

INCIDENCE AND SPATIAL ANALYSIS OF ROOT ROT OF *ACACIA MANGIUM* IN INDONESIA

R. S. B. Irianto¹, K. Barry^{2,3*}, N. Hidayati⁴, S. Ito⁵, A. Fiani⁴, A. Rimbawanto⁴, C. Mohammed^{2,3,6}

¹Forest and Nature Conservation Research and Development Centre, Forestry Research and Development Agency, Jl. Gunung Batu No. 5, Bogor, West Java, Indonesia

²School of Agricultural Science, University of Tasmania, Private Bag 12, Hobart 7001, Tasmania, Australia

³CRC for Forestry, Private Bag 12, Hobart 7001, Tasmania, Australia

⁴Centre for Biotechnology and Forest Tree Improvement, Forestry Research and Development Agency, Jalan Palagan T. Pelajar Km.15, Purwobinangun, Pakem, Sleman, Jogjakarta 55582, Indonesia

⁵Faculty of Bioresources, Mie University, 1515 Kamihama, Tsu, Mie 514-8507, Japan

⁶Ensis (CSIRO and SCION), Forest Biosecurity and Protection Private Bag 12, Hobart 7001, Tasmania, Australia

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IRIANTO, R. S. B., BARRY, K., HIDAYATI, N., ITO, S., FIANI, A., RIMBAWANTO, A. & MOHAMMED, C. 2006. Incidence and spatial analysis of root rot of *Acacia mangium* in Indonesia. Root rot caused by *Ganoderma* spp. is a serious concern in commercial plantations of *Acacia mangium* in Indonesia. This study surveyed root rot incidence and spatial arrangement in commercial plantations and trials. In second rotation commercial plantations in Sumatra and Kalimantan, root rot incidence was recorded between 3 and 28% in trees aged from three to five years old. The compartments surveyed in Riau province and East Kalimantan had significantly higher root rot than South Sumatra. In a progeny trial of *A. mangium* in Java, root rot incidence was surveyed twice, when trees were eight and again at nine years old. Root rot incidence was about 7 and 13% respectively. Spatial analysis by distance indices (SADIE) revealed that infected trees were randomly distributed at the first time point but tended to become aggregated by the time of the second survey. This highlights the probability of vegetative spread of the fungus after initial introduction to the site. Assessment of root rot incidence on the basis of *A. mangium* provenance did not reveal any statistically significant differences.

Keywords: Red root rot, *Ganoderma* spp., plantation, family trials, provenance, survey, basidiomycete

IRIANTO, R. S. B., BARRY, K., HIDAYATI, N., ITO, S., FIANI, A., RIMBAWANTO, A. & MOHAMMED, C. 2006. Insidens dan analisis ruangan bagi reput akar *Acacia mangium* di Indonesia. Reput akar yang diakibatkan oleh *Ganoderma* spp. merupakan masalah serius di ladang komersial *Acacia mangium* di Indonesia. Kajian ini meninjau insidens serta susunan ruangan reput akar di ladang komersial serta tapak ujian. Di ladang komersial pusingan kedua di Sumatra dan Kalimantan, insidens reput akar pada pokok berusia tiga tahun hingga lima tahun adalah sebanyak 3% hingga 28%. Kompartmen yang ditinjau di daerah Riau dan Kalimantan Timur menunjukkan kejadian reput akar yang lebih tinggi daripada Sumatra Selatan. Dalam ujian progeni *A. mangium* di Jawa, tinjauan untuk insidens reput akar dilakukan sebanyak dua kali iaitu ketika pokok berusia lapan tahun dan sembilan tahun. Insidens reput akar yang dicerap dalam tinjauan tersebut masing-masing adalah sebanyak 7% dan 13%. Analisis ruangan melalui indeks jarak (SADIE) menunjukkan bahawa pokok yang dijangkiti bertabur secara rawak pada tinjauan pertama namun menjadi teragregat pada tinjauan kedua. Ini menunjukkan kulat mungkin tersebar secara vegetatif selepas muncul pertama kali di tapak. Penilaian insidens reput akar atas dasar provenans *A. mangium* tidak menunjukkan sebarang perubahan signifikan.

INTRODUCTION

Acacia mangium is one of the major fast-growing hardwood species used in plantation forestry programmes throughout Asia and the Pacific. In

2000, the species accounted for over one million ha of landuse in South-East Asia (FAO 2000). Of the diseases identified in *A. mangium* plantations,

* Author for correspondence. E-mail: karen.barry@utas.edu.au

root rot is the most significant in terms of loss of productivity, resulting in tree death (Old *et al.* 2000). Since the 1980s, Indonesian private and state forestry companies have planted *A. mangium* extensively in Sumatra and Kalimantan. These plantations are now mostly second and some are third rotation.

Two main types of root rot diseases have been found in *A. mangium*; brown root rot caused by *Phellinus* spp. or red root disease caused by *Ganoderma* spp. Roots affected by red root rot have a wrinkled rhizomorphic (fungal) skin on the surface and the bark becomes red on the outside and white on the underside. Brown root rot is distinguished by rusty-brown velvety patches of fungal growth on the roots and in advanced stages, and honeycomb-like patterns of plates of hyphae can be seen in the rotting wood (Old *et al.* 2000).

Red or brown root rot of *A. mangium* plantations has been found to occur in several Asian countries, with reports from Malaysia (Lee 1993, 1997, 2000), the Philippines (Almonicar 1992, Militante & Manalo 1999), Papua New Guinea (Arentz 1996), India (Mehotra *et al.* 1996, Sharma & Florence 1997, Prasad & Naik 2002) and Vietnam (P. Q. Thu, pers. comm.). In Indonesia, the disease is widespread (Rahayu 1999, Old *et al.* 2000), but there are limited reports to date on the incidence and impact of root rot.

The incidence of root rot in *A. mangium* plantations is not uniformly distributed within a region or site and generally increases with plantation age (Lee 2000). Over successive rotations of *A. mangium*, the quantity of woody debris harbouring root rot fungi and the levels of mortality associated with high levels of fungal inoculum increase exponentially (Old 1997). While *Ganoderma* spp. and *Phellinus* spp. have been recognized as the main fungal genera causing root rot, little is known of their etiologies in *Acacia* plantations or the potential for phenotypic selection for host resistance.

In contrast, the etiologies of woody root rots in northern hemisphere forests is better understood, for example, *Heterobasidion annosum* and *Armillaria* spp. The primary route of infection for *H. annosum* is by airborne basidiospores, which infect freshly cut stumps and wounds. Subsequent vegetative spread to adjacent healthy trees takes place via root contacts and grafts. Roots of living trees that contact decaying roots of colonized stumps or diseased roots on living trees become

infected. In temperate regions of the world, *Armillaria* spreads mainly by vegetative means (via root-to-root contact or rhizomorphs) and not by basidiospores, though this may not apply to all *Armillaria* species (Rishbeth 1988). Studies of *G. boninense*, an important woody root disease of oil palm in Indonesia, suggested that for this species, both root-to-root contact and basidiospore dispersal play important roles in disease dissemination (Miller *et al.* 1999, Soepana *et al.* 2000). This could be of relevance to the mechanism of fungal spread in *A. mangium* root rot.

Woody root rot diseases are not easy to control and cost-effective options are often limited. For example, to control *H. annosum* root rot, removal of infected woody debris and roots has been shown to reduce disease incidence but this option is not economically feasible (Pratt 1998). The effectiveness of biocontrol and chemical agents vary for different root rot diseases and hosts. For example, the antagonistic white-rot fungus, *Phlebiopsis gigantea* is effective in controlling aerial stump infection by *H. annosum* root rot (Thor 2003). However, biological control of *Armillaria* spp. is more difficult (Hagle & Shaw 1991). Finding resistance to root rot is another control option but it is difficult to achieve, particularly within a susceptible species and is best done with the addition of other control measures such as reduction of inoculum or avoiding sites with a history of the disease (Hagle & Shaw 1991). For *A. mangium*, provenance differences in root rot incidence have been found in Malaysian studies (Lee 1997). It was found that root rot incidence was lowest in the West of Morehead and Oriomo River provenances from Papua New Guinea and the Broken Pole Creek provenance from Queensland.

The aims of the present studies were to (1) gain quantitative information on the incidence of root rot in *A. mangium* and relate this to factors such as region, climate and plantation management practices, (2) assess spatial spread of root rot over time; and (3) obtain a preliminary assessment of resistance to root rot at the progeny level. This was achieved by conducting independent surveys of industrial plantations in three regions of Indonesia (South Sumatra, Riau province, East Kalimantan) and by studying the spread of root rot in an experimental progeny trial of *A. mangium* in Central Java province.

MATERIALS AND METHODS

Commercial plantations

Site information

Root rot incidence surveys were conducted at two locations in Sumatra; two compartments were assessed for a forest company based in South Sumatra and three compartments for another forest company based in Riau, in November and October 2001 respectively. In addition, three compartments were surveyed in the Sebulu region of East Kalimantan in October 2002. All sites were planted with the second rotation of *A. mangium*, spacing was 3 × 3 m and no thinning was conducted. Further site details are included in Table 1.

Collections of fruiting bodies associated with root rot were made from these three regions in 2002. *Ganoderma philippi* was unequivocally identified as a causative agent of root rot and there was strong evidence to suggest that *G. mastoporum* and *Amauroderma rugosum* were also involved in root rot disease (N. L. Bougher, pers. comm.).

Survey

A survey was conducted by recording the incidence of root rot in two adjacent rows of *A. mangium*, skipping the next three rows and then repeating this survey pattern until a certain count was reached. This represented a 40% subsample of a randomly selected area of the compartment. In East Kalimantan for example, trees were assessed until a count of 200, representing a 40% subsample of 500 trees. Roots of trees that were standing dead, exhibited chlorotic/yellowing foliage, loss of foliage, or fruit bodies on the stem were uncovered and inspected for typical symptoms of root rot (red). Trees in which root rot was confirmed by this method, plus any missing trees were scored as a positive result. Missing trees were assumed to be dead due to root rot. Results were recorded as presence/absence of root rot, then the number in each of the two categories were summed and expressed as a percentage. During field surveys, observations on the pattern of root rot infections (i.e. size of root rot disease foci) were recorded.

Data analysis

Data were tested for significance with a single factor nested ANOVA using SAS for Windows Version 8 (1999). Plot (compartment) was nested within region and the health class was defined as root rot (1) or no root rot (0). While the box plot histogram and statistics indicated data was non-normal (due to a large count of 0) the central limit theorem states that as the sample size approaches infinity, the sample mean approaches normality. Type III SS was used due to the design being unbalanced.

Progeny trial

Site information

The progeny trial is located at Alas Ketu, Wonogiri, Central Java (latitude 7° 32'–8° 15' S, longitude 110° 4'–111° 18' E) and the elevation is 141 m asl. The site had been previously planted with *Dalbergia* spp., which were harvested and the stumps and roots removed.

The progeny trial was a randomized complete block design with seven blocks of 135 families. The progeny trial was planted in 1995 and originally consisted of four progenies per family in each block on 4 × 2 m spacing. The trial was uniformly thinned in 1997 and 1999 to leave only one progeny per family in each block (therefore seven progeny per family). It was these remaining trees which were mapped and assessed. Stumps from thinned trees were no longer present at the time of the survey. The families were from 14 different provenances, originating from Papua New Guinea and Australia.

Survey

Tree death in the progeny trial was first observed early in 2003. Surveys for root rot were conducted in February 2003 and repeated one year later. All trees were assessed for root rot disease. Trees were recorded as dead (either missing, a stump present, and with or without a fruit body) or alive (with a fruit body or alive without a fruit body). Apart from the missing trees, the roots of dead trees and stumps were inspected for symptoms of root rot (using the same method as for the commercial plantations). Trees in intermediate

stages of infection (i.e. evident by chlorotic or missing foliage) were not assessed and, therefore, the survey method did not correlate to the surveys in commercial plantations.

Management practice at this site was to remove trees which die from the trial. As such, dead trees were observable as stumps or were missing. In the first survey, some trees were missing from the trial and in some cases only stumps were present. It is assumed that in both cases, trees were killed by root rot and then removed from the plantation. In the second survey, all dead trees had been removed.

Statistical analysis

A spatial analysis by distance indices (SADIE) was carried out for the pattern of root rot within the progeny trial using the method described by Xu and Madden (2004). Binary data (tree death or no death) were analyzed in spatial sequence over the whole 1–48 columns of field trees, but limited to rows 1–18 (out of 23 rows) of the trial. SADIE can only analyze approximately 800 data entries and beyond row 18, the blocks were not adjacent; therefore, this was the best selection to exclude. Missing trees were accounted for in the analysis.

Statistical analysis of provenance differences was conducted using GenStat for Windows Version 5 (2000). Root rot presence or absence for the 2004 data was tested by provenance either as a fixed effect (fit analysis) or as a random effect (generalized linear mixed model analysis or GLMM).

Identification of fruiting body

Fruiting bodies were collected from the progeny trial during both the first and second surveys. They were identified on the basis of their macroscopic and microscopic characteristics by comparison to herbarium specimens and use of keys.

RESULTS

Commercial plantations

Trees exhibiting symptoms such as yellowing leaves or marked defoliation were generally recorded as being infected with red root disease. Similar signs of red root rot disease were also observed in the majority of dead trees. For all

regions surveyed, regardless of the stage of disease development, symptoms characteristic of the red root rot disease caused by *Ganoderma* spp. in which roots displayed a typical red rhizomorph over their surface were easy to recognize.

Root rot was usually observed in circular patches of five to eight affected trees. Incidence was considerably higher in compartments surveyed in Riau province and in East Kalimantan compared with South Sumatra, i.e. from about three- to nine-folds higher (Table 1). The incidence of root rot was significantly different ($p < 0.0001$) at the regional level and also, within region, a significant effect of variation between the compartments was observed ($p = 0.0001$). Root rot incidences in Riau and East Kalimantan were not significantly different but both were significantly higher than that in South Sumatra.

In the three-year-old plantations of East Kalimantan, many dying and standing-dead trees were observed. However, in the four- and five-year-old plantations, many trees were missing or in an advanced state of decomposition and assumed dead from root rot. Therefore, it appeared that the process of infection and death occurred quickly for *A. mangium* root rot. Dead trees appeared to quickly lose their crown and the stem became degraded due to termites and ants.

Progeny trial

The fruiting bodies associated with dying trees were identified as species from the *G. lucidum* complex. Two different morphotypes were present, named subspecies 1 and subspecies 2 for the purpose of this study. *Ganoderma lucidum* subspecies 1 caused typical red root rot symptoms. *Ganoderma lucidum* subspecies 2 was associated with a more rubbery rhizomorphic skin, which was yellow and brown on the outside with a blistered appearance and rubbery white on the inside when pulled away from the root wood.

The incidences of total root rot death approximately doubled (7 to 13%) between the first and second survey period (Table 2). Trees that were recorded as living but with a *Ganoderma* fruit body in 2003 were generally dead by the time of the second survey (all but five were dead and three of those no longer had a visible fruit body). Many more trees were observed to show defoliation but these were not systematically surveyed.

Table 1 Incidence of root rot in commercial plantations and site details

Region	East Kalimantan			South Sumatra		Riau		
	46B	35B	44D	6	21	196	10	204
Compartment								
Incidence (%)	19.0	28.5	14.5	4.5	3.2	26.5	18.0	20.4
n	200	200	200	300	253	391	322	378
Tree age	8	4	5	5	5	NA	NA	NA
Rotation	Second			Second		Second		
Seed source	Suban Jeriji			Suban Jeriji		Australian and PNG		
Annual rainfall (mm)	2000			2000		2400		
Previous vegetation	Forest			Forest		Forest		
Planting season	Continuous			Continuous		Continuous		

Note : NA = Not available

Table 2 Incidence of root rot at Wonogiri progeny trial

Tree Status	Number (2003)	Percentage ^x (2003)	Cumulative number (2004)	Percentage ^y (2004)
Dead—missing ^a	39	3.87	104	10.46
Dead—stump ^b	26	2.57	26	2.62
Dead with fruit body ^c	3	0.30	NA	NA
Alive with fruit body	28	2.67	2	0.20
TOTAL dead ^{a+b+c}	68	6.75	130	13.04

x total number of trees in plantation = 1008

y total number of trees in plantation = 994

Root rot was found throughout all replicates of the trial and in some cases, roughly circular patches of tree death were detected. In 2003, no more than three or four trees in direct proximity to each other showed root rot symptoms. Occasionally, single isolated trees were clearly infected or dead. In 2004, up to 10 adjacent trees were dead or infected by root rot. Trees that were missing in the first survey were assumed to be the first trees to have died in the trial from root rot.

Analysis by SADIE confirmed the random pattern of tree death in 2003. In 2004, a slightly aggregated (but not significantly aggregated) pattern was observed. There was a significant association between the two survey times, suggesting that diseased trees in 2003 were providing a source of inoculum to adjacent trees that were subsequently recorded as tree deaths in 2004. GLMM analysis showed that one replicate of the trial (block 2) had approximately a three-fold reduction in root rot incidence than the other six replicates ($p < 0.1$, results not shown).

Results collected from the second survey were collated on a per family and provenance basis (Table 3). All provenances were affected by the

disease to some extent, but not all families. As the provenances were not composed of the same number of families, the percentage the provenance represented in the trial was calculated and compared with root rot detected in the provenance. However, GLMM statistical analysis revealed there were no significant differences between provenances ($p = 0.353$).

DISCUSSION

Commercial plantations

A large range in root rot incidence was detected (3.2–28.5%) in the plantations surveyed. This is comparable with results from Malaysia where mortality was under 20% for five- to six-year-old first rotation plantations (Lee 2000) and in the Philippines where mortality was between 10–25% in six- to 10-year-old *A. mangium* (Militante & Manalo 1999). In older trees (nine to 14 years), more than 40% mortality of *A. mangium* trees had been recorded in India (Mehotra *et al.* 1996).

Our surveys showed that compartments in Riau and East Kalimantan provenance had higher root rot incidence compared with South Sumatra. Given that all compartments were

Table 3 Root rot incidence (%R) according to provenance and family and the proportion of the provenance (%P) it represents, at Wonogiri progeny trial

Provenance/family no.	Trees infected	Provenance/family no.	Trees infected	Provenance/family no.	Trees infected
Demise, PNG		Kini, PNG		105	1
2	0	50*	1	106	2
4	1	51*	2	107	1
5	1	52	1	108	0
% P ^x	2.22	53	2	109	0
% RR ^y	1.47	54	0	110	0
Deri-Deri, PNG		55	0	111	0
8	2	56	1	112	2
9	1	57	1	113	0
10	0	58	2	114	0
11	1	59	2	115	0
12	0	% P	7.41	127	1
13	1	% PR	8.82	128	0
14	0	Wilpim, PNG		129	0
15	0	60	1	130	0
16*	1	61	0	131	0
17	1	62	2	132	1
%P	7.41	63	0	133	1
%RR	5.18	64	0	134	2
Gubam, PNG		65	0	% P	25.18
18*	2	66	1	% PR	19.25
19*	1	67	0	Pascoe R. area, FNQ	
20	0	68	1	116	1
21	2	69	0	117	2
22	1	% P	7.41	118	1
23	0	% PR	8.67	119	1
24*	0	Nr. Coen, FNQ		% P	2.96
25	2	70	0	% PR	2.94
26	1	71	2	Pascoe R., FNQ	
27	1	72	0	121	1
%P	7.41	73	1	122	1
%RR	8.09	74	1	123	1
Bimadebun, PNG		75*	1	124	1
28	1	76	1	125	1
29	0	% P	5.19	126	2
30	2	% PR	4.41	% P	4.44
31	0	Claudie/Iron, FNQ		% PR	5.15
32	2	78	0	Cassowary, CRQ	
33	0	79	0	135	0
34	0	80	1	136	1
%P	5.19	81	0	137	0
%RR	3.67	82	0	138	1
Arufi Vill, PNG		83	1	139	2
35	3	84	0	140	1
36	2	85	2	141	2
37*	3	86	1	142	1
38	1	% P	6.66	143	2
39	4	% PR	3.68	144	0
40	1	Claudie R., FNQ		% P	7.41
41	1	87	1	% PR	7.35
%P	5.19	88	1	^x proportion represented by the provenance	
%RR	11.11	90	1	^y proportion of the root rot attributed to the provenance	
Boite, PNG		92	2	*double representation (i.e. 14 trees in trial, not 7)	
42	3	93	1		
43	0	94	2		
44	0	95	1		
45	1	96	0		
46	1	97	1		
47	0	99	2		
48	1	100	1		
49	1	101	0		
%P	5.93	102	1		
%RR	5.15	104	1		

planted as the second rotation of *A. mangium*, factors such as previous vegetation, climate and management practices could account for the difference. In Malaysia, root rot incidence of *A. mangium* tends to be highest in lowland areas of former rainforest due to greater inoculum levels (Lee 1997). Also, root rot was absent in *A. crassicarpa* grown in former grassland areas. Such findings show that previous vegetation possibly play a large role in root rot, as forest and grassland vegetation have different fungal compositions. For example, wood inhabiting fungi would be more abundant in a forest ecosystem (Risna & Suhriman 2003). In addition to previous vegetation, neighbouring vegetation is also likely to be a source of fungal inoculum to plantations. Unfortunately, we do not have information about the presence of *Ganoderma* in the previous forests of Riau and Kalimantan.

Planting practice may influence root rot incidence. Continuous planting (where there is only a short delay between harvesting and replanting) provides the fungus with a continuous network of roots to invade and infect. However, a gap in planting of only a few months would probably result in short-lived effects. Woody debris maintained in the soil would harbour the fungus but there is no current knowledge as to how long.

Progeny trial

The fact that incidence of tree death doubled between 2003 and 2004 showed that the disease was spreading rapidly in the trial. While the trial is a non-commercial plantation, this has implications for the potential rate of spread in plantations. In Malaysia, for first rotation *A. mangium* plantations grown on lowland ex-forest sites, mortality due to root rot could double within one year (Lee 2000). The incidence of other root rot fungi such as *Armillaria ostoyae* in pine forests have also been reported to double within one year (Lung-Escarmant & Guyon 2004).

In the progeny trial, root rot associated with the *Ganoderma* spp. was found to occur in a random pattern in 2003. Therefore, it is likely that sources of inoculum were distributed randomly throughout the trial. As the GLMM analysis showed that one replicate of the trial had approximately a three-fold lower incidence of root rot than the other six replicates, the

distribution of root rot has a tendency for uneven distribution.

The random pattern of root rot observed may be due to the random distribution of woody debris in the soil or old stumps from the previous tree crop which could harbour the fungi. Alternatively, the inoculum may have been introduced by spores from neighbouring vegetation. For example, thinning of the plantation in 1997 created many stumps throughout the plantation. Fungal basidiospores could then establish on the susceptible, dead stumps and infect the stump roots. The aggregated trend of tree deaths probably indicates that sites of initial infection are spreading further by fungal vegetative growth via rhizomorphs and root-to-root contact.

This epidemiological trend observed in this study followed a similar pattern that has been described for *G. boninense* of oil palm. In this case, primary infection has traditionally been considered to occur by contact of living roots with colonized debris within the soil (Turner 1981) but recent molecular studies showed a major role for basidiospore dispersal (Miller *et al.* 1999, Pilotti *et al.* 2003). The origin of *Phellinus noxius* root rot in *A. mangium* and *A. auriculiformis* can usually be traced to the vegetation which was present previously (Arentz 1996). In the present trial, fruit bodies of *Ganoderma* spp. may have been initially present somewhere near the site and spores spread may have introduced the main inoculum to the site. This can be tested in future studies by assessing the genetic relatedness between fungal cultures isolated from different root rot foci. That is, material introduced by basidiospores are likely to be genetically distinct from each other as evidenced by somatic incompatibility tests or mtDNA RFLP (Miller *et al.* 1999) while spread by vegetative growth will result in genetically identical material.

While some provenances were more affected by root rot than others (e.g. Arufi Village, PNG) there were no significant differences and no general trends between Australian and PNG sourced material. As the incidence of root rot became higher over time, trends in root rot resistance may become more apparent. Trends in the present study did not match the results reported in Malaysia, for example, the Claudie River provenance had high root rot incidence in the Malaysian study but was low in the present study compared with the other

provenances tested.

Ganoderma philippi appeared responsible for the majority of root rot in commercial *A. mangium* plantations in Indonesia (N. L. Bougher, pers. comm.). However, from this study it appeared that *G. lucidum* can cause a similar red root rot and also rapidly infect *A. mangium* stands. Identification of species from the *G. lucidum* complex is difficult. It has been argued that the name of *G. lucidum* is often misused and in South-East Asia all identifications of this species are likely to be *G. steyaertanum* (Smith & Sivasithamparam 2003). However, other researchers (Moncalvo *et al.* 1995, Gottlieb *et al.* 2000) have determined that of the collections that have been called *G. lucidum*, there may be six or more species represented on the basis of rDNA analysis. Therefore, future molecular studies of the *G. lucidum* subspecies collected at the progeny trial would be invaluable.

Suggestions for future research

Further surveys in commercial plantations are required to determine the rate of spread over time. Development of interpretive methods for remote sensing technologies to detect root rot patches will aid these surveys and allow extensive assessment of root rot spread. Quantification of root rot incidence throughout many regions and plantations will help managers assess the relative cost-benefit of potential control options.

Studies of genetic diversity of *Ganoderma* spp. foci within limited areas are necessary to determine the main mechanisms of fungal spread and their contribution to root rot disease. We suggest following the strategy and techniques of Miller *et al.* (1999) used for *G. boninense* in oil palm. This information will provide a better basis for control options such as stump treatments. Further research that will improve our understanding of the biology of the fungus, epidemiology and climatic impacts are also required. Research on resistance is warranted and assessment of the progeny trial described in this study will be continued in the future.

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