PATHOGENICITY OF *RIGIDOPORUS MICROPORUS* AND *PHELLINUS NOXIUS* AGAINST FOUR MAJOR PLANTATION TREE SPECIES IN PENINSULAR MALAYSIA

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MOHD FARID A, LEE SS, MAZIAH Z & PATAHAYAH M. 2009. Pathogenicity of *Rigidoporus microporus* and *Phellinus noxius* against four major plantation tree species in Peninsular Malaysia. *Rigidoporus microporus* and *Phellinus noxius* are destructive fungi in tropical forest plantations. Early stages of infection by these pathogens are often difficult to detect while trees bearing visible disease symptoms are mostly beyond treatment. Little research has been conducted to determine the aggressiveness of these two pathogens on different tree species. This study was conducted on 24-month-old saplings of *Acacia mangium, Azadirachta excelsa, Tectona grandis* and *Hevea brasiliensis.* Inoculum blocks of *R. microporus* and *P. noxius* were placed next to roots of saplings and left for 30 weeks for infection to take place. The study revealed that *R. microporus* and *P. noxius* were pathogenic to all hosts. However, the degree of pathogenicity varied. *Phellinus noxius* was more pathogenic to *H. brasiliensis* than to *A. excelsa, T. grandis* and *A. mangium,* while *R. microporus* was more pathogenic to *H. brasiliensis* and *A. excelsa* than to *A. mangium* and *T. grandis.* In terms of host susceptibility, *H. brasiliensis* was the most susceptible to *R. microporus* followed by *A. excelsa, T. grandis* and *A. mangium.* Against *P. noxius, T. grandis* was the most susceptible, followed by *H. brasiliensis, A. excelsa* and *A. mangium.* Saplings infected by both pathogens manifested similar aboveground symptoms, but symptoms belowground varied depending on the fungal species. The presence of adhering soil particles and dark brown mycelial crust on the root was common on saplings inoculated with *P. noxius,* while white rhizomorphs were usually present on the root surface of saplings inoculated with *R. microporus.*

Keywords: Host susceptibility, root disease, symptom, forest plantation

MOHD FARID A, LEE SS, MAZIAH Z & PATAHAYAH M. 2009. Kepatogenan kulat *Rigidoporus microporus* dan *Phellinus noxius* terhadap empat spesies pokok ladang yang utama di Semenanjung Malaysia. *Rigidoporus microporus* dan *Phellinus noxius* merupakan kulat pemusnah di ladang hutan di kawasan tropika. Peringkat awal jangkitan oleh kulat-kulat ini selalunya sukar dikesan manakala kebanyakan pokok yang telah pun menunjukkan gejala penyakit biasanya tidak dapat dirawat lagi. Tidak banyak kajian yang telah dijalankan untuk menentukan tahap keagresifan kedua-dua patogen tersebut terhadap pokok yang berlainan spesies. Kajian ini telah dijalankan terhadap anak-anak pokok *Acacia mangium, Azadirachta excelsa, Tectona grandis* dan *Hevea brasiliensis* yang berusia 24 bulan. Blok-blok inokulum kulat *R. microporus* dan *P. noxius* diletakkan pada bahagian akar anak-anak pokok tersebut dan dibiarkan selama 30 minggu supaya jangkitan boleh berlaku. Hasil daripada kajian ini mendapati kulat *R. microporus* dan *P. noxius* adalah patogenik terhadap kesemua perumah tersebut, tetapi tahap kepatogenan adalah berbeza. Kulat *P. noxius* didapati lebih patogenik terhadap *H. brasiliensis* berbanding *A. excelsa, T. grandis* dan *A. mangium* manakala kulat *R. microporus* lebih patogenik terhadap *H. brasiliensis* dan juga *A. excelsa* berbanding *A. mangium* dan *T. grandis.* Dari segi kerentanan perumah, *H. brasiliensis* merupakan perumah yang paling rentan terhadap jangkitan kulat *R. microporus* diikuti oleh *A. excelsa, T. grandis* dan *A. mangium.* Sebaliknya, *T. grandis* merupakan perumah yang paling rentan terhadap jangkitan *P. noxius,* diikuti oleh *H. brasiliensis, A. excelsa* dan *A .mangium.* Anak-anak pokok yang dijangkiti kedua-dua patogen ini menunjukkan gejala serupa pada bahagian silara tetapi gejala berbeza pada bahagian akar bergantung kepada spesies kulat. Kehadiran partikel-partikel tanah dan kerak miselium berwarna coklat kehitaman pada permukaan akar sering kelihatan pada anak pokok yang dijangkiti oleh kulat *P. noxius* manakala rizomorf putih biasanya hadir pada permukaan akar anak-anak pokok yang telah diinokulasi dengan kulat *R. microporus.*

INTRODUCTION

Although agroplantations have existed in Peninsular Malaysia since the 1880s, plantations of forest tree species are still relatively recent. The majority of our older forest plantations were established by the British as a reforestation effort, mainly of forest destroyed during World War II

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(Krishnapillay & Appanah 2002). In the 1980s, the acreage of forest plantations grew rapidly due to the forecasted shortage of timber from the natural forest (Chong 1979). Thus large-scale forest plantations of fast-growing exotic species were established for general utility wood. With the increase in acreage of forest plantations, pest and disease problems have become a greater concern, especially since very little information or data are available about them. In many cases, failures during the early phase of plantation establishment have been linked to serious pest and disease problems (Wingfield 1999). The susceptibility of a particular species to pests and diseases will usually determine its success in the future (Gibson 1979, Ivory 1987, Harrington & Wingfield 1998).

Disease surveys conducted in several forest plantations in Peninsular Malaysia have shown that root disease is the main cause of mortality in these plantations (Lee 1997, Mohd Farid *et al.* 2005). Root disease fungi such as *Rigidoporus microporus* and *Phellinus noxius* are known to be pathogenic and destructive to trees in the tropics. These pathogens cause significant economic losses in rubber, cocoa, tea and fruit tree plantations (Fox 1977, Johnston 1989). Trees are rapidly killed by these pathogens and detection during early stages of infection is difficult. In the field, the aboveground symptoms associated with both pathogens are almost similar but belowground they differ. Normally, the presence of aboveground symptoms indicate that the trees are mostly beyond treatment and recovery, as rapid progress of infection makes death imminent (Ismail & Azaldin 1985). However, information on the susceptibility and aggressiveness of these pathogens on forest plantation species in Malaysia remains scanty. Therefore, the aims of this study were, firstly, to assess the susceptibility of four hosts, namely, *Acacia mangium, Azadirachta excelsa, Hevea brasiliensis* and *Tectona grandis* against white root (*R. microporus*) and brown root (*P. noxius*) diseases using foliar symptoms. Secondly, we aimed to improve skills in detecting incidence of white root and brown root diseases on the selected hosts based on observation of root symptoms in the field.

MATERIALS AND METHODS

A pathogenicity study was conducted on 24-month-old healthy plants of *A. mangium, A. excelsa, H. brasiliensis* and *T. grandis.* The two root disease fungi used in this study were *R. microporus* (isolate 590) obtained from the Lembaga Getah Malaysia (LGM—Malaysian Rubber Board), Kuala Lumpur and *P. noxius* (isolate FRIM154) obtained from the Forest Research Institute Malaysia (FRIM) collection. Prior to inoculation, young rubber tree branches obtained from within the FRIM campus were cut into smaller blocks measuring approximately 8 × 1.5–2.0 cm diameter. Six to eight debarked blocks were placed into individual autoclavable plastic bags and 50 ml of 2% malt extract (ME) were added. The mouth of each bag was fitted with a PVC ring and stoppered with a cotton wool plug, covered with aluminium foil and autoclaved at 121 °C for 15 min. The autoclaved bags were transferred into a culture room to cool. Four to five mycelial discs taken from the edge of one-week-old actively growing cultures with a sterilized 5-mm diameter cork borer were used to inoculate the sterilized wood blocks in each plastic bag. The inoculated bags were then incubated in the dark at 28 ± 2 °C for about a month to allow good colonization of the wood blocks by the fungi.

In the pathogenicity test, all plants used were obtained from known sources. *Acacia mangium* and *A. excelsa* seeds collected from healthy mother trees at FRIM's campus were germinated and raised at the FRIM nursery. For *H. brasiliensis* and *T. grandis* clone T16, seedlings were obtained from the LGM nursery, Sg. Buloh, Selangor and FRIM substation, Mata Ayer, Perlis respectively. The plants were maintained at the FRIM nursery until they were 24 months old and transferred into plastic pots measuring 60 cm diameter × 45 cm height. They were left for a month in the open in the nursery for hardening.

Experimental layout and data analysis

Arrangement of the saplings was according to randomized complete block design (RCBD) with three replications. Two treatment combinations were assigned in each replicate with four types of plant species, two types of fungi and one control. Each treatment consisted of five healthy and uniform saplings. Each sapling was inoculated with six to seven inoculum blocks placed in contact with the taproot. Uninoculated plants were used as controls. Plants were observed at two-weekly intervals for the appearance of aboveground disease symptoms for up to

30 weeks. Evaluation of disease infection was conducted according to Nandris *et al.* (1983) with the following modification: the scale used for rating the severity of attack aboveground was 1 (healthy), 2 (yellowing of foliage), 3 (wilting), 4 (defoliation) and 5 (death of plant). The data were then subjected to Fisher's exact test (2-tail) to determine the interaction between pathogens and symptom development on the hosts using SAS (Statistical Analysis System) package, Version 6.03 (1989). Prior to the test, frequency of symptom appearance was grouped into three categories, namely, healthy (scales 1 and 2), moderate (scales 3 and 4) and severe (scale 5).

Plants bearing disease symptoms were brought to the laboratory and fungal isolation was made from the roots to confirm the presence of causal pathogens. Diseased tissues were directly transferred onto *Ganoderma* selective media (GSM) (Ariffin & Idris 1992) and incubated at room temperature. The diseased tissues were then transferred onto malt extract agar (MEA) and left to grow. Identification of the causal organisms was done based on cultural and microscopic characteristics as described by Stalpers (1987).

RESULTS

Comparison of the pathogenicity of *R. microporus* and *P. noxius* for each host

Results of this study revealed that all uninoculated control saplings with the exception of *A. excelsa* were healthy without any symptoms of root disease infection until the experiment was terminated (Figures 1a, d and g). Belowground, all uninoculated saplings possessed vigorous and clean root systems compared with inoculated saplings. Roots of symptomatic saplings could be separated into two categories depending on the inoculated pathogens. For saplings inoculated with *R. microporus,* rough white rhizomorphs were present on the surface of roots of all symptomatic saplings. In contrast a dark brown mycelial crust with soil particles covering the roots was found on diseased saplings inoculated with *P. noxius.* Table 1 shows the percentage of saplings exhibiting root symptoms for all treatments.

All *H. brasiliensis* saplings inoculated with *R. microporus* exhibited characteristic white root disease symptoms (Table 1). In contrast all *A. excelsa* and *T. grandis* saplings and 93% of *H. brasiliensis* saplings inoculated with *P. noxius* exhibited characteristic brown root disease symptoms. *Acacia mangium* had the fewest symptomatic saplings against both pathogens. Further detailed results, particularly of the aboveground symptoms for each species, are described below.

Acacia mangium

Results indicated that *P. noxius* was more pathogenic than *R. microporus* on *A. mangium* saplings (Figures 1b and c). At week 8, although the percentage of saplings with yellowing symptoms was similar (13.3%) for saplings inoculated with *P. noxius* and *R. microporus,* progression of disease symptoms was generally more rapid on saplings inoculated with *P. noxius* than with *R. microporus.* In addition, severe symptoms such as defoliation and death of saplings appeared thereafter on saplings inoculated with *P. noxius.*

Sapling death caused by *P. noxius* and *R. microporus* was first observed at week 20 and week 22 respectively. At the end of 30 weeks, *P. noxius* inoculated plants suffered 20% mortality compared with 13% for plants inoculated with *R. microporus* (Figure 1c).

Hevea brasiliensis

Yellowing symptoms on *H. brasiliensis* saplings inoculated with *P. noxius* were observed on 13.3% of saplings at week 4. Symptom severity increased rapidly and by the 12th week, all

Table 1 Percentage of uninoculated control saplings and saplings inoculated with *Rigidoporus microporus* or *Phellinus noxius* showing belowground symptoms

Pathogen	*Acacia mangium*	*Azadirachta excelsa*	*Tectona grandis*	*Hevea brasiliensis*
R. microporus	13.33	86.67	53.34	100
P. noxius	46.67	100	100	93.33
Uninoculated control	0	0	0	0

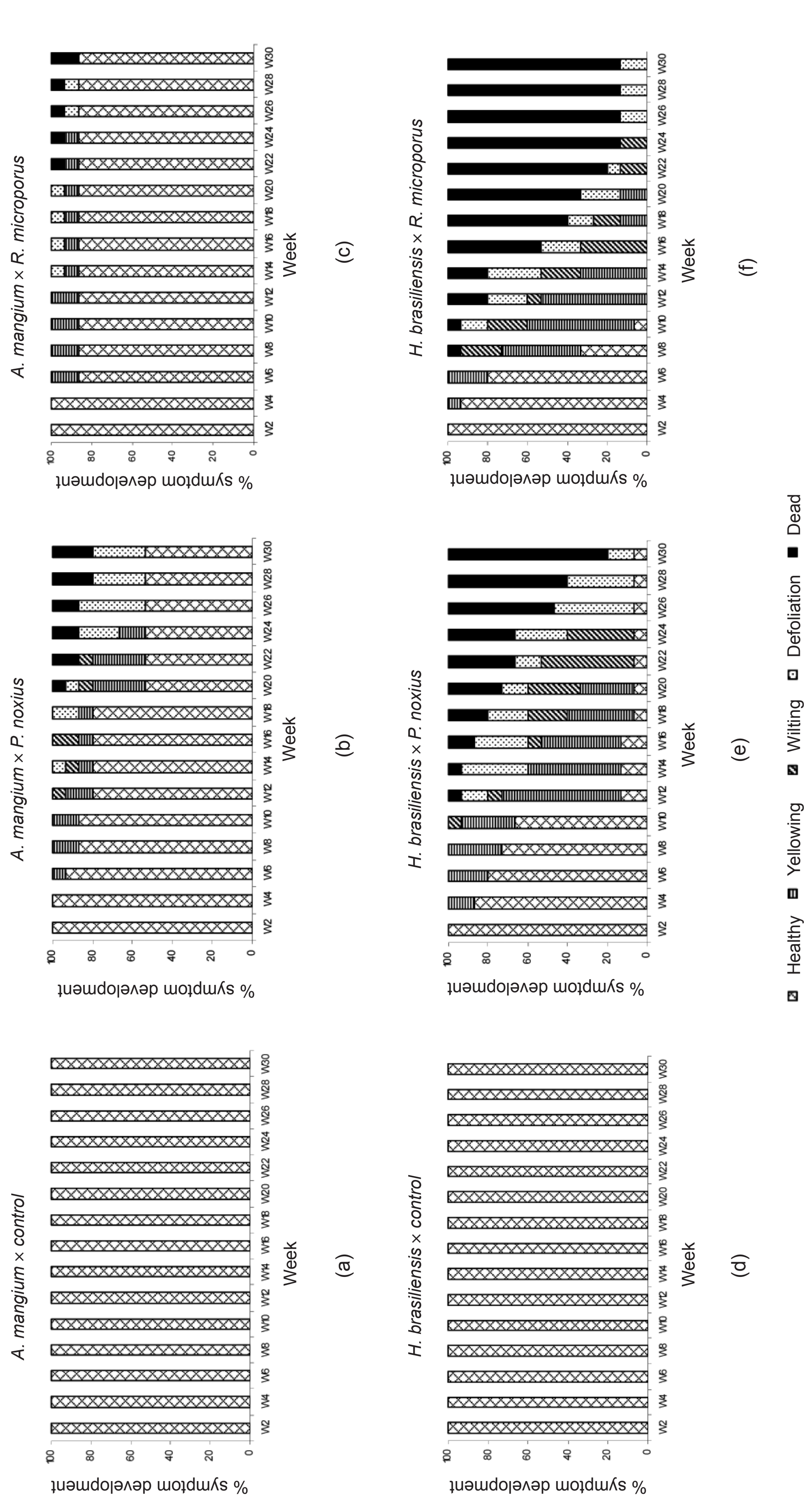
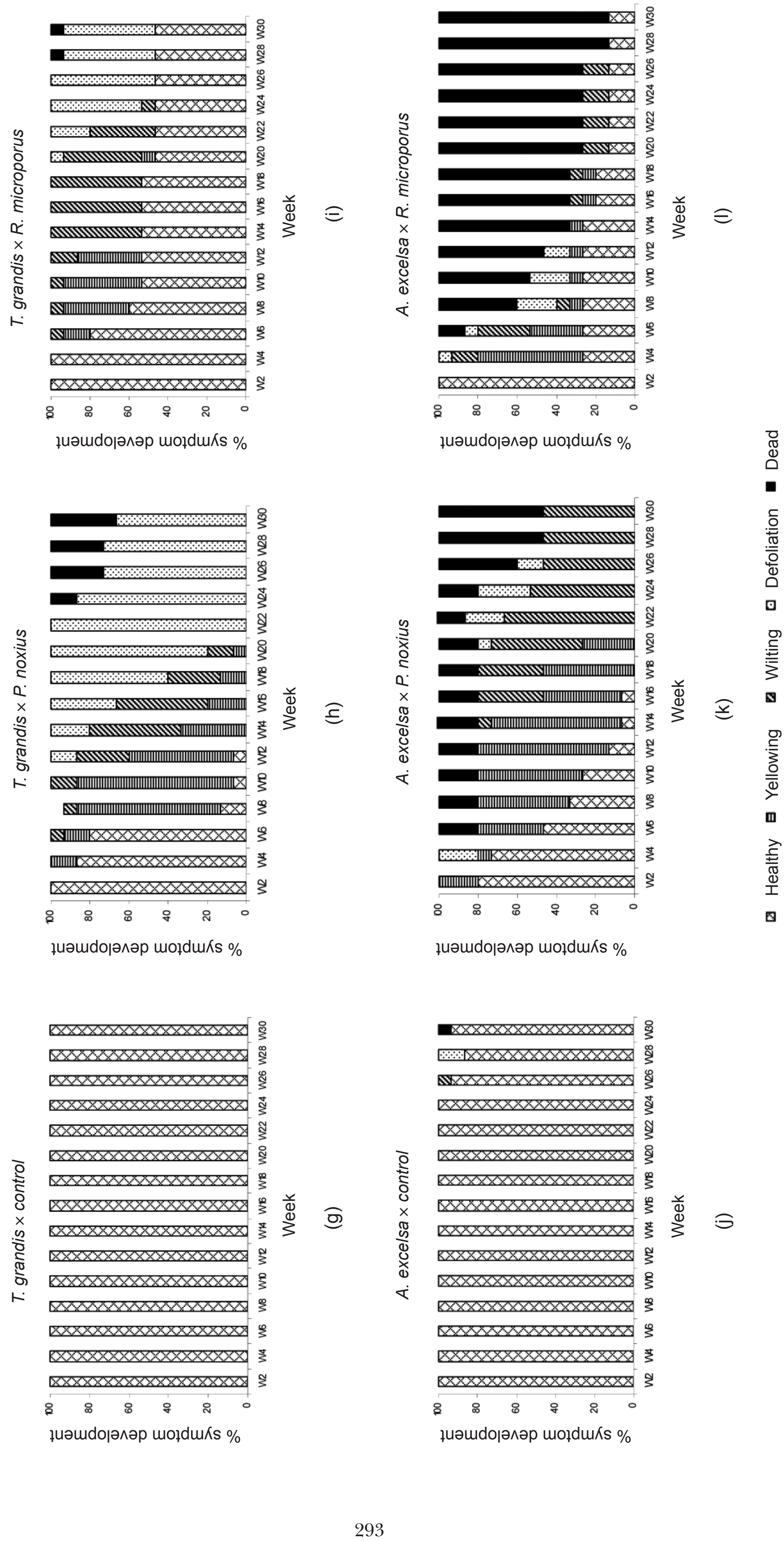


Figure 1 Development of symptoms on saplings of *Acacia mangium*, *Hevea brasiliensis*, *Tectona grandis* and *Azadirachta excelsa* inoculated with *Rigidoporus microporus* and *Phellinus noxius*

(continued)

Figure 1 (continued)



the yellowing saplings had died (Figure 1e). Symptom development was more severe on saplings inoculated with *R. microporus,* where 6.7% of saplings which developed yellowing symptoms at week 4 had died by week 8 with mortality increasing to 20% by week 12 (Figure 1f). By week 24, 86% of the saplings inoculated with *R. microporus* had died. Although the rate of increase in mortality caused by *R. microporus* infection was faster compared with *P. noxius* (Figure 1f), at the end of the experiment, the percentage of saplings killed by *R. microporus* was only slightly higher (86%) than that killed by *P. noxius* (80%).

Tectona grandis

Progression of disease symptoms on *T. grandis* inoculated with *P. noxius* and *R. microporus* was less severe than that observed on *H. brasiliensis.* Symptom progression was faster on saplings inoculated with *P. noxius* than with *R. microporus.* Yellowing of saplings inoculated with *P. noxius* was observed at week 4. This was followed by wilting and defoliation at week 12 (13.3%). Defoliation increased rapidly to 100% by week 22. Death of saplings was first observed at week 24 (13.3%) and by the end of the experiment, 33.3% of the saplings were dead (Figure 1h).

In comparison with saplings inoculated with *P. noxius,* all saplings inoculated with *R. microporus* were healthy at week 4. At week 6, a few of the saplings exhibited yellowing (13.3%) and wilting (6.67%). Although defoliation was first observed at week 20 (6.67%), the percentage of saplings showing this symptom increased rapidly to 20% two weeks later. At the end of the experiment, 46.7% of the saplings were defoliated and 6.7% dead (Figure 1i).

Azadirachta excelsa

For saplings inoculated with *P. noxius,* 20% saplings exhibited symptoms of yellowing as early as the second week and two weeks later defoliation occurred. By week 6, sapling death was observed. Mortality was gradual and by the end of the experiment, 53.3% saplings were dead (Figure 1k). In contrast, saplings inoculated with *R. microporus* started to show symptoms of yellowing, wilting and defoliation simultaneously at week 4. Sapling mortality was first observed at week 6 and by week 8, 40% of saplings were dead. The rate of mortality was rapid and by the end of the experiment, 86.7% of the saplings were dead (Figure 1l). This showed that *R. microporus* was more pathogenic to *A. excelsa* compared with *P. noxius*. A few of the uninoculated control saplings showed wilting and defoliation followed by death at week 26 onwards (Figure 1j) due to mechanical injury during weeding.

Based on the disease scale, Fisher's exact test showed that there was a very high probability that development of aboveground symptoms on all tested saplings was caused by the inoculated fungi (Table 2). *Acacia mangium* saplings inoculated with these pathogens showed P(F) = 8.34×10^{-4} significant difference, whereas *H. brasiliensis, A. excelsa* and *T. grandis* showed 1.57×10^{-15}, 6.08×10^{-15} and 2.11×10^{-11} significant differences at 95% confidence level respectively.

Table 2 Fisher's exact test showing very high probability that development of aboveground symptoms 30 weeks after inoculation was caused by the tested pathogens

Host	*A. mangium*			*H. brasiliensis*			*A. excelsa*			*T. grandis*		
Pathogen	H	M	S	H	M	S	H	M	S	H	M	S
Control	15	0	0	15	0	0	14	0	1	15	0	0
P. noxius	8	4	3	1	2	12	0	7	8	0	10	5
R. microporus	13	0	2	0	2	13	2	0	13	7	7	1
Total	45			45			45			45		
Fisher's exact test (2-tail): probability	8.34×10^{-4}			1.57×10^{-15}			6.08×10^{-15}			2.11×10^{-11}		
P(F)*	0.001			0.000			0.000			0.000		

H= Healthy; M= moderate; S= severe; * = $p \leq 0.05$

Comparison of the susceptibility of the four hosts to each pathogen

In general, the pathogenicity test revealed that saplings of *A. mangium, H. brasiliensis, T. grandis* and *A. excelsa* were susceptible to both *P. noxius* and *R. microporus*. However, their degree of susceptibility differed. Against *R. microporus*, both *H. brasiliensis* and *A. excelsa* were the most susceptible followed by *A. mangium* and *T. grandis*. Against *P. noxius*, *H. brasiliensis* was the most susceptible followed by *A. excelsa, T. grandis* and *A. mangium* (Figure 1).

Acacia mangium was equally susceptible to *P. noxius* and *R. microporus* but *T. grandis* was more susceptible to the former than to the latter (Figure 1). In contrast, *A. excelsa* was more susceptible to *R. microporus* compared with *P. noxius*. In general, *H. brasiliensis* was the most susceptible host to both pathogens, whereas *A. mangium* was the least susceptible host.

DISCUSSION

Results of this study showed a good association between the appearance of foliar symptoms and damage to roots belowground. Nevertheless, observation of foliar symptoms is generally more important for tree disease diagnosis as it is often difficult, costly and time consuming to excavate roots for observation of symptoms. Foliar inspection was often relied upon in identifying infected trees in the field (Nandris *et al.* 1987, Ann *et al.* 2002). In the present study, all saplings inoculated with *R. microporus* and *P. noxius* appeared to exhibit almost similar foliar symptoms. Progress of disease was generally observed first as yellowing followed by wilting, defoliation and finally death of the host. In addition, progress of these symptoms concurs with those observed by Mohd Farid *et al.* (2001, 2006a, b) who worked on root diseases of *A. excelsa* and *T. grandis*. It has been reported that foliar symptoms in *Swietenia macrophylla, Cordia alliodora* and *Gmelina arborea* infected by *P. noxius* progressed rapidly from yellowing to wilting and finally death of trees (Ivory 1990). Similar observations have also been recorded on *H. brasiliensis* infected by root diseases where general discoloration of the foliage was linked to the interruption in root function (Anonymous 1974, Nandris *et al.* 1987). In root disease, wilting and defoliation of leaves are considered symptoms of advanced disease development. At this stage, chemical treatments are usually not effective as the presence of these symptoms indicate that the tree is beyond the point of recovery (Ismail & Azaldin 1985, Chee 1986).

Although the infected hosts exhibited very similar foliar symptoms, root symptoms differed depending on pathogens. Roots of saplings inoculated with *R. microporus* had white rhizomorphs on their surface while those inoculated with *P. noxius* had encrustations of rusty brown mycelia with soil particles on the root surface. Similar observations were also reported by Holliday (1980), Nandris *et al.* (1987), Lee (2000), Ann *et al.* (2002) and Mohd Farid *et al.* (2005). Furthermore, these symptoms were consistent for the fungus irrespective of the host species. The high probability of Fisher's exact test P(F) showed that symptoms developed on saplings inoculated with *R. microporus* and *P. noxius* were due to the effect of the inoculated fungi and most certainly not the effect of chance.

Planters could use these symptoms as a guide to detect and control the spread of root disease infection in the field. However, emphasis should be given on trees showing yellowing symptoms as these are considered to be at an early stage of infection. Fungicide treatments to combat rubber root disease are recommended for newly infected trees or trees with mild infection levels (Ismail & Shamsuri 1998). Thus, it is vital to conduct regular disease monitoring in plantations so that fungicide treatments can be applied on yellowing trees as early as possible.

The high mortality of *A. excelsa* (86.67%) and *H. brasiliensis* (86.67%) saplings inoculated with *R. microporus* indicated that the pathogen was equally aggressive in killing both hosts and that both hosts were very susceptible to the white root pathogen. *Hevea brasiliensis* is well known to be very susceptible to *R. microporus* in Malaysia, Sri Lanka, Indonesia and Gabon (Anonymous 1974, Fox 1977, Nandris *et al.* 1987, Liyanage 1997, Rajalakshmy & Jayarathnam 2000, Semangun 2000, Guyot & Flori 2002), while *A. excelsa* had previously been reported to be susceptible to white root disease in plantations established on areas previously planted with *H. brasiliensis* (Mohd Farid *et al.* 1999, 2005).

The study also revealed that *A. mangium* and *T. grandis* were less susceptible to *R. microporus* as indicated by the lower mortality rate. Furthermore, the white rhizomorphs often associated with infection by this pathogen were seldom found on the root surface of infected saplings compared with those of *H. brasiliensis* and *A. excelsa*. This suggests that the presence of rhizomorphs may be directly linked to susceptibility of the host to the pathogen. Similar observations have been made for root disease caused by *Armillaria ostoyae* (Omdal *et al.* 1995, Prospero *et al.* 2006) where dense rhizomorphs were often observed on the root surface of trees killed by the pathogen. This could indicate that production of rhizomorphs is strongly correlated with the ability to cause disease. Rhizomorph production was a prerequisite in assessing pathogenicity and virulence (Morrison & Pellow 2002). Susceptibility and damage by root disease fungi vary with tree species (Filip 1999). These results appear to indicate that both *A. mangium* and *T. grandis* may have some resistance to *R. microporus*.

In this study, cumulative mortality was used to assess host susceptibility since it could show the aggressiveness of pathogens in killing their hosts, particularly in the plantations. The overall results suggested that *P. noxius* was a less aggressive pathogen to *H. brasiliensis* and *A. excelsa* than *R. microporus* as it took a longer time to kill the test plants. Similar results have been reported by Nicole *et al.* (1986) and Mohd Farid *et al.* (2001).

The inoculated saplings that were not infected by the disease were probably vigorous enough to withstand the infection as evidenced by the presence of more vigorous, greener and denser canopy prior to infection. The difference in disease severity is often attributed to differences in tree vigour (Rosso & Hansen 1998). This could suggest that hosts need to be weakened before infection can occur. This is in agreement with a study that reported that pathogenicity of *Armillaria* sp. was often greater when trees were somewhat weakened and stressed (Wargo & Harrington 1991). This also indicates that trees under stress are more susceptible to infection by a pathogen (Agrios 2005). Another possible reason for the lack of disease development in some of the inoculated saplings could have been due to the lack of viable inoculum in some wood blocks. This could have occurred in wood blocks which had been buried too shallowly in the soil, thereby becoming exposed to sunlight and drying out.

CONCLUSIONS

In the field, brown root disease caused by *P. noxius* can be recognized by the presence of adhering soil particles and dark brown mycelial crust, while white root disease caused by *R. microporus* is usually identified based on the presence of rough white rhizomorphs on the tree root surface. Although results presented in this study are limited to cultivars of the four tree species, it is likely that similar symptoms will be observed in other tree species as well.

The pathogenicity test confirmed that both *R. microporus* and *P. noxius* were pathogenic to *H. brasiliensis, A. excelsa, T. grandis* and *A. mangium* saplings. In terms of host susceptibility, *H. brasiliensis* and *A. excelsa* were more susceptible to *R. microporus* followed by *A. mangium* and *T. grandis*. Against *P. noxius, H. brasiliensis* was again the most susceptible followed by *A. excelsa, T. grandis* and *A. mangium*. Overall, *H. brasiliensis* was the most susceptible and *A. mangium* was the least susceptible to root rot disease caused by these two pathogens. Results from the present study suggest that *A. excelsa* is not suitable for planting in areas with a previous history of white root and brown root diseases, particularly in ex-rubber plantations, as it is extremely susceptible to both diseases. Hence, planting *A. mangium* and *T. grandis* trees in these areas could be an alternative since they show a lower degree of susceptibility to the diseases. Although *A. mangium* trees were least susceptible to white root and brown root disease, interplanting *H. brasiliensis* and *A. mangium* is not recommended. *Hevea brasiliensis* trees may not perform well in the field due to the high competitiveness of the fast-growing *A. mangium* for light and space. Consequently, latex production from *H. brasiliensis* may be reduced considerably.

Since all infected saplings exhibited identical foliar symptoms which are generally detected too late for efficient treatments, cultural practices would be more practical to reduce and/or prevent incidence of root disease in the future. Good land clearing, regular disease monitoring and implementation of effective control measures are still required to prevent the spread of root disease in plantations. It may also be advisable to identify and treat the diseased areas with suitable chemicals before planting.

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