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Effect of soil types on root infection of *Acacia mangium* and *Eucalyptus pellita* by *Ganoderma philippii* and *Pyrrhoderma noxium*

HERU INDRAYADI[1,2,♥], MORAG GLEN[2], FAHRIZAWATI[1], BAYO ALHUSAERI SIREGAR[1], ANTO RIMBAWANTO[3], MARDAI[1], BUDI TJAHJONO[1], CAROLINE MOHAMMED[2]

[1]Corporate R&D Sinarmas Forestry. Jl. Minas-Perawang Km. 26, Perawang, Siak 28772, Riau, Indonesia. Tel.: +62-761-9000200, ♥email: heru.indrayadi@gmail.com

[2]Tasmanian Institute of Agriculture, University of Tasmania. Life Sciences Building, Level 2, College Rd, Sandy Bay TAS 7005, Australia

[3]Research Centre for Plant Conservation, Botanic Garden and Forestry, National Research and Innovation Agency. Jl. Kaliurang, Pakem, Sleman 55582, Yogyakarta, Indonesia

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Abstract. *Indrayadi H, Glen M, Fahrizawati, Siregar BA, Rimbawanto A, Mardai, Tjahjono B, Mohammed C. 2023. Effect of soil types on root infection of* Acacia mangium *and* Eucalyptus pellita *by* Ganoderma philippii *and* Pyrrhoderma noxium*. Biodiversitas 24: 2358-2364.* *Acacia mangium* Willd. and *Eucalyptus pellita* F.Muell. are susceptible to red root-rot disease, caused by *Ganoderma philippii* (Bres. & Henn. ex Sacc.) Bres., has led to the demise of planted *A. mangium* as a commercial pulpwood in Indonesia. A second root-rot pathogen, *Pyrrhoderma noxium* (Corner) L.W.Zhou & Y.C.Dai (syn. *Phellinus noxius* (Corner) G.Cunn.), also attacks both species. The objective of this study was to investigate the effect of different soil types on the root infection of *A. mangium* and *E. pellita* by *G. philippii* and *P. noxium*. The effect of three contrasting soil types (mineral R41, R51 and peat soil) used in a plantation estate in Riau province on disease development was investigated in a pot trial using four isolates, two of *G. philippii* and two of *P. noxium*. Two models of inoculation were used, in one of which inoculated wood blocks were placed against the seedling roots (Model 1), the second where the blocks were 10-20 cm from the roots (Model 2). Fifty weeks after the experiment was established, mortality was significantly higher in the soil of low than high clay content, in *A. mangium* than *E. pellita*, and in Model 1 than Model 2; there was no difference between the two *G. philippii* isolates, and *P. noxium* did not cause any mortality. *G. philippii* inoculum could survive in all three soil types for over 9 months; hence, the effect of soil type was not mediated by inoculum longevity. Alternatives should be pursued to limit further development of root-rot diseases in existing *E. pellita* estates.

Keywords: *Acacia mangium*, *Eucalyptus pellita*, *Ganoderma philippii*, inoculum longevity, inoculation model, root-rot diseases, wood block

INTRODUCTION

The risk of development of root-rot diseases is influenced by soil properties (Hardie et al. 2018). *Acacia mangium* Willd. was planted extensively across a wide range of mineral soils in Sumatra in the 1990s and 2000s, but through successive rotations their growth and survival were increasingly compromised by red root-rot disease caused by *Ganoderma philippii* (Bres. & Henn. ex Sacc.) Bres. (Francis et al. 2014). To gain a better understanding of the factors that contribute to infection and plant death, this study examines whether the rate of disease development is related to the physical and chemical properties of three contrasting soils that supported large areas of this plantation estate.

Soil texture is one of the factors associated with the expression of root-rot diseases. For example, disease incidence caused by *Pythium* spp. was significantly greater in slit soils than those in clay soils. (Ayundra et al. 2022). In oil palm (*Elaeis guineensis* Jacq.), fine sand content was positively correlated with the spread of basal stem rot caused by a *Ganoderma* species (Mih and Kinge 2015). For *G. philippii*, survival in soil may depend on its ability to produce chlamydospores as they appear to enhance the tolerance of pathogen to stress. (Chang 2003). In an earlier study, the mortality of *A. mangium* trees caused by *G. philippii* was found to be related with soil family (Hardie et al. 2018). However, the nature of the data set meant that it was not possible to consistently link tree mortality to soil and topographical variables.

The level of incidence of root-rot disease in *A. mangium* in field caused by *G. philippii* varies with the site (Francis et al. 2014). The rate of tree mortality increases with each successive rotation (Francis et al. 2014) and this is associated with the build-up of inoculum load in stumps and coarse roots (Mohammed et al. 2014). The fruiting bodies that develop on stumps are a potential source of spores and their spread may be influenced by the genetic variability of *G. philippii* in the field (Page et al. 2020).

Eucalyptus pellita F.Muell is replacing *A. mangium* in forest plantations in Sumatra and Kalimantan (Inail et al. 2019; Mendham et al. 2020). However, *E. pellita*, as well as *A. mangium*, can be the host for *G. philippii*; a second root-rot pathogen, *Pyrrhoderma noxium* (Corner) L.W.Zhou & Y.C.Dai (syn. *Phellinus noxius* (Corner) G.Cunn.), also attacks both species (Agustini et al. 2014). Although *E. pellita* appeared to be less affected in these growing environments (Mohammed et al. 2014), both tree species were grown across the same range of soil types.

Experiments that attempt to separate out the combined effects of soil and pathogen type can only be undertaken in controlled conditions due to inoculum load (Mohammed et

al. 2014), host susceptibility and the aggressiveness of the pathogen (Jazuli et al. 2022). Infection by pathogen inoculum in the field is also unpredictable (Jazuli et al. 2022). To best represent field conditions and to avoid bias, root-rot pathogen inoculations are conducted in pots containing representative soils (Cui et al. 2015). Artificial inoculum using wood blocks are prepared from branches of a suitable woody species, and the pathogenic fungi first cultured in agar media before being transferred to the wood block (Hidayati et al. 2014). The objective of this study was to investigate the effect of different soil types on the root infection of *A. mangium* and *E. pellita* by *G. philippii* and *P. noxium*.

MATERIALS AND METHODS

Soil preparation and analysis

Two type of mineral soils and one type of peat soil were collected from the hardwood plantation forests in Sumatra were used for the experiment (Table 1). The soil was collected from the A horizon only and mixed thoroughly, and no pretreatment of soil was done before use. Approximately 100 Liter of volume of each soil was placed into 50 cm diameter and 50 cm height of plastic pots that were set 1 m apart in an open area at the R&D Corporate PT Arara Abadi, Plant Protection Research Laboratories at Perawang in Riau Province, Sumatra, Indonesia (0.2933°N, 101.7068°E). Forty-eight pots were allocated to each soil.

For soil analysis, a 100 cm^3 ring was used to remove three representative samples to a depth of 5 cm from the A horizon at each site. The samples from each site were mixed and oven-dried at 105°C for 18-20 h. Soil texture was analysed by the pipette method and the class division was based on the USDA texture triangle. Soil bulk density (BD, g/m^3) was calculated as the ratio of soil air-dry weight divided by solid volume. Soil pH was measured using a glass electrode in a soil sample mixed in distilled water (ratio 1:5) at 200 rpm for 30 min and after settling for 30 min. Cation exchange capacity (CEC) was measured by an Atomic Absorption Spectrophotometer (AAS-Analytic Jena AG-ContrAA 700) using an air-dried soil sample that had been passed through a 20-mesh sieve; 2.5 g of the sample was weighed and put into a 125 mL Erlenmeyer flask and 25 mL 1N NH_4OAc, pH 7.0 was added. The sample was shaken for 15 min and the solution filtered prior to analysis. Organic C, and total N and total C were measured by Flash Elemental Analysis (EA-CHN628 LECO) using a 40-60 mg oven-dried (at 50-60°C) and ground soil sample.

Inoculum preparation

For this, total four isolates, two strains (based on location) each of *G. philippii* and *P. noxium*, were used (Table 2). The isolates were cultured on agar plate and incubated for four weeks in 9 cm diameter petri dishes, each dish containing 20 mL of 2% malt extract agar (MEA) that incorporated 230 ppm thiabendazole, 25 ppm polymixin B sulphate, 50 ppm streptomycin and 50 ppm penicillin. For each isolate, thirty pieces of *A. mangium* woodblock of approximately 10 cm length by 3-4 cm diameter were wrapped in sterilized plastic bags and autoclaved at 121°C for 30 min. Each wood block was then inoculated with one of the (1×1 cm slices) fungal isolates. The blocks were then incubated in plastic bags at 27°C for 6 weeks. The wood blocks were divided into a total of 552 pieces of inoculum for the treatments and 576 pieces for survival test and stored in plastic bags.

Plant preparation

Two species, namely *E. pellita* clone EP0077AA (resistance clone) from tissue culture and *A. mangium* freshly germinated seedlings from Bimadebun provenance were grown in planting tubes in nursery media for three months. The plants tested were chosen based on their uniformity.

Inoculation model

At the age of three months, two plants of the same species were placed into a planting hole in each pot. Two models of inoculation were used: (i) two inoculum blocks were placed vertically in each planting hole, with each block touching the seedling roots just below the soil surface; (ii) five inoculum blocks per pot were placed vertically at a distance of 10-20 cm from the plant, just below the soil surface (Figure 1). The inoculum blocks were covered with soil to prevent exposure to sunlight.

Twenty-four additional pots were used to check for inoculum survival in the absence of plants. There were two pots per soil type per isolate. Each pot contained 24 inoculum wood blocks covered with soil to a depth of 5 cm. Two wood blocks per pot, i.e. four wood blocks per isolate, were removed monthly for re-isolation of pathogens over 9 months. Eight pieces of each block were cultured in petri dishes, one per block, containing MEA medium and incubated for 2 weeks. Re-isolated fungi were identified on the basis of morphological s and microscopic characteristics of mycelia.

Table 1. Soil types used in the pot experiments

Soils	Texture standard*	Origin
R41	Sandy clay loam-to-sandy loam: sand >45%, clay 18 – 34%	Minas Rasau Kuning District P. RSKA175A
R51	Loamy sand-to-sand; clay <18%	Minas Rasau Kuning District Kampung Nias area
Peat	Ombrogenous deep peat (>3 m)	Minas Rasau Kuning District BF9

Notes:*based on the standards used for soil analysis at the Soil Laboratory of PT Arara Abadi Sinarmas Forestry

Table 2. Fungal isolates used in pathogenicity test

Species	Isolates	Locations of sampling
G. philippii	Gp5D	Deras, South Sumatra
G. philippii	Gp6LS	Logas South, Riau
P. noxium	Pn4D	Deras, South Sumatra
P. noxium	PnLC	Rasau Kuning, Riau

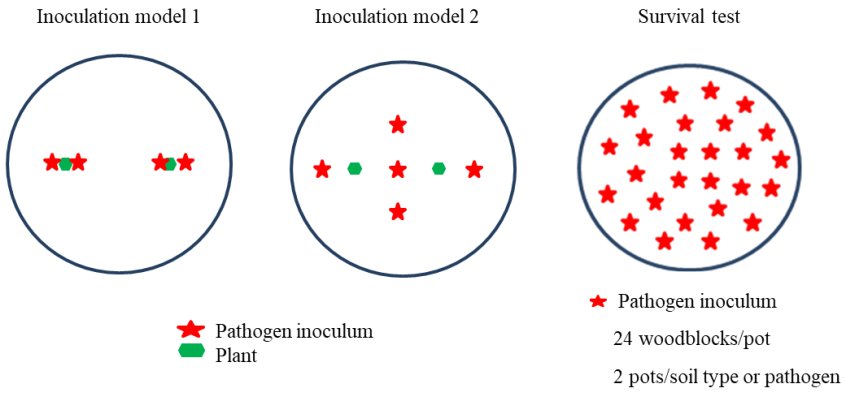

Figure 1. Schematic diagram of the two different inoculation models and survival test

Maintenance and disease assessment

Pots were watered twice daily except on rainy days. At planting, 50 g granular N: P: K fertilizer (16:16:16) was placed into a hole of 5-cm depth, 15 cm from the plants; this was repeated 16 and 32 weeks later. Two hypotheses were tested: the first hypothesis that sandy soil was associated with an increased risk of root-rot disease and the second hypothesis that disease incidence was influenced by the longevity of inoculum. Disease incidence was assessed weekly by recording leaf symptoms and the week of plant death. At 50 weeks, all plants were carefully removed from the pots and roots examined for infection by the pathogen.

Experimental design and data analysis

The trial used a factorial design with four factors: three soil types, two hosts, four inoculum types (from two different pathogens) and two inoculation models. Inoculation model 1 was represented by two pots (four plants) and inoculation model 2 by three pots (six plants), plant as a replicates. The percentage of plant death in each treatment combination was recorded 50 weeks after inoculation. Treatment differences and species by isolate interactions were examined using a generalised linear model with binomial error and a logit link function. The analysis was performed using the GENMOD procedure of SAS/STAT version 9.3 (SAS institute, Cary NC, United States).

RESULTS AND DISCUSSION

Soil type, isolates, host, and inoculation model

It was observed that plant death commenced at approximately the same time in all soils but was subsequently affected by soil type and disease progression. Plant death in soil R51 was faster than in other soils (Figure 2.A). As there was no mortality associated with *P. noxium* isolates, statistical analysis was done only for *G. philippii* isolates. During the early part of the experiment, Gp6LS had a higher rate of tree death than Gp5D (Figure 2.B); however, there were no significant differences between isolates of Gp6LS and Gp5D ($P=0.79$) at week 50.

Both mineral soils were dominated by sand (R41=72.3%; R51= 87.9%); however, the clay content of Soil R41 was much higher than that of Soil R51 (21.2 *vs.* 3.7%) (Table 3). The mineral-free peat soil had substantially higher total N, total C and CEC, and smaller bulk density than the mineral soils, while pH was low for all soils.

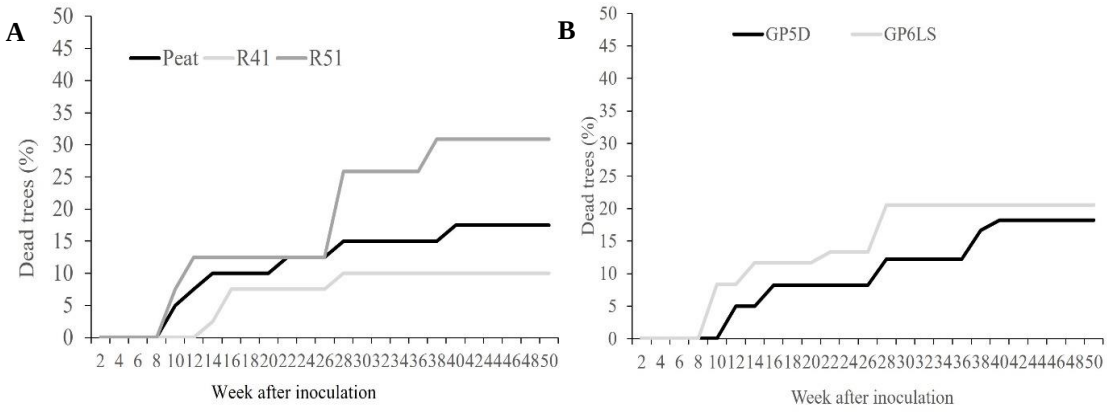

Figure 2. Progression of percentage of dead trees based on the soil types (A) and Isolates (B)

Table 3. Result of soil analysis

Parameters	R41	R51	Peat
Sand (%)	72.30	87.90	-
Silt (%)	6.60	8.30	-
Clay (%)	21.20	3.70	-
Total N (%)	0.19	0.19	0.95
Total C (%)	2.49	2.87	38.74
CEC	15.50	11.80	88.22
pH	4.15	4.23	3.87
Organic C (%)	2.75	2.77	*
Bulk density	1.14	1.11	0.25

Note: *Not available included because of errors in the measurement system

At week 50, mortality in Soil R51 was significantly different and higher than Soil R41 (P=0.017), whereas in peat, mortality was not significantly different from mineral soil (Figure 3.A). At week 50, mortality was significantly different between species and was higher in *A. mangium* than *E. pellita* (*P=0.023*) (Figure 3.B). In both species, the first plant death was recorded at 10th week 10 and no plants died after 40th week .

Inoculation model affected plant death, and at week 50 mortality was significantly greater ($p<0.0001$) in model 1 than in model 2 (Figure 3.C). The rate of plant death in inoculation model 1 was five times higher than in model 2.

It was also noted that there was a significant interaction between isolate and species (P<0.05). The percentages of dead *A. mangium* and *E. pellita* trees did not differ significantly when exposed to isolate Gp5D, with 33.3% mortality of *A. mangium* trees compared to 6.7% dead *E. pellita* trees (Figure 3.D).

Disease assessment

Disease development by *G. philippii* isolates can be assessed by observing symptoms ranging from brown color changes to yellowing, leaf drop and plant death (Figure 4.A). In the 8th week of observation, the most rapid plant death occurred.

Inoculum survival

For all soil types, the inoculum remained viable for at least 9 months (Table 4), although no pathogen growth was observed from samples recovered from two blocks retrieved from pots of R51 soil, and one block from peat soil; *Gliocladium* sp. was isolated from the latter block. *Gliocladium* sp. and *Trichoderma* sp. were also isolated from four other pots. At the end of inoculum trial, roots had penetrated most inoculum blocks in pots containing plants but there was no sign of pathogen infection or mycelial growth on roots of surviving plants (Figure 4.B).

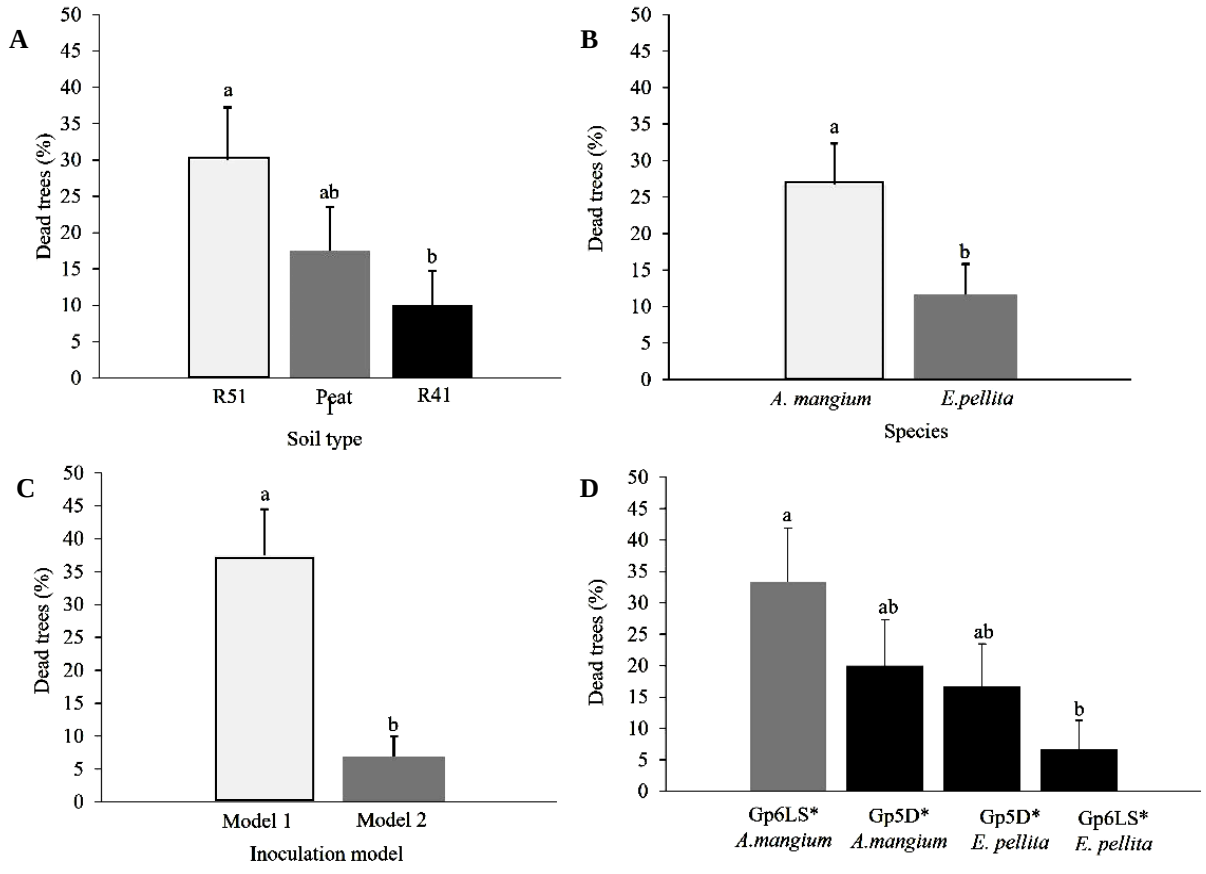

Figure 3. Mortality differences of trees based on the soil type (A); Species (B); Inoculation model (C); Interaction isolate*species (D). The different letters 'a' and 'b' indicate a significant difference between the two isolates at significance level α=0.05

Table 4. Re-isolation of fungal pathogens from the two inoculum blocks incubated in soil for 9 months

Isolates/inoculum codes	Pot/block	Soil type and number of isolates recovered from 8 pieces		
		Peat	R51	R41
Gp6LS	1/1	8 *Ganoderma* sp.	6 *Ganoderma* sp. 2 did not grow	5 *Ganoderma* sp. 3 *Gliocladium* sp
Gp6LS	1/2	8 *Ganoderma* sp.	8 *Ganoderma* sp. 2 *Gliocladium* sp.	8 *Ganoderma* sp.
Gp6LS	2/1	8 *Gliocladium* sp.	7 *Ganoderma* sp. 1 did not grow	8 *Ganoderma* sp. 6 *Gliocladium* sp.
Gp6LS	2/2	8 *Ganoderma* sp.	8 did not grow	8 *Ganoderma* sp.
Gp5D	1/1	8 *Ganoderma* sp.	3 *Ganoderma* sp. 5 *Gliocladium* sp.	6 *Ganoderma* sp. 2 did not grow
Gp5D	1/2	8 *Ganoderma* sp.	6 *Ganoderma* sp. 2 did not grow	8 *Ganoderma* sp.
Gp5D	2/1	8 *Ganoderma* sp. 2 *Trichoderma* sp.	5 *Ganoderma* sp. 3 did not grow	8 *Ganoderma* sp.
Gp5D	2/2	8 *Ganoderma* sp.	8 *Ganoderma* sp.	6 *Ganoderma* sp. 2 did not grow
Pn4D	1/1	8 *Phellinus* sp.	7 *Phellinus* sp. 1 did not grow	8 *Phellinus* sp. 2 unidentified
Pn4D	1/2	8 *Phellinus* sp.	8 did not grow	6 *Phellinus* sp.
Pn4D	2/1	6 *Phellinus* sp., 2 did not grow	8 *Phellinus* sp.	8 *Phellinus* sp.
Pn4D	2/2	8 *Phellinus* sp.	7 *Phellinus* sp. 1 did not grow	8 *Phellinus* sp.
PnLC	1/1	8 *Phellinus* sp.	8 *Phellinus* sp.	8 *Phellinus* sp.
PnLC	1/2	7 *Phellinus* sp. 1 did not grow	8 *Phellinus* sp.	6 *Phellinus* sp. 2 did not grow
PnLC	2/1	8 *Phellinus* sp.	6 *Phellinus* sp.	8 *Phellinus* sp.
PnLC	2/2	8 *Phellinus* sp.	7 *Phellinus* sp. 1 *Gliocladium* sp.	5 *Phellinus* sp. 3 did not grow

Figure 4. Disease symptoms and plant death (A, circle); *G. philippii* around the inoculum block and penetrating roots 50 weeks after inoculation (B)

Discussion

The effect of soil types, including mineral and peat soils, on the development and spread of fungal mycelia was investigated in the present study. Mortality was three times greater in the mineral soil with low clay content compared to that with high clay content. Fungi generally grow better in soils offering a large network of pores (Ritz and Young 2004) and increasing clay content is associated with decreasing pore volume and interconnectedness between pores (Boivin et al. 2004). Accordingly, fungal mycelia spread further and faster in mineral soils of low clay and high sand content (Gill et al. 2000). Peat soils show a lower mortality rate than mineral soils with low clay content. Contributing factors may be peat's very low bulk density as this discourages mycelial development (Zhang et al. 2017); the large cracks and bio pores in peat soil also diminish survival of fungal mycelium and inhibit its spread (Otten et al. 2001). Although pH may influence the pathogenicity of fungal pathogens (Ayundra et al. 2022), in this study it was similar between the soils. However, although the effect of clay content was significant, it is recognised that soil structure and edaphic condition as well as root architecture can also affect the development of fungal root-rot disease (Haus et al. 2020).

In this study, each caused similar rates of tree death 50 weeks after inoculation, although Gp6LS was initially more aggressive than Gp5D. Thus, both isolates ultimately expressed the same level of virulence. *G. philippii* isolates taken from different areas can be genetically different (Page et al. 2020). It has been previously reported that the death of *A. mangium* plantations caused by *G. philippii* was not related with the origin of inoculum source (Coetzee et al. 2011).

A. mangium was more susceptible than *E. pellita* to *G. philippii*, a similar results were observed by Coetzee et al. (2011) where *G. philippii*, was the primary pathogen causing root-rot disease in *A. mangium* species. However, *E. pellita* is not immune to *G. philippii* (Agustini et al. 2014; Yuskianti et al. 2014) but does appear to be more tolerant than *A. mangium* (Eyles et al. 2008; Hardie et al.

2017). This study suggests that it is associated with a slower rate of disease development. The tolerance of plant species to root-rot disease can be related to the formation of wound periderm following infection by the pathogen (Cleary et al. 2012). However, in this study, *E. pellita* was infected more quickly than *A. mangium*, supporting the view that tolerance of *E. pellita* is unrelated to wound periderm formation (Gill et al. 2016).

Different host species can respond in different ways to the same *G. philippi* isolate. This could be influenced by the variety of factors, such as different secondary metabolites produced against pathogen attack (Sahebi et al. 2017), and the quantity of pathogenic inoculum required to initiate infection (Jazuli et al. 2022). Previous studies have highlighted the different host responses to *G. philippii* infection in *E. pellita* and *A. mangium* (Gill et al. 2016). The rate of wood degradation *in vitro* by *G. philippii* also varies among tree species (Paula and Brioso 2021) and may reflect the structure or chemistry of the host species.

In the present study no plant deaths were recorded by *P. noxium*. This pathogen causes brown root disease (Liu et al. 2022), in a wide range of hosts including *A. mangium* and *E. pellita* (Agustini et al. 2014), and ornamental trees (Hsiao et al. 2019); it also causes heart-rot disease in *A. mangium* (Farid et al. 2023). Recent studies have shown that it has high genetic variability (Kozhar et al. 2022) and a broad array of enzymes associated with degradation of plant cell walls (Caballero et al. 2020). The limitation of an appropriate food source in the soil that is a requirement for the fungus to grow and colonize, in this case the wood block inoculum, may also influence the capacity of a fungal pathogen to infect the host (Fatima and Senthil-Kumar 2015).

Placing the inoculum blocks against the roots increased the rate of mortality. The function of the mycelium is to seek out nutritional resources (Ritz and Young 2004), and for plant pathogens like *Ganoderma* and *Phellinus*, the plant roots are the target food source. Thus, the closer the distance of the inoculum source to the root, the faster the fungal infection (Rees et al. 2009). In addition, there is less opportunity for other microorganisms to compete with the pathogen. Intimate contact between the pathogens and the roots also appears to be essential for rapidly initiating infection and accelerating the rate of infection (Rees et al. 2009). In the context of this experiment, this relied on placing the inoculum blocks against the basal stem of plant.

The inoculum in the blocks of wood remained viable for a minimum of nine months. *G. philippii*, this ability may be linked to their production of chlamydospores which survive for more than two years in wood blocks (Chang 2003). Furthermore, chlamydospores are the resting structures that allow the fungus to persist in adverse environmental circumstances (Chang 2003; Loyd et al. 2019). This may explain why no tree mortality was observed after week 38 and why many roots penetrated the block inoculums without infection. In the preview study, inoculum consisting of a rubber (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.) wood block culture of the pathogen, placed within a polythene bag, started showing symptoms 10 weeks after inoculation (Gafur et al. 2015).

The present experiment confirmed that physical properties of soils can affect the rate of mortality and *G. philippii* was more vulnerable to cause infection in *A. mangium* than *E. pellita*, also the distance of inoculum from the root base determines the time to first infection. The results proved the first hypothesis, that the risk of disease was increased in sandy soil, both mineral soils had high sand content; however, the higher clay content reduced the risk. In the second hypothesis, disease incidence was stimulated by the viability of inoculum and the host; however, for *G. philippii*, viability was less important than the distance of inoculum from the target host.

Eucalyptus pellita and its hybrids have replaced *A. mangium* over large parts of the plantation forests in Sumatra and Kalimantan; by 2018, 1.2 Mha had been planted (Harwood 2018). the demonstrated vulnerability of *E. pellita* to red root disease shown in this study and the build-up of inoculum load following successive rotations of *A. mangium* (Francis et al. 2014) are likely to be the main reasons why this eucalypt species is succumbing to this disease in Indonesia and Malaysia as previously report by Lee (2018). As is currently the case with *A. mangium*, though to date without a commercial solution for this species, it is clearly important to examine what options are available for controlling this disease in *E. pellita* as currently there are no other alternative species available to meet the demand for pulpwood in Indonesia.

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