide timber for a growing domestic wood processing industry. With the transition of plantation management from the production of pulpwood to solid-wood with longer rotation, the risk of pests and pathogens causing diseases such as heartrot are being addressed by actively challenging breeding populations (Thu et al. 2021). One of the more promising solutions for managing heartrot disease is to select genotypes that tolerate the pathogens which cause disease leading to decay. However, little attention has been paid to creating disease-tolerant genotypes within the progeny trials of this species in Southeast Asia. This study aimed to determine the feasibility of using genetic improvement to develop a heartrot tolerant breed of *A. mangium* using two third generation progeny trials in the Tuyen Quang and Quang Tri provinces of Vietnam.

## MATERIALS AND METHODS

### Genetic material, experimental design and site description

Based on growth and/or wood shrinkage, 112 families of *A. mangium* were selected from second-generation progeny trials and seed production areas of Pongaki provenance in Vietnam. These families were then used to establish the progeny trials in 2019 in Tuyen Quang and Quang Tri province. Design of both trials were a Row-Column incomplete block design generated by the software program CycDesign, with 8 replicates and 4 trees per family row plot planted with initial spacing of 3 m × 2.5 m. The seedlings of 3-month-old were planted into 40 × 40 × 40 cm pits; and just before planting 200 g of NPK (5:10:3) was placed at the pit base. The three ha trials were established in Trang Da commune, Tuyen Quang city, Tuyen Quang province in northern Vietnam and Cam Hieu commune, Cam Lo district, Quang Tri province in central Vietnam. In both sites, the mean annual temperature is 22.5–24.5 °C and the average rainfall is 1650–2350 mm per year. Hot dry winds often occur from April to September in Quang Tri. The terrain is flat and a little stony and overlies gravel in Tuyen Quang, but hilly and heavy lateritisation in Quang Tri. The red-yellow feralit soil in both sites is > 50 cm in depth.

### Measurements and inoculation of heartrot pathogen

#### Growth traits and stem straightness

Total tree height, diameter at breast height over bark (DBH) and stem straightness were measured for all trees of families in the trials. Straightness was scored using a scale with 5 classes as described by Hai et al. (2015).

#### Damage incidence of heartrot decay

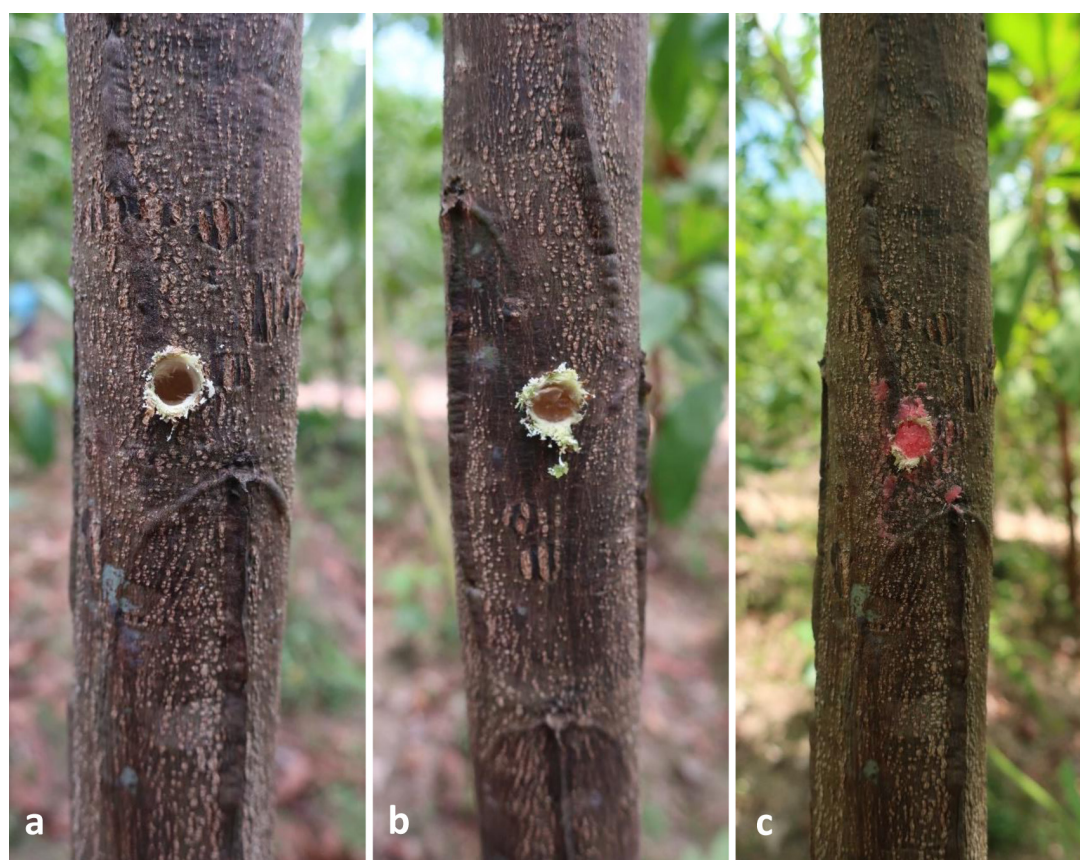
The damage incidence (P%) was evaluated by the method of Gilbert et al. (2016) and Zuhri et al. (2018) using an acoustic tomograph (ArborSonic 3D®) for all trees when they were 3 years of age after out planting. The procedures were as follows: (1) eight transducers per tree,

with equal distance around the trunk, were driven through the bark at 1.3 meters above the ground using a mallet; (2) the distance between the transducers were measured with a pair of callipers and recorded; (3) each transducer was tapped with a small steel mallet to generate sound waves; and (4) the internal sound-velocity distribution of the tree was used to estimate the percentage of the cross sectional area that was decayed. The damage incidence (P%) is the percentage of an area of wood decay compared to the total area of the internal trunk.

### *Fungal inoculation*

In each trial, three replicates were selected for inoculating with an isolate 62.1.2 of the fungus associated with heartrot, *Perenniporia tephropora*. This isolate was the most pathogenic heartrot fungus in in-vitro studies (Duong et al. 2022) and when inoculated into 1.5-year-old *A. mangium* trees in a nursery (Chi et al. 2022). The isolate

was grown on PDA media to produce sufficient plates to inoculate 2664 branches of *A. mangium* trees in two progeny trials in late September 2022. An eastern branch of each tree was chosen about 1 m above the ground and a wound was created by drilling a 0.6 cm hole and depth of 1.5–2.0 cm into the middle of branch 60 cm away from the main stem. Pieces of mycelia agar was cut out and packed into each hole on the branches and paraffin was then used to seal the hole (Figure 1). The tools used for creating the wounds were sterilised between trees with 90% alcohol. After 60 days of incubation, the inoculated branches were cut and split to measure lesion length. Disease resistance or heartrot tolerance of each tree was evaluated using the length of lesions in the branches as a surrogate variable. An ordinal score with five levels was used, namely: (4) lesion length < 5 cm, very strong resistance; (3) lesion length from 5 to < 10 cm, strong resistance; (2) lesion length from 10 to < 15 cm, moderate resistance; (1) lesion length from 15 to < 20 cm,



**Figure 1** Inoculation for *Acacia mangium* trees using heartrot pathogen  
 a. a hole was made on the branch  
 b. agar plate containing mycelium of *Perenniporia tephropora* was put into the hole on the stem  
 c. paraffin was used to seal the hole



weak resistance; (0) lesion length > 20 cm, susceptible (Chi et al. 2022).

### Data analysis

Stem straightness data were not normally distributed and scores were transformed into asymptotic ‘normal scores’ following Gilbert and Norton (1981) to adjust for non-adequate or variable spacing of classes and to improve the efficiency of subsequent analyses (Ericsson & Danell, 1995).

The statistical analysis was conducted in two steps; 1) univariate analyses were used to derive variance components for each trait, and 2) bivariate analyses were used to estimate variances and covariances between pairs of traits. The statistical analysis was based on individual tree observations using a linear mixed model (Equation 1):

$$y = X_B m + Z_W w + Z_N n + Z_T t + Z_F f + e \quad (1)$$

where  $y = (y'_1, y'_2, \dots, y'_n)'$ ,  $m = (m'_1, m'_2, \dots, m'_n)'$ ,  $w = (w'_1, w'_2, \dots, w'_n)'$ ,  $n = (n'_1, n'_2, \dots, n'_n)'$ ,  $t = (t'_1, t'_2, \dots, t'_n)'$ ,  $f = (f'_1, f'_2, \dots, f'_n)'$ ,  $e = (e'_1, e'_2, \dots, e'_n)'$ ,  $X = \Sigma_{\oplus} X_{Bi}$ ,  $Z_W = \Sigma_{\oplus} Z_{Wi}$ ,  $Z_N = \Sigma_{\oplus} Z_{Ni}$ ,  $Z_T = \Sigma_{\oplus} Z_{Ti}$  and  $Z_F = \Sigma_{\oplus} Z_{Fi}$ ,  $\Sigma_{\oplus}$  denotes the direct sum and  $i$  the number of traits from 1 to  $n$ .  $y$  is the vector of individual observations for the different traits,  $m$  is the vector of fixed effect of replicate,  $w$  is the vector of random row within replicate effect,  $n$  is the vector of random column within replicate effect,  $t$  is the vector of random plot within replicate effects;  $f$  is the vector of random family by trait effects, and  $e$  is the vector of random residuals. and are incidence matrix relating  $m$ ,  $w$ ,  $n$ ,  $t$  and  $f$  to  $y$ .

ASReml software was used to estimate the importance of genotype by environment interaction (G×E) on family breeding values across trials, individual heritability and coefficients of additive genetic variation for the studied traits of families (Gilmour et al. 2009). Family variance ( $\sigma_f^2$ ), phenotypic variance ( $\sigma_p^2$ ), row-replicate variance ( $\sigma_w^2$ ), column-replicate variance ( $\sigma_n^2$ ), plot variance ( $\sigma_t^2$ ), and environmental variance ( $\sigma_e^2$ ) for different traits were also estimated using ASReml. The estimated variance components were used to calculate the narrow-sense heritability for the

traits under consideration. Since the families came from open-pollinated parent trees in the progeny trials, the additive genetic variance ( $\sigma_a^2$ ) was estimated as three times the family variance component. The additive genetic variance ( $\sigma_a^2$ ) (Equation 2), total phenotypic variance ( $\sigma_p^2$ ) (Equation 3), and individual heritability ( $\hat{h}^2$ ) (Equation 4) estimates were calculated as follows:

$$\sigma_a^2 = 3\sigma_f^2 \quad (2)$$

$$\sigma_p^2 = \sigma_f^2 + \sigma_w^2 + \sigma_n^2 + \sigma_t^2 + \sigma_e^2 \quad (3)$$

$$\hat{h}^2 = \frac{\sigma_a^2}{\sigma_p^2} \quad (4)$$

The coefficient of additive variation ( $CV_a$ ) (Equation 5) was estimated as:

$$CV_a = \frac{100\sigma_a}{\bar{X}} \quad (5)$$

where  $X$  is the phenotypic mean;  $\sigma_a^2$  is additive genetic variance.

$$r_a = \frac{\sigma_{a_1 a_2}}{\sigma_{a_1} \sigma_{a_2}} \quad (6)$$

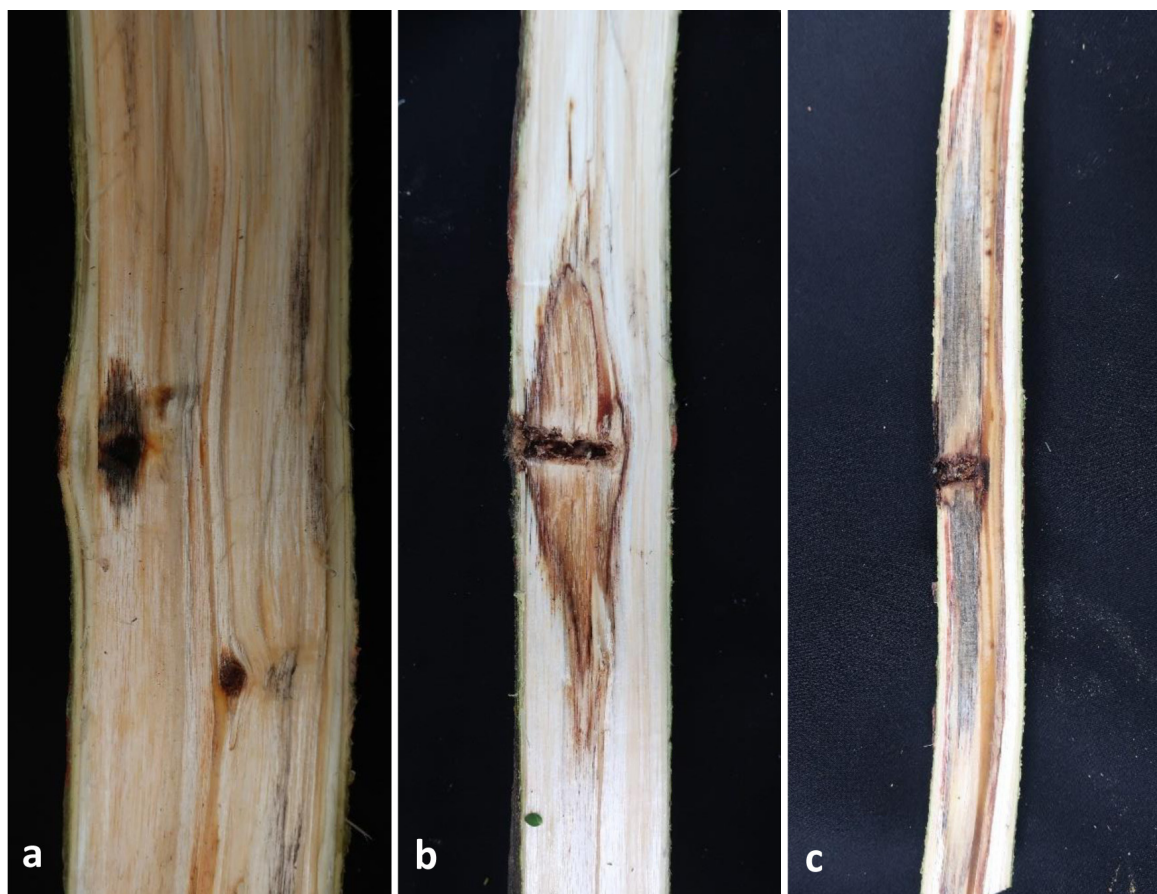
where  $\sigma_{a_1 a_2}$  is the genotypic covariance between two traits, respectively. and  $\sigma_{a_1}$ ,  $\sigma_{a_2}$  are the genotypic standard deviations of trait 1 and trait 2.

Genetic correlations between sites were also estimated based on multivariate REML analysis, by treating measurements from different sites as different traits based on model 1. To test the significance of genotype by environment (G × E) interactions, all off-diagonal elements of variance-covariance matrices were assumed to be zero for combinations of traits measured in different trials. Log likelihood ratio tests were used to test if the correlations were different from one, and also to test if the correlation was different between pairs of trials.

## RESULTS

### Disease tolerance of 3-year-old *Acacia mangium* families

Differences in damage incidence assessed with acoustic tomography and lesion length measurements among the families in both trials. Dissection of the branches after 60 days



**Figure 2** Lesions on the branch of *Acacia mangium* trees after 60 days of artificial inoculation  
 a. family KTT113  
 b. family KTT67  
 c. family KTT9

of artificial inoculation provided obvious lesions for assessment (Figure 2b & 2c), with clear differences between susceptible and tolerant families (Figure 2c). In these trials, one family in Tuyen Quang and two in Quang Tri presented no symptoms with no lesion present after artificial inoculation (Figure 2a).

### Heritabilities and coefficients of additive variation

Heritability estimates for damage incidence and lesion length were moderate ( $\hat{h}^2 = 0.25\text{--}0.38$ ). Coefficients of additive variation ( $CV_a$ ) for damage incidence and lesion length ranged from 5.7–9.5% (Table 1). In this study, genetic correlations between growth and disease traits were not different than zero, but there was a significant and strong positive genetic correlation between damage incidence and lesion length (Table 2).

### Genotype by environment interactions

Genotype by environment interactions ( $G \times E$ ) were important for growth traits assessed in Tuyen Quang and Quang Tri, with low correlations among family breeding values across sites. This contrasted with the estimates of damage incidence and lesion length, where genetic correlations were strongly positive across sites (0.62–0.81) (Table 3).

### DISCUSSION

Improvements in growth and wood quality that have been made in *A. mangium* will not be converted into more profitable plantations if wood quality is degraded or trees are lost to disease (Harwood et al. 2015). Damage from disease, which tends to occur in patches spreading from the initial plantings of *Acacia* species, has compromised the results of many

**Table 1** Mean values, heritability estimates and coefficients of additive variation for disease associated traits within *A. mangium* families at age of 3 years in Tuyen Quang and Quang Tri provinces

| Site        | Trait            | Unit | Mean values | Heritability ( $h^2$ ) | CV <sub>a</sub> (%) |
|-------------|------------------|------|-------------|------------------------|---------------------|
| Tuyen Quang | Damage incidence | %    | 2.30        | 0.38 ± 0.08            | 9.5                 |
|             | Lesions length   | cm   | 9.29        | 0.31 ± 0.03            | 5.7                 |
| Quang Tri   | Damage incidence | %    | 1.58        | 0.36 ± 0.10            | 7.9                 |
|             | Lesions length   | cm   | 6.61        | 0.25 ± 0.02            | 7.2                 |

**Table 2** Genetic correlations and standard errors between growth traits and damage incidence in the progeny trials of *A. mangium* at age of 3 years

| Site           | Lesions length             | Damage incidence            |
|----------------|----------------------------|-----------------------------|
| Tuyen Quang    |                            |                             |
| DBH            | -0.33 <sup>ns</sup> ± 0.18 | -0.21 <sup>ns</sup> ± 0.21  |
| Height         | -0.15 <sup>ns</sup> ± 0.14 | -0.12 <sup>ns</sup> ± 0.17  |
| Lesions length |                            | 0.79 <sup>***</sup> ± 0.05  |
| Quang Tri      |                            |                             |
| DBH            | -0.03 <sup>ns</sup> ± 0.14 | -0.01 <sup>ns</sup> ± 0.15  |
| Height         | -0.05 <sup>ns</sup> ± 0.15 | -0.07 <sup>ns</sup> ± 0.16  |
| Lesions length |                            | 0.89 <sup>***</sup> ± 0.004 |

ns = non significant difference; \*\*\* = significant difference

**Table 3** Genotype by environment interactions (G×E) of damage incidence and lesion length between the progeny trials of *A. mangium* in Tuyen Quang and Quang Tri provinces

| Trait            | G × E                      |
|------------------|----------------------------|
| Damage incidence | 0.81 <sup>***</sup> ± 0.05 |
| Lesion length    | 0.62 <sup>***</sup> ± 0.08 |

genetic trials, making determination of genetic parameters difficult in Southeast Asian countries. The use of tomography to quantify decay in trees along with artificial inoculation provided evidence that these tools may be used to aid the breeding and selection of *A. mangium* with the ability to restrict the development of decay and constrain heartrot-associated pathogens.

This is the first study to evaluate the genetic control of tolerance to the heartrot associated pathogen *Perenniporia tephropora* using artificial inoculation in progeny trials of *A. mangium*. Our results showed that there were significant differences in lesion length and damage incidence (P%) among families in both trials. Lesion length among families was highly

variable, ranging from 0.0 to 35.9 cm at 60 days after inoculation, and the percentage of the stem impacted by decay ranged from 0.0 to 18.9%. Three families with small lesions were found in the progeny trials. These families also showed high levels of tolerance to inoculation with heartrot pathogens in a previous nursery experiment (Chi et al. 2022). These disease-tolerant genotypes may be effectively mass-propagated using clonal family forests (CFF) to maintain favorable allelic profiles in a similar manner to what has been done with *A. mangium* and *A. crassicarpa* (Griffin et al. 2010). Mass-propagating seed from tolerant selections of full-sib families using CFF provides options to deploy material from these trials for commercial

use. CFF approaches involving multiplication of *A. mangium* families through tissue culture of seedlings rather than from planted seedling hedges are currently under evaluation in Vietnam (Hai & Duong 2024) and might overcome problems experienced with CFF to date.

After artificial inoculation, the symptoms inside the affected branches (Figure 2b & 2c) were similar to those observed in trees with heartrot disease in plantations (Duong et al. 2022) and nurseries (Chi et al. 2022). The symptoms showed discoloration of the wood, similar to the discoloration of the wood of infected trees as described by Lee et al. (1988) and Chi et al. (2022). A similar artificial inoculation approach was used by Brawner et al. (2020) to screen tolerance of acacias to *Ceratocystis maguecans* wilt. Screening at the family or clone level is often more definitive when performed using genetically diverse populations of many different provenances. Screening of provenances of *A. mangium* using artificial inoculation with heartrot pathogens (*Pycnoporus sanguineus* and an unknown isolate) showed non-significant differences among populations in Indonesia (Barry et al. 2006). Furthermore, heartrot diseases affect the wood inside the trees and symptoms cannot be observed unless the tree is dissected or broken, complicating the identification of genotypes that are tolerant to heartrot pathogens that severely impact the profitability of plantations managed for sawn timber.

Unlike other native forest plant species in Vietnam, where the origin is often unclear due to the dispersal of the mother trees (Dell et al. 2022), the origin of the acacia populations used to develop the Vietnamese breeding programs is very clear (Barry et al. 2006, Hai et al. 2015, Brawner et al. 2020). In Vietnam, provenance and family trials for *A. mangium* have been conducted for many years, with hundreds of families from 16 provenances selected for superior growth across a range of trials. The techniques developed in this study have identified a promising path to improve the wood quality and wood recovery of *A. mangium* through the deployment of genetic material that constrains pathogen development and has a lower incidence of stem decay.

Sonic tomography is a useful scanning method for quantifying wood decay in living

trees (Win et al. 2015, Gilbert et al. 2016). Damage incidences were previously assessed with tomography in 9 year-old *A. mangium* trees and showed high accuracy ( $r^2 = 0.77$ ) compared to direct methods of Caroline (2006) and Duong (2019). In this study, artificial inoculation and tomographic estimates of heartrot were assessed in pedigreed progeny trials to provide direct estimates of the genetic correlation among these assessment techniques. We found a strong genetic correlation between damage incidence assessed using acoustic tomography and lesion length after inoculation with *Perenniporia tephropora*. This wood decay fungus has been identified as the causal pathogen of heartrot disease and isolated from a range of trees with heartrot in *A. mangium* plantations in Vietnam (Chi et al. 2022, Duong et al. 2022). We provide evidence that damage incidence measured using acoustic tomography on standing trees is a direct and highly reliable method for quantifying decay in standing trees. Given the strongly positive correlation with lesion length, this suggests that selecting for damage incidence (P%) may provide a trait for indirect selection of disease tolerance in *A. mangium* that may be integrated into breeding programs.

The weak genetic correlation between growth traits, damage incidence and lesion length in both progeny trials is unfavourable. Independence between growth and disease tolerance has also been reported for fast-growing acacia and eucalypt clones evaluated for susceptibility to wilt disease (Chi et al. 2023). Selection of families for growth and disease tolerance will not be antagonistic and may be integrated into a selection index that focuses on both traits to improve sawn timber plantation productivity (White et al. 2007). Because canker and wilt pathogens have led to the abandonment of *A. mangium* planting in some Asian countries (Harwood et al. 2015), resistance to major disease threats are high priority traits for incorporation into a selection index that balances growth, disease resistance and wood quality (Hai et al. 2015).

In the present study, the individual heritability ( $\hat{h}^2$ ) of damage incidence and lesion length of *A. mangium* in this study were moderate,  $\hat{h}^2 = 0.25$ – $0.38$  and higher than what were observed for *Dalbergia tonkinensis* ( $\hat{h}^2 = 0.02$ – $0.03$ ) by Dell et al. (2022). High heritability and coefficient



of additive variation of the disease-tolerant characteristics in both the Tuyen Quang and Quang Tri trials suggests that damage incidence and lesion length data are traits that may be used to select for improved tolerance to a heartrot pathogen impacting plantations of *A. mangium* in Vietnam.

Because of different climates (especially humidity and temperature) in two sites, the correlation between the two sites for damage incidence and lesions length was high and there was little evidence of G×E for these traits. This shows stability of disease tolerance when families are inoculated with the same pathogen but grown at different locations. Stability in tolerance to wilt disease has been reported in *Acacia* spp. (Brawner et al. 2020). Management of heartrot disease in *A. mangium* plantations has been tested using biological agents or chemicals to limit the pathogens (Prasad & Naik 2002). However, screening for tolerant genotypes is a priority in Vietnam that will be pursued to ensure sustainability in the development of forest plantations.

## CONCLUSION

Significant levels of genetic variation for damage incidence and lesion length were found in *A. mangium* progeny trials at age of 3 years in Tuyen Quang and Quang Tri province. High individual heritability estimates and coefficients of additive variation of for the traits assessed in these trials indicate they will be useful for selecting heartrot tolerant genotypes, which can be integrated into breeding programs and new plantation establishment. Genetic correlations also indicate that the damage incidence estimated using acoustic tomography on standing trees could be an indirect and highly reliable method to quantify genetic variation for this trait in *A. mangium*. High levels of genotype-environment interactions for damage incidence and lesion length suggest that the most suitable families will be similar in terms of deployment into northern and central Vietnam. Incorporating assessments of traits that are associated with tolerance to heartrot disease into the *A. mangium* breeding program will be used to identify material that may be deployed to improve the productivity and health of plantations managed for sawn timber.

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