Australian Government

Australian Centre for International Agricultural Research

# Final report

*project*

## Management of fungal root rot in plantation acacias in Indonesia

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| *project number* | FST/2003/048 |
| *date published* | March 2012 |
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| *approved by* | Dr Tony Bartlett, Research Program Manager for Forestry, ACIAR |
| *final report number* | FR2012-06 |
| *ISBN* | 978 1 921962 50 9 |
| *published by* | ACIAR<br>GPO Box 1571<br>Canberra ACT 2601<br>Australia |

# Contents

# 1 Acknowledgments

We gratefully acknowledge all the help and assistance of the company and staff of FORDA, PT AA, PT MHP, PT RAPP, PT SRH and SDN GP.

# 2 Executive summary

ACIAR project FST/2003/048 "Management of fungal root rot in plantation acacias in Indonesia" delivered on all four project objectives and achieved the overall aim of offering potential management solutions which can be explored to reduce losses in productivity due to these diseases in pulpwood crops in Indonesia.

### *Identification of the main causal agent(s) of root-rot disease and characterisation of their field biology*

At the start of the project '*Ganoderma*' was the commonly used generic name for any root-rot disease in hardwood plantations. An output of the project is the identification of several fungal species consistently associated with root-rot disease in *Acacia* **and** *Eucalyptus* plantations. We extended the scope of the project to include an investigation of the biology of root-rot disease in *Eucalyptus pellita* planted on ex-*Acacia mangium* sites because two of the three companies associated with the project shifted from planting *Acacia mangium* to *Eucalyptus pellita* during the lifetime of the project. This change is linked to a perceived lower risk of this eucalypt species to root-rot disease.

The root-pathogens that we identified differ in their abundance in plantations, their pathogenicity and biology. They therefore pose different challenges to management. The most frequently encountered species was *Ganoderma philippii* but *Phellinus noxius*, *G. mastoporum*, *G. steyaertanum* and an additional three *Phellinus* species new to science were also encountered. The project carried out the first successful re-isolation of *G. philippii* during pathogenicity tests proving this fungal agent to be the cause of red root-rot disease. Illustrated guides to identification have been developed for the different root-rot pathogens. These distinguish between root symptoms, and include a general outline of the mycological methods required for fungal diagnostics. All fungal root-rot pathogens have been characterised by DNA profiling and cultural morphotyping. We developed rapid DNA based diagnostic techniques for the more common root rot pathogens. Our outputs allow the accurate identification of root-rot fungal agents in both *Acacia* and *Eucalyptus*, the accurate monitoring of disease incidence/severity and the development of management strategies against specified root-rot pathogens.

We investigated the field biology of *G. philippii*, the causal agent of the frequently observed red-root rot disease. Pathogen spread through vegetative transmission predominates in disease gaps especially in first rotation crops. A gap tends to expand at a relatively constant rate. New genotypes do appear in a disease gap although population diversity within a gap is generally low except in old and large second-rotation compartments. Since we found clear evidence of spore dispersal it is possible that new disease gaps could arise from stump infection. Management strategies preventing stump infection by spores are likely to be useful in preventing new infection centres but are unlikely to reduce the size of existing gaps. The study came to the preliminary conclusion that the tree mortalities due to root-rot by the end of rotation are related to the number and size of disease gaps, and their relative closeness to one another at the time of measurement relative to the remaining time until harvest. Thus many, evenly dispersed disease gaps would result in higher end-of-rotation losses than the same number of gaps very closely spaced (because coalescence would occur earlier) or fewer gaps of equivalent or larger sizes (because of smaller effective perimeter size).

### *Identification of site factors*

The project demonstrated that sites at high risk of root-rot can be identified and that plantation managers could reduce this risk through management decisions such as reducing rotation length and not planting at spacings less than 2.5 X 3.0 metres. A simple prototype "site risk assessment tool" was developed. This was based on data pertaining to broad-scale disease-incidence and site characterisation provided by our project partners. Second and third rotation sites are more prone to root rot than first-rotation

sites; sites with stands older than age six years have a higher risk of disease incidence than younger stands; stands with trees spaced at < 2.5 x 3 m have a higher risk; soil types vary in risk and, when ranked, risk increase more or less linearly for the set of soil types examined with sandy-clay soils the worst affected; compartments with a slope > 31% have a slightly higher risk; root-rot risk generally decreases across a west to east gradient in Indonesia but, north to south, is highest closest to the equator. However average monthly rainfall patterns do not appear to explain disease progression curves.

### *Management*

The project reviewed those biological and silvicultural control options that are most likely to provide operationally and economically feasible root-rot management solutions in both industrial and small-holder plantings. Root-rot management options including chemical and biological control were compared to those employed for similar root and stem rot pathogens in the oil-palm and rubber industries. All review information has been made available via flexible delivery mechanisms including the project web-site. Combined meetings were held with ACIAR Project FST/2004/058 "Realising genetic gains in Indonesian and Australian plantations through water and nutrient management". This allowed a wider perspective when interpreting project results and how they could be integrated into the silvicultural management of root-rot.

Mortality rates for root-rot infected areas were shown to increase rapidly as a plantation ages. Harvesting at 5 years on root-rot diseased sites with a mortality rate of 20-25% would mean that landowners that in certain cases a landowner would harvest a greater volume than if harvest is delayed.

Trials were established to investigate the efficacy of stump removal, burning and biological control. Treatment of planted trees with a species of *Cerrena*, new to science, discovered by project partner PT Arara Abadi and identified by the project, is associated with substantial reductions in mortality due to root rot caused by *G. philippii* (75%) and *P. noxius* (40%) in pot and field trials and is currently being deployed. However its application is cumbersome as it can only be applied as wood blocks in the ground. The project discovered another potential biocontrol agent (*Phlebiopsis*) that might be deployed as a spore solution and could be applied as a spray to stumps. *Cerrena* and *Phlebiopsis* isolates warrant further investigation.

A cross rotational examination of the effects of slash removal by burning coupled with stump removal was set-up and monitored. Somatic incompatibility tests between the different cultures of *G. philippii* isolated from the trial showed that the burn carried out was not an effective control measure; genotypes present in the second rotation were present and killed trees post-burn in the third rotation. The possible influence root architecture on root-rot incidence was explored by ground-penetrating radar technology applied in *A. mangium* and *E. pellita*. Images were difficult to interpret as this tool was hampered by the rough terrain in plantations, but its potential to explain and support root-rot research trials warrants further investigation.

### *Training*

The capacity of industry partners and FORDA to identify and manage forest diseases has been transformed by the training activities of the project. The application of mycology and the microbiological aspects of plant pathology to the protection of Indonesia's forests were areas of relatively underdeveloped expertise prior to the project.

The project supported three John Allwright Fellows from the Indonesian Forestry Research and Development Agency (FORDA) and another senior FORDA scientist was hosted at the University of Tasmania for a four week training period. Project participants from P.T. Riau Andalan Pulp and Paper and P.T. Arara Abadi also visited Australia for a period of collaboration investigating risk assessment, biological control and remote sensing in terms of managing for root-rot. Four FORDA staff received intensive instruction in the various methodologies used for mycological molecular biology and a further four

were trained in mycological methods including field survey techniques, culture management, microscopy and the identification of fungal sporocarps.

The establishment by the project of a Mycology Laboratory at the FORDA Centre for Forest Biotechnology and Tree Improvement (CFBTI) will provide the capacity for mycological expertise to be fostered and maintained into the future. The close synergy developed during the project between the CFBTI's mycology and molecular biology laboratories greatly enhances the scope of research activities at CFBTI. The personnel trained during the project at FORDA (CFBTI) laboratory in Yogyakarta are now able to offer commercial diagnostic services to Industry project partners.

An additional training activity funded by AusAID, run by the University of Tasmania and CSIRO proved very beneficial to the ACIAR project. A workshop was organised to build capacity in forest disease management strategies. This allowed project staff to showcase the advances made by the project and to network with other industries facing similar root-rot issues. We were able distribute to a wide audience information about root-rot disease management options. The workshop also served to alert policy makers in the Ministry of Forestry and in Indonesian Quarantine to the threat posed by forest pests and diseases, especially root-rot, to hardwood plantation industries in Indonesia.

### *In conclusion*

Indonesian capacity in the different aspects of forest mycology and pathology was increased significantly. Fungal root-rot pathogens especially *Ganoderma philippi* were described and their biology determined. Losses from root-rot at disease sites increase across rotations, smaller tree spacing incurs higher risk, soil types vary in risk and soil texture may be important, compartments with a slope value of 5 have a slightly higher risk, plantations older that 5-6 years have higher risk and delayed harvesting could mean significant economic loss. A prototype site-risk assessment tool was developed. Additional root-rot disease surveys and detailed site-factor analyses are necessary to enhance, expand and validate the project's risk assessment tool to the point where it can reliably inform management decisions by industry.

Management options for root-rot were reviewed, information disseminated and research trials established and monitored during the life of the project. Results from silvicultural trials indicate that options show no or variable levels of control i.e.de-stumping; burning between rotations; slash removal; the use of more tolerant break crops for *Acacia mangium* such as *Eucalyptus pellita;* the natural regeneration of *Acacia mangium* as opposed to planting seedlings. Both major pulpwood crops (*E. pellita* and *A. mangium*) are susceptible to *G. philippi*. The relative susceptibility to root rot of *E. pellita* versus *A. mangium* should be better quantified to allow an informed cost/benefit analysis for this alternative pulpwood crop. A species of *Cerrena* applied in the form of infested wood blocks into planting holes appears to be an effective biocontrol agent over a short initial period of testing. The full biological and taxonomic characterisation of *Cerrena* and other promising biological control agents (such as *Phlebiopsis*) and the development of suitable production / application methods are necessary to achieve the greatest impact in biological control.

The Ministry of Forestry of Indonesia has proposed a 9M ha expansion of hardwood plantation forestry in Indonesia with approximately 60% or 5.4M ha of this area is earmarked for small-holder growers. This project has shown the extent to which root-rot disease is a very significant threat to the long-term viability of *Acacia* plantations and that the diseases poses a less certain, but real threat to *Eucalyptus* plantations. During its nearly four-year term, the project made substantial progress in understanding the complex root-rot pathosystem threatening pulpwood forestry in Indonesia and towards reducing this threat.

# 3 Background

## 3.1 Partner country and Australian research and development issues and priority

Indonesia now reports a total of 3.359 million hectares of planted forest in the FAO Global Forest Resource Assessment 2010. *Acacia mangium* represents a large proportion of the forest-plantation industry in Indonesia: currently about 1.6 Mha of plantings to this species (Rimbawanto, pers.comm). A second acacia species, *A. crassicarpa*, is planted on peat soils to which it is better adapted than *A. mangium*. *Acacia mangium* is fast-growing and of medium density. Acacia plantations are primarily managed for pulp wood.

The major forest companies have large concession areas of public land granted to them by the Government of Indonesia for the establishment of acacia plantations. The ownership of substantial parts of this land is claimed by the communities who live and work there because it is land that has traditionally been used to support, and provide income to, these communities. Although these claims have no legal status, the companies choose to engage community support for the planting of *A. mangium* through outgrower schemes or share-farm agreements as part of their policy of resolving land claims in a socially responsible manner. Through these agreements, the companies guarantee to buy the wood over a period of 40 years (six rotations) and provide materials and wages for plantation establishment and tending during the growing cycle. There is no coercion to plant *Acacia* only. Indeed the companies offer training schemes in other types of plant culture. The benefits inevitably work in both directions. These agreements offer the companies security to operate in the area where their concession is located and a means of meeting their requirements for wood. As the communities recognise that their welfare and security is based on these agreements, so the incidence of arson and illegal logging decreases in those areas where the schemes operate. Thus it is a system that helps to resolve conflict, promotes mutual benefit and plays a substantial role in poverty alleviation, as well as delivering environmental positives. Collectively PT. Arara Abadi, PT. MHP, and PT Riau Andalan Pulp Paper already access more than 150,000 ha of acacia plantations through outgrower schemes, some outgrowers managing parcels of land as little as 2 ha. Annual rates of planting of over 30,000 ha per annum through the schemes point to the increasing importance of outgrowers and the demand for wood supplies in Indonesia. Such schemes are set to become much more important to the wood supply for the mills. The Ministry of Forestry (MoF) plans to double the production of pulp to 16M t per year by 2020, requiring an additional 9M ha of plantations, of which around 60% (~5.4M ha) is to be grown by smallholder farmers.

As the growing of *A. mangium* is starting to represent a primary source of income for communities in the rural sector in Sumatra and Kalimantan, any threats to the productivity and sustainability of this resource is of concern. Root rot caused by decay fungi currently represents the highest priority of all diseases in *A. mangium* plantations because it causes tree death, which directly affects the ability of outgrowers to supply wood to the industries they serve. Levels of tree death are relatively low (around 5%) in the first rotation. However, the build-up of inoculum load associated with stump retention and the accumulation of woody debris over successive rotations is already associated with much higher (>15% tree death) by the third rotation. Yields are clearly not sustainable under such a scenario. These losses could be considerable for individual small holders with planted areas of around 2ha for whom even a few large disease gaps could represent the loss of up to half their production.

In 2005 the idea for the root-rot project was suggested by Indonesian growers of *A. mangium* during the period of the fungal heart-rot project (FST/2000/123) in response to the severe impact of root rot disease observed by both industry and heart-rot project staff. The networks and expertise were already in place to switch from the study of heart-

rot to root rot. ACIAR's Forestry Program in Indonesia targets the sustainability of wood-based industries: root rot was perceived and remains today a major threat to sustainability which if not managed to acceptable levels significantly impacts outgrowers and the rural communities that rely on this resource for their income and welfare. The three industrial companies, PT. Arara Abadi, PT. MHP and PT-RAPP that rely most heavily on local forest communities for part of their wood supply supported this project with substantial in-kind contributions. Project outcomes are directly aligned with the ACIAR focus area "Sustainable management of tropical plantations" within Theme 2: Improving the productivity and efficiency of food crop and forestry systems.

## 3.2 Research and/or development strategy and relationship to other ACIAR investments and other donor activities

*Project development strategy*

Project development and the subsequent research in FST/2003/048 greatly benefited from a previous ACIAR project - FST/2000/123, completed in 2006. The latter project focused on the prevention of heart-rot in *Acacia mangium* produced as saw-logs but also carried out preliminary research into root-rot when it became evident that this was a serious problem. An end-of-project workshop for the heart-rot project in February 2006 was used to also develop the root-rot project. All industrial partners were present at the workshop and this milestone meeting brought together all three partners for an open discussion and kick-started the root-rot project. Project FST/2003/048 therefore started from a solid basis of knowledge about *A. mangium* and its associated decay organisms, a network of interested parties and an excellent rapport with the industry participants (PT. MHP, PT. Arara Abadi and PT. RAPP) and their networks to the outgrowers.

Many fungi cause root rots and most belong to the Basidiomycete division. Basidiomycetes encompass fungi with sexual spores borne on macroscopic fruiting structures bearing gills or pores. Basidiomycete fungi usually exist as endemic saprophytes in the natural forest causing little, if any, problem. They can be saprotrophic (surviving on dead woody material or debris) or pathogenic (invading living plant tissues). Basidiomycetes capable of causing woody root rot can be both saprotrophic and pathogenic. When land is cleared for plantation establishment, these fungi are able to survive saprotrophically on root remains, tree stumps and other woody debris in the soil and then some species can pathogenically invade roots of newly planted trees. Fungal pathogens and diseases are spread by spore invasion of woody debris such as freshly cut stump surfaces or by the contact of healthy roots with inoculum in the form of infested stumps or infected roots. The general life cycle of woody root rot basidiomycetes is outlined in Figure 1 using a tropical root rot fungus *Phellinus noxius* as an example.

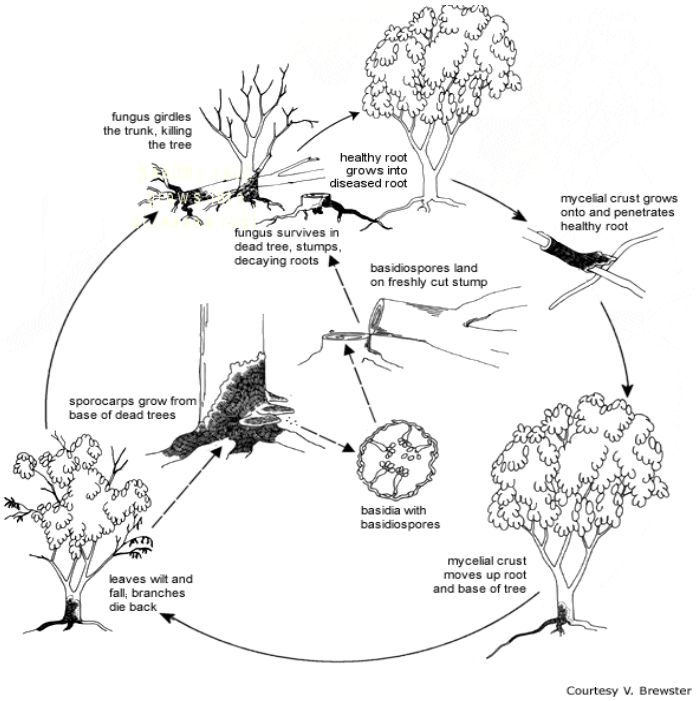


**Figure 1: Life cycle of a tropical root rot pathogen (*Phellinus noxius*)**

In general, the control and prevention of root rot diseases in different tree crops worldwide has been achieved through a combination of methods including the use of resistant species, avoidance of hazardous sites, cultural manipulation, chemical application and biological methods (Woodward *et al.* 1998). Root rot is difficult to manage but strategies employing the different types of disease control do exist for tropical tree crops such as oil palm in SE Asia (Arrifin et al. 2000) and rubber in Malaysia and Africa (Nandris et al. 1987).

The Indonesian companies in 2006 had started to test the application of treatments known to be effective treatments for other types of basidiomycete root rot in both temperate and tropical forestry. This entirely pragmatic approach was intended to deliver short-term commercial benefit. It included biological (*Trichoderma*-based) and chemical treatments to soil and stumps which harbour sources of inoculum. In 2006 in South-East Asia, knowledge about the identities, biology, aetiologies and control of the causal agents responsible for root rot disease in *A. mangium* was sparse (Lee 2000). For tree crops such as rubber and oil palm with a longer history of forest disease management than *A. mangium*, cost effective reduction of root rot incidence has only been achieved through gaining a thorough prior understanding of the biology and aetiology of the pathogens involved in causing disease. The poor performance of the biological and chemical treatments applied by the Indonesian companies at the start of the project which attempted to reduce inoculum was almost certainly due to their non-specificity caused by a poor understanding of the disease organisms and their behaviour in plantation monocultures.

There was, however, at the start of the project a general recognition that the build-up of inoculum load is linked to the presence of stumps and woody debris and that the potential for these substrates to accumulate in plantations that are harvested on a 6-to-7 year cycle is very high and increases when rotation length is reduced. The way forward was to embrace treatments that reduce the quantity of stumps and woody debris and that can easily carried out as part of the harvesting operation.

Stump and debris removal are operationally expensive treatments even for large companies to contemplate and are entirely unsuited to the outgrower sector. Therefore management strategies of possible relevance and adoption by outgrowers are;

a) Plantation establishment (preventative) e.g. matching species selection to site risk, reducing inoculum by giving debris sufficient time to break down, rotating susceptible and non-susceptible hosts b) Chemical control c) Biological control

Biological control of root-rot fungi can be afforded by the use of non-pathogenic fungi that either breakdown woody debris, occupy the same resource as the pathogen, compete for nutrients, produce inhibitory compounds or are mycoparasites. The potential use of non-pathogenic lignolytic basidiomycetes, both as a natural means to eliminate the woody debris and to destroy/outcompete pathogens represents is an attractive alternative to less environmentally friendly chemical-based control. The commercial formulation based on *Phlebiopsis gigantea* is however the only successful biological control available for basidiomycete root-rot disease. This widely distributed wood-rotting basidiomycete, *P. gigantea* has been used throughout pine and spruce forests in Europe and North America as a biological control agent for the control of *H. annosum* (Rishbeth 1979). *P. gigantea*, a white rot fungus, competes with *H. annosum* for the woody resource within conifer stumps and has been used as a control option for over 40 years. For reasons of biosecurity and environmental safety any biological control of root rot in *A. mangium* must be developed specifically from and for the *A. mangium* environment and will have a long lead time to commercial development.

*Project research strategy*

The research providers had combined skills (many developed during ACIAR project heart rot project (FST/2000/123)) which allowed them to broaden and strengthen the pragmatic approach to root rot control taken by the companies; the morphological and molecular identification of disease organisms especially wood-rotting fungi; the development of biological control strategies; tree disease physiology; tree crown health assessment/monitoring and risk management.

This project set out to

- Determine which decay species cause disease and how disease spreads within and between trees
- Examine the variables that drive disease build up. The distribution of root rot varies between and within sites, as does the rate of disease build-up. This suggested at the start of the project that disease presence and disease expression are a function of edaphic, environmental and silvicultural factors. Stress caused by disease is often observed as changes in crown condition and the physiological behaviour of the crown. In some cases e.g. *Phytophthora* such changes (e.g. development of water stress) are often not observed until disease is well advanced. Red root-rot disease (*Ganoderma*), the major focus of this project, develops quite rapidly and is associated with changes in crown condition (thinning of crowns, smaller leaves and chlorosis). The trees appear to be killed before being completely girdled by the fungus. These observations suggest that physiological changes may be early indicators of disease presence.

The fundamental scientific understanding from the above activities was applied to:

**Developing effective systems for the reduction of root rot investigating all feasible options based on a genetic, silvicultural, biological and/or chemical approach**

This project put a very strong emphasis on its training and information dissemination components e.g. technical information sheets written in Indonesian, company officer training so that this training could be extended to smallholder farmers.

*Relationship to other ACIAR investments and other donor activities*

ACIAR FST/2004/058 (Realising genetic gains in Indonesian and Australian plantations through water and nutrient management) was a project that was developed and was active at the same time as the root rot project FST/2003/048. Both projects involved scientists from CSIRO and Dr. Sadanandan Nambiar participated in both projects to facilitate co-ordination between the two projects. This facilitated effective communication

between the projects, joint meetings and combined reviews so that root-rot management strategies could be integrated into best practice silvicultural management.

SDN Grand Perfect in Sarawak, Malaysia, opted to support project by facilitating the incorporation of site characteristic and root-rot incidence data to expand the regional usefulness and applicability of the project's research.

AusAID funded a training workshop under its Public Sectors Linkage Program funding scheme. This was a generic workshop in forest disease management strategies but also explored root and stem-rot pathogens in oil palm, rubber and hardwood plantations.

# 4 Objectives

*AIM:* **To manage fungal root- rot diseases in plantation acacias in Indonesia and reduce losses in productivity caused by these diseases**

### *Objective 1: Identify the main causal agent(s) of root-rot disease and characterise their field biology*

**Activity 1.1** Identification of fungi associated with root rot disease.

**Activity 1.2** Determination of mode of dispersal for root- rots pathogens

**Activity 1.3** Assessment of fungal pathogenicity and host susceptibility

### *Objective 2: Identification of site factors influencing the development of disease symptoms and root-disease distribution*

**Activity 2.1:** Ground based surveys of root- rot disease incidence and severity

**Activity 2.2:** Monitoring the symptoms of root rot disease development

**Activity 2.3:** Development of site-risk rating systems

### *Objective 3: Development of simple and cost-effective root rot reduction strategies*

**Activity 3.1:** Evaluation of existing and new biocontrol and chemical agents

**Activity 3.2:** Evaluation of stump application technologies for the delivery of biocontrol agents.

**Activity 3.3:** Field testing of control options. Combined output of all activities in 3 is the field testing of interventions that can be applied simply in an integrated management plan for root rot reduction

### *Objective 4: Training, information packaging, dissemination and adoption of outcomes*

**Activity 4.1:** Training; will be ongoing throughout the project (q.v. 5.2 and 5.3 for specific training plans)

**Activity 4.2:** Delivery of root-rot management strategies to outgrowers

# 5 Methodology

## 5.1 Indonesian Activities

### 5.1.1 Summary of Activities

The activities of the project were carried out via a suite of different experimental procedures.

- Permanent plots to intensively understand and monitor the biology and spread of the root-rot pathogen.
- Broad-scale surveys of root rot incidence
- Opportunistic sporocarp collections in the field to collect herbarium specimens and possible biological control agents
- Mycological studies in the laboratory applying traditional and molecular techniques
- Shade house based studies to test the pathogenicity of hardwood species to *G. philippii*
- Industry partner trials to test different management strategies including biological control

### 5.1.2 Permanent plots

#### *Plot locations, establishment and monitoring frequency*

The permanent plots allowed us to intensively monitor the spread of the root rot pathogen. Disease progress curves for northern temperate basidiomycete root-rotting fungi have been modelled. However this is the first time in the world that such a fine scale observation of the behaviour of this particular root-rot pathogen (*Ganoderma philippi*) has been undertaken.

The semi-permanent plots were 10 × 10 tree plots 'centred' around a dead tree confirmed as having red root rot (e.g. Figure 1).

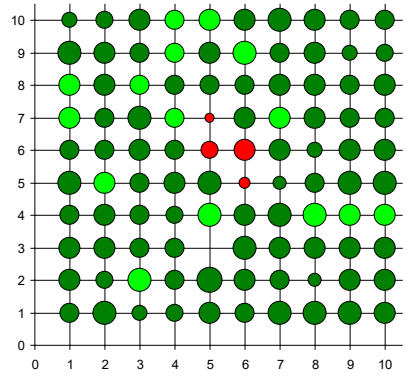


**Figure 1: Diagrammatic representation of a semi-permanent plot. Red circles indicate dead trees confirmed as having red root rot, green circles are other living trees included in the plot.**

Three to five such plots were established at each of five sites spread across three provinces: Riau (RAPP: Langgam, Nov. 2006; Logas South, Feb. 2007), South Sumatra (MHP: Deras, Dec. 2006; Selibing, Feb. 2007) and East Kalimantan (SRH: Sebulu, September 2007).

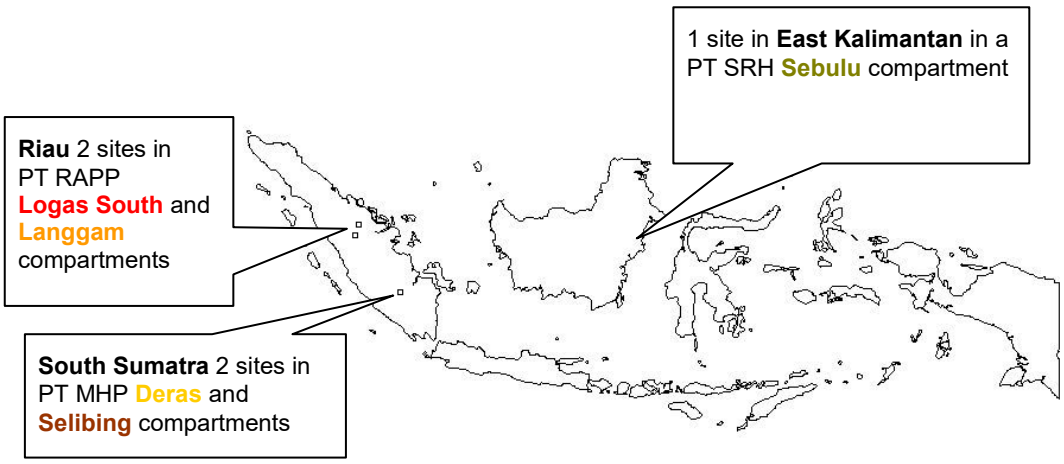


**Figure 2: The plantations used in this experiment were at five locations in three provinces of Indonesia: Riau province (Langgam Lat. 0.13ºN, Long 101.6 ºE; Logas South Lat. 0.29ºS, Long 101.27 ºE); South Sumatra province (Deras Lat. 3.31ºS, Long 103.58 ºE; Selibing Lat. 3.25ºS, Long 103.54 ºE); and East Kalimantan province (Sebulu Lat. 0.01ºS, Long 117.10ºE).**

The dates the permanent plots were established and subsequently monitored are presented in Table 1. The number in brackets next to the Site Name is the number of plots initially laid down at that site which were identified by a letter between 'A' and 'E'.

**Table 1: Details of five permanent sampling plot sites, provinces, planting dates and the dates of subsequent monitoring occasions.**

| Site Name and no. of plots | Province | Rotation no. | Planting | Plot established and monitored T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|
| Deras (4) | South Sumatra | 2nd | 1/12/ 2001 | 7/12/ 2006 | 8/8/ 2007 | 8/2/ 2008 | | |
| Langgam (3) | Riau | 2nd | 1/12/ 2003 | 1/12/ 2006 | 29/5/ 2007 | 27/5/ 2008 | 4/12/ 2008 | 23/6/ 2009 |
| Logas South (4) | Riau | 1st | 1/2/ 2006 | 21/2/ 2007 | 28/8/ 2007 | 29/5/ 2008 | 3/12/ 2008 | 24/6/ 2009 |
| Selibing (4) | South Sumatra | 1st | 1/2/ 2006 | 28/2/ 2007 | 14/8/ 2007 | 8/6/ 2008 | 29/10/ 2008 | 3/7/ 2009 |
| Sebulu (5) | East Kalimantan | 3rd | 1/9/ 2002 | 6/9/ 2007 | 16/6/ 2008 | 19/11/ 2008 | 24/6/ 2009 | |

Several additional plots were laid down at Sebulu and Deras:

- A fifth 10 × 10 tree plot ('E') at Sebulu was centred around a dead tree identified as having black root rot rather than red root rot to make and initial investigation of this form of root rot;
- Four additional 10 × 10 tree plots ('E' to 'H') were laid down at Deras in August 2007 to allow for replication in a planned burning trial; and

- On the boundary of each of three Deras plots ('A', 'C' & 'H') a 10 × 30m plot of additional trees was added to accommodate a stump removal experiment.

Deras plots were harvested in August 2008. Following the harvest of the original monitoring plots at Deras and the imposition of the burning and stump removal treatments four new plots were established on the site of the initial plots 'A' to 'D'. These plots established post-burn are referred to in this document as 'New Deras'.

### *Monitoring methodologies*

Above and below ground variables were assessed during each monitoring event (Figure 3). Roots were uncovered using a hoe before inspection and sampling. They were then covered until the next monitoring event.

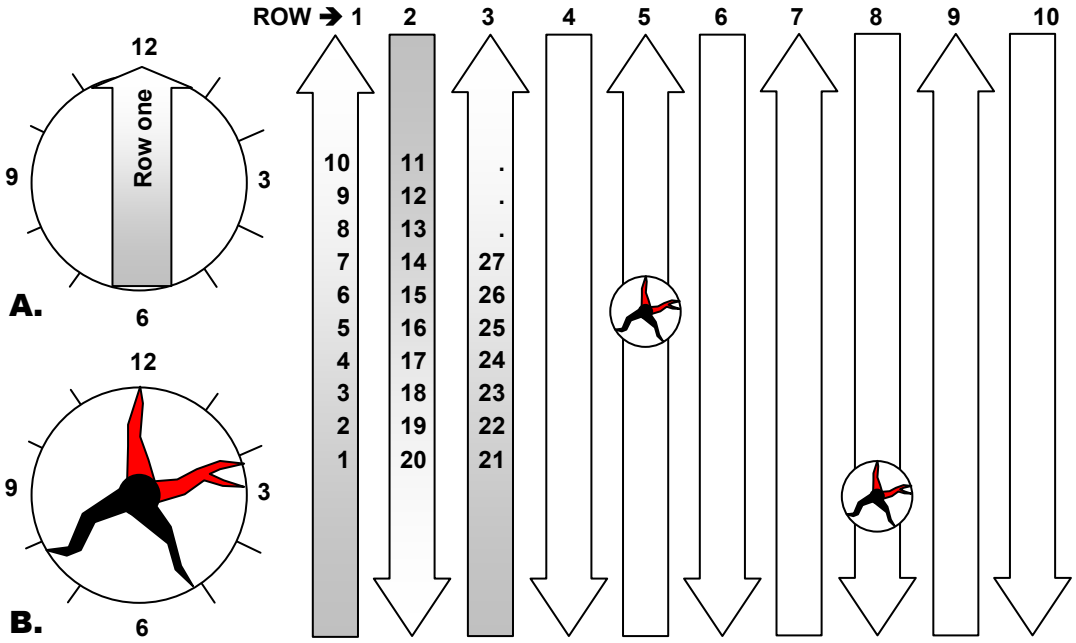


**Figure 3: Diagrammatic representation of the order in which the trees in the permanent plots were numbered and monitored and of the clock-face system of scoring the roots. The trees in each plot were numbered consecutively from one to approximately 100 by 'snaking' up and down the rows as indicated by the arrows and numbers above (illustrated in rows one to three). The 'clock face' (A. above) shows how for every tree, 12 o'clock was orientated by the direction of row one, so in B. the infection would be localised between 12 and 3 o'clock and this would be the same if the tree were in either row 5, which runs the same direction as row one, or in row 8 which runs in the opposite direction.**

### *Above-ground variables*

Above-ground variables monitored included:

- Diameter at breast height (DBH).
- Vitality (alive, dead or missing).
- Crown colour (green, green-yellow or yellow).
- Crown density (Five classes indicating the percentage of the average maximum density for a plot: 1. 0-25%, 2. 25-50%, 3. 50-75%, 4. 75-99% and 5. 100%).
- The presence of any visibly infected wounds, other identifiable causes of damage or death, or the presence of any fungal fruiting bodies.
- Leaf size (either normal or reduced).

***Below-ground variables***

Below ground variables were recorded for each tree on roots exposed to 20 to 50cm from the base of the tree. Variables recorded included:

- The presence or absence of any kind of root rot,
- The morphotype of any rot (red, black, *Phellinus*-type), and
- The distribution of infected roots on a 'clock face' (as illustrated in Figure 3) in order to give some indication of any directionality in infection.

Root samples were also taken. Initially this was from every tree on every monitoring occasion. Following analysis of the results of this sampling strategy this was limited to only sampling from obviously infected roots.

### 5.1.3 Broad-scale surveys of root-rot incidence

The broad-scale surveys carried out by P.T. Riau Andalan Pulp and Paper (RAPP) and Grand Perfect Sdn. Bhd. (GP) in Malaysia provided information on the root rot incidence.

***RAPP***

- Once-off purposive sampling specifically for root-rot incidence
- Survey plots were 400 tree plots with the number of plots dependant on the size of the compartment
- Surveys were only carried out in root rot affected areas where 400 tree plots were initiated near the first root rot infected tree encountered.

***GP***

- Surveys carried out in random permanent sampling plots (PSPs) established for inventory purposes, without reference to root rot incidence or other types of mortality
- 20 × 15m plots, orientated north-south

***Project staff***

The broad-scale survey carried out by project staff provided a uniform assessment of root rot presence and incidence across the estates of all three industry partners and over 3 Provinces of Indonesia.

A suite of three to four year old, second rotation *Acacia mangium* compartments were identified that reflected a range of site characteristics. The information on site characteristics was not available to stratify the sites consistently across all industry partners. A unilateral best practice in terms of site characterisation in particular, soil classification would assist future research.

Because there is no industry wide standard for site characterisation, the methodology for site stratification varied between industry partners as follows:

RAPP - sites stratified by soil type and slope

SRH - sites stratified by soil family

MHP - sites stratified by productivity class

We carried out a preliminary analysis to determine optimal sampling size required to detect a 0.5% change in tree mortality across a 20 ha compartment. This figure was based on a plot based sample of only the dead trees in the plot.

Given the economic and time constraints of the project's broad-scale sampling program it was not possible to carry out the sampling design as planned. Constraints included: time,

number of dead trees, number of trained staff, and thickness of undergrowth. Three replicates of 30 tree transects were run in six compartments in each of three estates of each company visited.

### 5.1.4 Laboratory studies

#### *Cultural identification of target genera*

A mycology/pathology laboratory was set up at FORDA in Jogjakarta and training given in the different types of laboratory procedures. Isolations from infected wood and sporocarps were carried out at this laboratory and 4644 isolates were stored. The target fungi of interest (wood-rotting fungi) produce enzymes (laccase and tyrosinase) capable of degrading lignin. An initial screening was carried out with cultures obtained from isolations. Sporulating contaminants and isolates that tested negative for laccase and/or tyrosinase were discarded. The identity of the remaining isolates was established by:

- Their morphology although this is very media dependant
- Molecular analyses

The synergistic use of molecular identification allowed project staff to validate their morphological identification skills. Target pathogenic genera are now readily recognised in culture reducing the need for molecular pre-screening of isolates.

#### *Morphological identification of sporocarps collected in the field*

Fungal sporocarps were collected on the permanent monitoring plots and also opportunistically during visits to the estates of all three Indonesian industry partners. Fungal taxonomy is essentially determined by the morphology of the fruiting body. A morphological analysis must be carried out before a taxonomic name can be assigned to a fungus collected in the form of a sporocarp from the environment. Our collections allowed the morphological identification of the major pathogenic types of fungi found and these sporocarps are conserved as vouchered herbarium specimens.

The sporocarps were tentatively assigned to two groups based on their morphology:

- Pathogenic genera including *Ganoderma*, *Phellinus* and *Inonotus*.
- Non-pathogenic genera including *Antrodiella* (closely related to *Cerrena*).

Vouchered herbarium specimens can be linked by their DNA to cultures obtained from sporocarps or from wood material. The cultures can then be assigned a taxonomic name.

#### *Molecular identification and diagnostics*

DNA was extracted from a square of mycelium, approx 0.25 cm2, which was cut from the growing edge of the medium, placed in a 1.5 ml microcentrifuge tube and stored at -20°C until used for DNA extraction. DNA was extracted and purified using a glassmilk method (Glen et al., 2002) and an aliquot diluted 1/20 in TE buffer (10mM Tris-Cl, pH 8, 1 mM Na2EDTA) for use in PCR.

Given the large number of isolates that required molecular verification it was not possible within budget constraints to sequence all cultures that were positive with enzyme tests and therefore a putative wood-rotting fungus. A species-specific test for the most frequently encountered root-rot pathogen (*G. philippii*) was developed based on DNA sequences obtained from a diverse range of cultures from this species. The species-specific test allows a cheaper and quicker screening of isolates. Only those isolates that are not *G. philippi* need to be identified by sequencing.

**Internal Transcribed Spacer (ITS)-Polymerase Chain Reaction (PCR) and DNA sequencing**

PCR reactions contained reaction buffer [67 mM TRIS-HCl pH 8.8, 16.6 mM(NH4)2SO4, 0.45% Triton X-100, 0.2mg/mLgelatine] (Fisher Biotec, W. Perth, Western Australia), 2

mM MgCl2, 0.2 mg/mL bovine serum albumin (Fisher Biotec), 0.2 mM dNTPs (Promega Corp., Madison, WI, USA), 0.25 mM primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990) and 1.1 U TTH+ DNA polymerase (Fisher Biotec) with 5 µL DNA template in a final volume of 25 µL. The thermocycler program consisted of: 2 minutes at 95°C, 35 cycles of (30 s at 95°C, 60 s at 56°C, 90 s at 72°C), followed by 7 min. at 72 °C in a PTC 100 (MJ Research Inc.) or a PCR System 2720 (Applied Biosystems) thermocycler. PCR products were electrophorised on a 1.5% agarose gel at 10 V/cm for 30 min., stained in 0.1 µg/ml EtBr for 15 minutes and visualised under UV (Vilber-Lourmat). DNA sequencing was carried out by Macrogen Inc, Seoul, N. Korea.

Chromatograms were viewed and sequences edited in the Seqman module of the DNAStar package. Sequence similarity searches of GenBank were carried out using BLASTn and isolates grouped according to the accession number of the best match. Sequences from all isolates within each group were aligned using Clustalw (ref) to assess the degree of 'intraspecific' variability. Phylogenetic analyses were conducted using DNAML of the Phylip package (Felsenstein, 1989).

**Development of the *G. philippii* species-specific test**

ITS sequences from the two *Ganoderma spp.* most commonly found in *A. mangium* plantations were aligned with additional species of *Ganoderma* and *Amauroderma* (Glen et al., 2009) that had the highest sequence similarity. The alignment was scanned for regions with high interspecific variability and primers designed according to (Dieffenbach et al., 1993). Public DNA databases were searched with the primer sequences to determine whether there were any significant matches to non-target fungi. Selected primers were manufactured by Geneworks (Adelaide).

Primers were tested at a range of annealing temperatures with concentrations of all components as for ITS PCR. The thermocycler program was: 2 minutes at 95°C, 35 cycles of (30 s at 95°C, 30 s at annealing temperature, 30 s at 72°C), followed by 7 min. at 72 °C; where the annealing temperatures tested were 60, 61, 62 or 63 °C.

Two sets of primers proved specific for *G. philippii* and three sets of primers for *G. mastoporum*. They were tested against a wider range of fungi (isolates from known sporocarps found in *A. mangium* plantations including those species likely to be found on roots). The annealing temperature for the test was optimised and the test was adopted for routine screening.

### *Somatic incompatibility tests*

Somatic incompatibility tests were carried out between a wide range of cultures obtained within a single site, at different sites in the same area and geographically distant sites. These tests are used to investigate methods of dispersal; a high degree of compatibility between cultures indicates clonal or vegetative spread; a large number of incompatibility reactions point toward spore dispersal and genetic variability.

Cultures were isolated from roots onto Malt Extract Agar (MEA) 1% (10g / L) with the addition of 50 ppm each of penicillin and streptomycin, 25 ppm of polymyxcin, and 230 ppm of the fungicide thiabendazole which inhibits common fungal contaminants.

*Ganoderma philippi* isolates were selected for the tests had their identity verified by DNA analysis. All cultures for testing were subcultured from stock cultures at the same time and then incubated for 2 weeks at 25 ° C in the dark so that all isolates were of uniform age and vigour.

Small culture blocks (3 mm ²) were taken from the initial inoculum plate about then placed with a distance of approximately 1-2 mm between the two isolates to be tested for their compatibility. The isolates selected from Riau (Table 2) for the somatic incompatibility tests were paired in each possible combination including self-pairings with 3 replications of each pairing. A similar process was repeated for the cultures from Deras. Five isolates from Deras had been collected from a 1st rotation crop before harvesting at this site and four had been collected from dying trees in the second rotation. Culture plates were

incubated at 25 ° C in the incubation chamber and observed every 2 days to determine growth, contamination and to score the tests.

Somatic incompatibility was evaluated after 2 weeks. The numbers 0-2 are used to score the degree of compatibility/antagonism between the isolates, i.e. 0 = compatible (no reaction), 1 = incompatible without pigmentation (reaction occurs without the formation of pigmentation line), 2 = incompatible with pigmentation (reaction occurs with the formation of pigmentation lines).

**Table 2: List of isolates used for somatic incompatibility testing**

| Logas (Riau) 1st Rotation | | Deras (South Sumatera) 2nd rotation (A-E) and 3rd rotation (F-I) | |
|---|---|---|---|
| Name of isolate | Coding given for SI tests | Name of isolate | Coding given for SI tests |
| 6-LS-3-A-36(M).3 | LS-A | 4-D-2-A-42(M)-A.2.1 | D-A |
| 6-LS-3-A-44(M).3 | LS-B | 4-D-2-A-63(M)-C.1.1 | D-B |
| 6-LS-3-A-45(M).3 | LS-C | 4-D-2-A-64(M)-B.1.2.1 | D-C |
| 6-LS-3-A-54(M).3 | LS-D | 5-D-3-A-48.1 | D-D |
| 6-LS-3-A-55(M).2 | LS-E | 5-D-3-A-72.1 | D-E |
| 6-LS-3-A-66(M).3 | LS-F | 8-ND-6-A-56(M)-A.1 | D-F |
| 6-LS-3-A-76(M).3 | LS-G | 8-ND-6-A-68(M)-A.1 | D-G |
| 6-LS-3-A-77(M).1 | LS-H | 8-ND-6-A-87(M)-A.1 | D-H |
| 6-LS-3-A-78(M).3 | LS-I | 8-ND-6-A-90(M)-A.2 | D-I |

### *Antagonism tests*

Isolates of *Phlebiopsis* and *Cerrena* (*Cerrena* 2) were isolated from *Ganoderma* infected eucalypt wood by project staff by project staff. Initial antagonism tests have been carried out on agar media.

### *Pathogenicity tests*

Extensive pathogenicity tests have been carried out by project partners but the isolates that were used are no longer available and were not characterised by a description of morphology or DNA fingerprint.

Pathogenicity tests were therefore repeated by the project team at FORDA Jogjakarta with isolates that have been DNA fingerprinted. Additionally itis the first time that Koch's postulates have been confirmed with *Ganoderma philippi.* Koch's Postulates form a basic plant pathology procedure in which it is proved (by artificial inoculation, observation of disease symptoms caused by the pathogen and its subsequent re-isolation from diseased tissue) that the pathogen actually is the causal agent of a disease.

Three fungal isolates of *Ganoderma philippi* were selected from different experimental sites, namely Riau (Logas South), South Sumatra (Deras), and East Kalimantan (Sebulu) and are available in the culture collection held at CFBTI, Jogjakarta. These isolates are:

G1 = isolate 6-LS-3-D-56(M).3 with DNA code 081224-06 from Logas South – Riau

G2 = isolate 6-SU-2-A-24(M)-A.2 with DNA code 081202-40 from Sebulu – East Kalimantan

G3 = isolate 5-D-3-C-12(M) with DNA code 090901-14 from Deras – South Sumatra

All fungal isolates were sub-cultured from storage and maintained on plates of malt extract agar (MEA) 2% at 25˚C. Cultures were used in the inoculum production process approximately 14 days after sub-culturing.

Inoculum was made using branch segments of *Acacia mangium* which had been autoclaved for 30 minutes at 121˚C and 1 atm. These branch segments were put into 10.5-cm-diameter flasks and the tops covered with aluminum foil. MEA medium was poured onto flasks with autoclaved branches, and then the flasks were autoclaved again for 20 minutes at 121˚C and 1 atm. Branch segments were then cultured with each of the 3 isolates selected for testing. After one month the fungus has grown on all the branches and they were ready to use for inoculation.

Three tree species, namely *A. mangium*, *Eucalyptus pellita*, and *Alstonia scholaris* were used in the pathogenicity tests. *E. pellita* and *A. scholaris* seedlings were obtained from CFBTI Nursery. They were maintained in 10-cm-diameter plastic pot for 3-6 months before being transplanted into 40-cm-diameter trial pot filled with soil. Seedlings of *A. mangium* (Am66) were grown from seed sown specifically for the experiment and originating from the CFBTI seed orchard, Wonogiri, Central Java. Three-month-old *A. mangium* seedlings were transplanted into 40-cm-diameter pots filled with soil. All seedlings were kept in an open-shade nursery before and after inoculation, and they were watered using a manual sprayer twice per day (morning and afternoon). Seedlings were maintained in pots for 5 months before inoculation. NPK fertilizer was applied 3 times at approximately 20 g/plant.

To inoculate three inoculum branch segments were placed into the pot around each seedling in the experiment making sure that the inoculum touched large roots near the root collar. The branches were put at 10-cm-depth from the surface, covered with soil, adding a top soil until covering the full surface of the trial pot. To inhibit and reduce the high transpiration and retain the soil moisture, rice husk was added covering the full surface.

A control was set up where branches without inoculum were buried for each host species treatment. The total number of experimental units was 72 (3 isolates plus control x 3 host species x 6 replicates).

Monitoring was conducted periodically over a 10 month period to observe symptoms of disease. When seedlings died, roots were excavated, pictures were taken and a sample taken for re-isolation. The entire trial was harvested at 10 months and roots examined for infection.

### *Ground Penetrating Radar*

A preliminary investigation of the usefulness of this technology was carried out comparing root architecture and distribution under six conditions: two *Eucalyptus pellita* trials and four *Acacia mangium* trials.

**Table 3: Highlighting the general findings from the Ground Penetrating Radar work.**

| Test Area | Maximum Root Depth (mm) | Common Root Depth (mm) | Root Size Range (mm) |
|---|---|---|---|
| Coppice (*E. pellita*) | 1630 | 100-1300 | 40-120 |
| Cuttings (*E. pellita*) | 900 | 100-400 | 40-100 |
| T1R5 (*A. mangium*) | 400 (1300?) | 100-400 | 40-90 |
| T1R6 (*A. mangium*) | 700 | 100-400 | 40-100 |
| T2R5 (*A. mangium*) | 1100 | 100-400 | 50-100 |
| T2R6 (*A. mangium*) | 1200 | 100-500 | 40-90 |

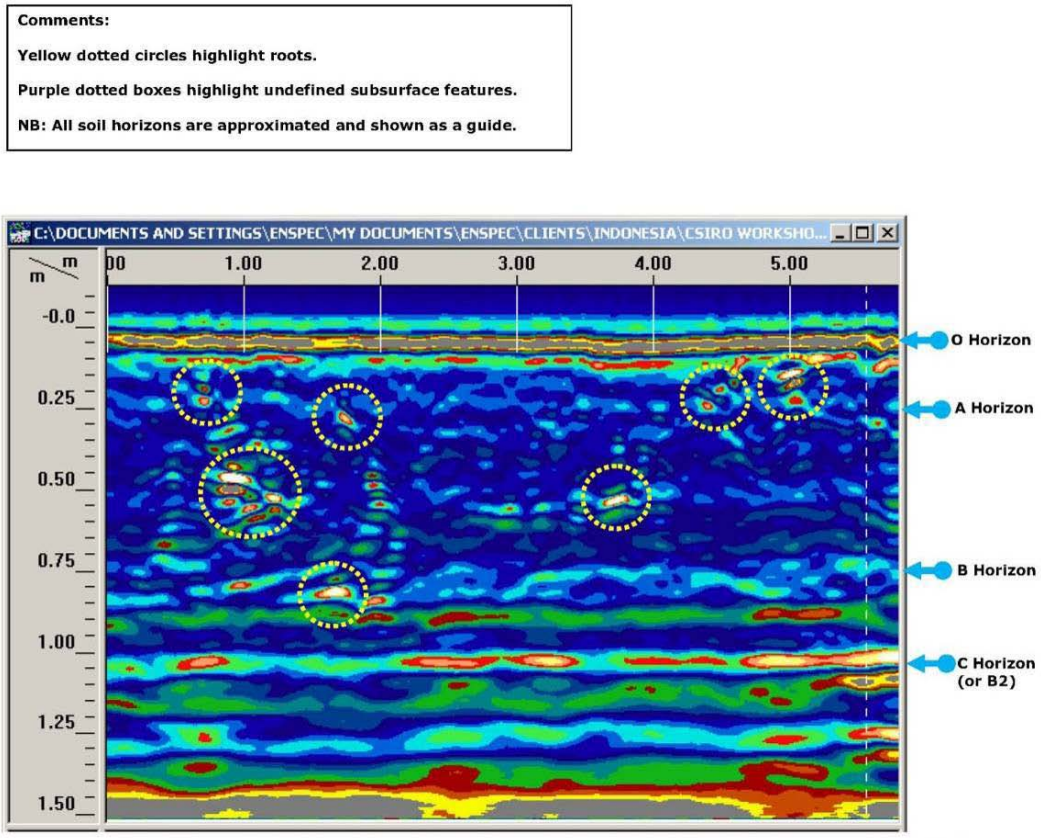


**Figure 4: Vertical section taken in from *E. pellita* coppice trial site.**

### 5.1.5 Industry partner trials to test different management strategies

T. Riau Andalan Pulp and Paper (RAPP) and Arara Abadi established several field trials. These trials were discussed with Australian project staff although it was not always possible to establish trials to a particular experimental design. Due to the severity of the root rot disease problem Indonesian pathologists were under considerable pressure to find a solution. Some trials were even started before the root rot project.

At RAPP field trials focused on the application of *Trichoderma* and similar biocontrol agents; the removal of stumps and assessing the impact of natural regeneration. Trials at Arara included pathogenicity tests and the application of a basidiomycete as a biocontrol. While we were given experimental data we are not formally permitted to write up the results of these experiments due to the sensitivity surrounding losses from root rot.

## 5.2 Australian Activities

### 5.2.1 Summary of Activities

- Experimental design of root rot investigation plots in far North Queensland hoop pine plantations. These investigations are on-going.
- Training of Queensland DPI staff in morphological mycological diagnostic methods and in molecular diagnostics
- Development of reliable infection systems for testing tree species susceptibility to *Phellinus noxius* root rot.
- Discovery of resupinate *Phellinus noxius* fruiting bodies in urban environments in Queensland answering the question of infection sources. Investigation of *Phellinus* root rot in native rainforest and Avocado plantations.
- Investigation of root and heart rot issues in northern Western Australian sandalwood plantations.

# 6 Achievements against activities and outputs/milestones

### *Objective 1: To identify the main causal agent(s) of root-rot disease and characterize their field biology*

| No. | Activity | Outputs<br>- milestones | Completion date | Comments |
|---|---|---|---|---|
| 1.1 | Identification of fungi associated with root rot disease. | A list and fingerprinting of main fungal agents present in Indonesian *A. mangium* plantations<br>- At least 6 sites with root rot disease visited and samples collected<br>- Specimens from these 6 sites identified to genus if not species level. Internal report on fungi identified from plantations | Broader-scale collection of pathogenic and non-pathogenic sporocarps: June 2008<br><br>Last collection of root samples / sporocarps from semi-permanent sampling plots: Jun 2009<br><br>Sequence information complete: Feb 2010 | *G. philippii* is the most common fungus isolated from *Acacia mangium* root rot samples examined in this project and the most commonly encountered species fruiting on standing dead *Acacia mangium*.<br><br>Summary of Molecular work below, full details in Results sections 7.1.1 & 7.1.2<br>- Total cultures processed: 9578<br>- Active cultures; 4644<br>- Total molecularly identified cultures: 2713<br>- Molecularly identified active cultures: 2348<br>- Molecularly identified *G. philippii* cultures: 2009<br>- Primary subcultures identified molecularly: 1330<br>- Primary *G. philippii* cultures identified molecularly: 991 (these are isolates from individual sampling dates, trees or locations not including duplicates)<br><br>124 vouchered fungal sporocarp collections have been made and identified and are to be lodged with Herbarium Bogoriense (BO).<br>- A full list of fungal samples identified by the project is presented under Results section 7.1.1<br>- A visual guide to the most common pathogenic and non-pathogenic species found growing on living or dead A. mangium and E. pellita has been produced and incorporated into the field guide.<br><br>Paper published: Glen et al. (2009) (ref 8 section 10.2.1), a second paper is in preparation. |

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|---|---|---|---|---|
| 1.2 | Determination of mode of dispersal for root- rots pathogens | Description of mode of dispersal and relative importance of spore and vegetative dispersal (healthy root contact with diseased roots or stumps)<br>- At least 3 sites selected for intensive survey<br>- Cultural SI tests<br>- Molecular SI tests<br>- Report on cultural and molecular SI tests<br>Publication e.g. "How are root rot fungi spread and how this influences possible control strategies" | Last collection of root samples / sporocarps from semi-permanent sampling plots: Jun 2009<br><br>Cultural SI tests completed: Dec 2009<br><br>Molecular assessment of SI groups: Mar 2010 | - 25 semi-permanent sampling plots established at five sites in three Indonesian provinces and monitored for up to two years as per methods (section 5.1.2).<br>- Discoveries about root-to-root spread and rates of disease spread and incremental loss.<br>- Red root rot infections were observed apparently having travelled along un-grafted roots and natural root grafts.<br>- Somatic incompatibility tests recovered compatible isolates from several trees within a disease gap suggesting that a single isolate had infected all trees in that gap via root contact. Compatibility groups were determined within a single second rotation disease gap suggesting multiple isolates infecting multiple trees by root to root contact within a single disease gap. The somatic compatibility of isolates from pre- and post-harvest surveys suggests that root to root/debris contact was responsible for the transmission of these isolates.<br>- Results presented in a series of talks, the paper is in preparation: see section 10.2.1: communications 1, 4 & 7<br>10.2.2.: communications 2, 5, 7, 8, 9 & 13 and Trip report for fieldwork in February and June 2008.<br>- SI tests showed high variation between disease patches and low variation within disease patches for sites from Langgam and Deras (Results section 7.1.2) with variation in the SI groups present before and after clearing at Deras. These patterns consistent with spore dispersal playing an important role in the establishment of new disease centres and in introducing genetic diversity to established disease centres such as those at Deras.<br>- Somatic incompatibility tests on isolates obtained from Deras before and after the harvest and burning trial carried out at that site indicate that isolates present in one rotation are indeed responsible for tree deaths in the following rotation.<br>- Molecular examination of SI groups attempted but, for the suite of 20 primers tested no variation was observed between somatically incompatible isolates. |

| | | | | |
|---|---|---|---|---|
| 1.3 | Assessment of fungal pathogenicity and host susceptibility | Description of degree of pathogenicity for each major root rot pathogen identified against major species and hybrids of tropical hardwoods<br>- Trials established<br>- Data collected, analysed & interpreted<br>- Information disseminated e.g. "The major root rot pathogens in Indonesian plantations of *A. mangium* and susceptibility of important tropical plantation hardwoods" | Final pot trial completed April 2010 | - Pot trials have indicated the pathogenicity of *Ganoderma philippii*, *Phellinus noxius*, *G. mastoporum* and *G. australe* against both *Acacia mangium* and *Eucalypts pellita*.<br>- A pathogenicity trial completed in April 2010 with three isolates of *G. philippii* and three tree species (*A. mangium*, *E. pellita* and *Alstonia scholaris*), conclusively re-isolated *Ganoderma philippii* from infected, symptomatic *A. mangium* and demonstrated that both *E. pellita* and *Alstonia scholaris* could maintain latent infections without showing obvious above-ground symptoms of infection.<br>Observations in operational *Eucalyptus pellita* (clone) plantations indicate that there are sizeable disease gaps present in some plantations exhibiting signs of red root rot and associated with *Ganoderma philippii* and *G. mastoporum*. Disease gaps in *Eucalyptus pellita* plantations have also been observed affected by *Phellinus noxius*. These observations indicate that eucalyptus pellita and *Alstonia scholaris* will not provide a break crop in the sense that they will still harbour pathogens lethal to *Acacia mangium*. |

*PC = partner country, A = Australia*

## *Objective 2: To identify site factors influencing the development of disease symptoms and root-disease distribution*

| No. | Activity | Outputs<br>- milestones | Completion date | Comments |
|---|---|---|---|---|
| 2.1 | Ground based surveys of root-rot disease incidence and severity | Mapping of root rot disease and incidence at a regional level and also in association with particular sites<br>- Data sheet ready to use<br>- A map of site locations<br>- Indonesian staff capable of undertaking surveys independent of Australian assistance<br>- Data collated from surveys<br>- Map and discussion of disease incidence and severity out in public arena | Broad-scale survey across 3 industry partners completed: November 2008<br><br>Company broad-scale data analysed: May 2009 | - Two broad-scale investigations of root rot incidence were carried out, one a single company purposive survey and 2 a blocked random sample survey across all three industry partners (see methods section 5.1.3 & 0)<br>- The approximately linear trends observed for tree mortality on the semi-permanent plots suggest that the average level of root rot across the plantation may not be a good representation of the level of disease by the end of rotation. These results suggest that because of pathogen spread a better indicator of end-of-rotation losses may be the number and size of discreet disease gaps qualified for time since planting.<br>- Presentation: Francis, AA (2009) Risk management for *Ganoderma* root rot in *Acacia mangium* plantations: a case study. Presented at "Workshop on Disease Management Strategies in Plantations", Yogyakarta, Indonesia, May 4th-8th 2009. |

| | | | | |
|---|---|---|---|---|
| 2.2 | Monitoring the symptoms of root rot disease development | Description of early symptoms of root rot infection in the crown and the influence of infection on tree physiology<br>- Site locations mapped<br>- Indonesian staff capable of undertaking surveys independent of Australian assistance<br>- Data collated and preliminary report<br>- System for ground based monitoring developed including a strategy for dissemination to outgrowers<br>- Workshop presents ground based root rot monitoring system | Last collection of root samples / sporocarps from semi-permanent sampling plots: Jun 2009 | - The same 25 semi-permanent sampling plots (see methods section 5.1.2) used in Activity 1.2 were used for this activity.<br>- Symptoms described in pot-trials and in the field<br>Root-rot monitoring system presented as part of the field guide. |
| 2.3 | Development of site-risk rating systems | Simple site risk analysis based on site characteristics (DSS or check sheet)<br>- Preliminary report highlighting relevance of existing data to site risk modelling<br>- Training workshop held. At least 3 local staff using & capable of modifying the model<br>- Selection of most effective model(s)<br>- Publication of model including a strategy for dissemination to outgrowers in Yr 3 end of project workshop | | - The risk assessment tool developed by the project indicates that management choices can affect the level of mortality due to root rot at the end of rotation (particularly as age is a major factor in the level of mortality). The following factors interact in making a site high or low risk:<br>- Second rotation sites are more prone than first<br>- Sites older than around 6 years have higher risk<br>- Spacing < 2.5 x 3 m (or 3 x 2.5 m) have a greater risk<br>☐ - Soil types vary in risk (listed in order of increasing risk below) and, when ranked, risk increases more or less linearly for the set of soil types examined ($R^2$ = 0.97).<br>- Compartments with a slope value of 5 have a slightly higher risk<br>- Root-rot risk generally decreases as one travels east but is highest closest to the equator as one travels north to south. (Rainfall / temperature information was not included as it is only collected at the estate level. It does not appear that average rainfall is related to measured changes in the rate of infection over the life of the project) |

*PC = partner country, A = Australia*

## *Objective 3: To develop simple cost-effective root rot reduction strategies*

| No. | Activity | Outputs<br>- milestones | Completion date | Comments |
|---|---|---|---|---|
| 3.1 | Evaluation of existing and new biocontrol and chemical agents | A list and description (including fingerprinting) of existing commercial or semi-commercial biocontrols that could be applied to stumps at harvest.<br>Biocontrol agents that maybe more specific to root rot control and hence effective including basidiomycete and non basidiomycete candidates.<br>An information leaflet about the possibilities of environmentally friendly chemical stump treatments.<br>- Data collated and structured in a fashion relating to the intent of the review<br>- Categorical identification fingerprint for putative biocontrol agents<br>- Report listing biocontrol agents, including their fingerprinting and a strategy for their field testing<br>- Map of sites and description of bait trials at different sites<br>- Two progress reports over time period stipulated for task<br>- List of and molecular identity of potential agents including strategies for further testing of their suitability before field testing<br>Report to ACIAR | | - A list of available methods was published in a review by Eyles et al. (2008) and revised in the position paper for the workshop "Disease management strategies in plantations" hosted in Yogyakarta, Indonesia May 4-8, 2009<br>- Identification of promising biocontrol agents including one developed by project partner P.T. Arara Abadi and three promising species discovered during project surveys. The biological control developed by P.T. Arara Abadi (*Cerrena sp.*1) is currently undergoing field testing and reduced mortality in *Acacia mangium* seedlings challenged with *Ganoderma philippii* from 75 to 25% in pot trials.<br>- A strategy for rigorously testing and developing these species is included in a proposal for a new ACIAR funded project FST2009/051. |

| | | | | |
|---|---|---|---|---|
| 3.2 | Evaluation of stump application technologies for the delivery of biocontrol agents. | A list and description of stump application technologies for existing commercial or semi-commercial biocontrols and their cost/suitability for outgrower use<br>- Report submitted to ACIAR<br>- Meeting held and reports submitted to ACIAR | | - A list of available methods was published in a review by Eyles *et al.* (2008) and revised in the position paper for the workshop "Disease management strategies in plantations" hosted in Yogyakarta, Indonesia May 4-8, 2009. |
| 3.3 | Field testing of control options. | Control treatment(s) for outgrowers of A. mangium<br>- Field trial sites selected and trials designed<br>- Report on trials established<br>- Report on preliminary results<br>- Report / Publication on outcomes including a strategy for dissemination and adoption | | - P.T. Arara Abadi are currently field testing their biological control identified through the project. These trials are currently only one year old, however disease levels with the *Cerrena* biocontrol are roughly half those in the untreated plots. |

*PC = partner country, A = Australia*

## *Objective 4: Training, information packaging, dissemination and adoption of outcomes*

| No. | Activity | Outputs<br>- milestones | Completion date | Comments |
|---|---|---|---|---|
| 4.1 | Training | Trained project staff in morphological and molecular mycology, site risk assessment, disease biocontrol<br>- Training complete and objectives attained | | - 8 FORDA staff and two representatives from project industry partners were involved in Activity 4.1of the project. Training outcomes are detailed under Results and Discussion section 7.4.1. |
| 4.2 | Delivery of root-rot management strategies to outgrowers | Package of information (manuals and software) for adoption by outgrowers delivered to extension staff and direct to outgrowers by demonstration trials<br>- Production of at least 6 leaflets produced and disseminated by FORDA<br>- Report on risk assessment training workshop and project meeting<br>- Report to ACIAR "Strategies for technology transfer" including discussion of dissemination medium (software, manual etc.) and linkages with project FST/2004/058<br>- Report on feedback to ACIAR (representative of ACIAR to be present at field workshops)<br>- Final project report | | - No direct outgrower contact<br>- Reliance on company transmission to small-holders / outgrowers<br>- Julien DeMayer attended the project organised workshop on Disease Management Strategies in Plantations (May 2009, Yogyakarta, Indonesia)<br>Leaflets have not been produced. However FORDA articles were published internally on a) the identification of *Ganoderma philipii* in the field and in the laboratory; b) somatic incompatibility tests; c) a report of the outcomes of the ACIAR. Another is under preparation about pathogenicity trials, how to carry these out and the results from a trial at CFBTI in Jogjakarta.<br>Discussions are in progress on how to convert these four publications to leaflets for a wider audience. There is also enough information available for additional leaflets. |

*PC = partner country, A = Australia*

# 7 Key results and discussion

- *Eucalyptus pellita* is susceptible to root rot caused by both *Ganoderma philippii* and *Phellinus noxius* and can suffer disease gaps as large as those seen in *A. mangium*.
- Mortality rates for root-rot infected areas can be in the range of 40-60% between ages 5 and 7 years on certain sites.
- The 'average level of root rot' across a plantation is not a good representation of the real root-rot problem - to fully appreciate the real financial cost one must extrapolate out to end of rotation.

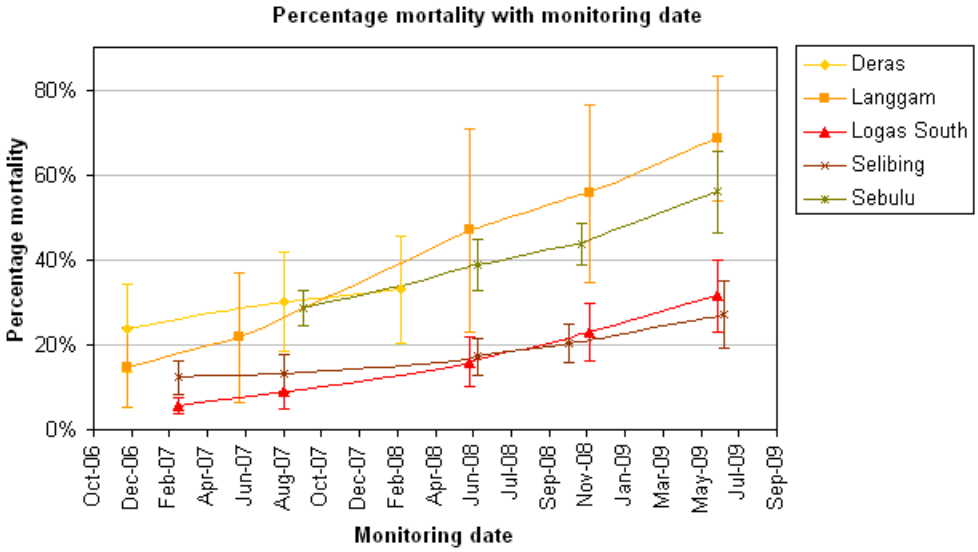



**Figure 5: Average percentage mortality for all plots at each site against monitoring date. Error bars are plus and minus one standard deviation.**

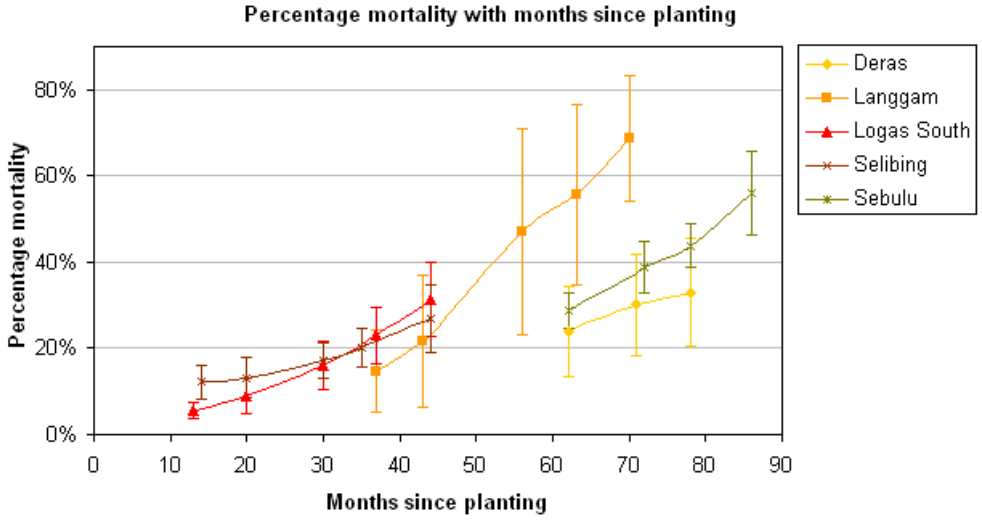



**Figure 6: Average percentage mortality for all plots at each site here against monitoring date represented as months since the monitored trees were planted. Error bars are plus and minus one standard deviation.**

- The *Cerrena sp.* infested wood blocks deployed by PT Arara Abadi is a promising biological control species showing activity against both *Phellinus* and *Ganoderma spp.* pathogens and has been field tested by this project partner by the application of *Cerrena* infested wood blocks into planting holes. However the form of application is cumbersome and relatively expensive but if this fungus is applied in another form it does not survive. The *Phlebiopsis* sp. isolate discovered is a significant find as *Phelbiopsis gigantea* in the northern hemisphere is used to control an aggressive root-rot pathogen of conifers and can be produced and applied successfully as a spore solution to spray onto debris or stumps. While the Indonesian isolate is not the same species it warrants further investigation and has shown antagonism towards isolates of *Ganoderma* and *Phellinus* in agar media. The isolate of Cerrena found by project staff also shows antagonism towards isolates of *Ganoderma* and *Phellinus* in agar media.
- Somatic incompatibility: The morphological characteristics on agar medium of somatic compatibility/ incompatibility reactions were described for the first time for the fungus *Ganoderma philippi*. Although these tests were limited in scope due to time constraints, the usefulness of this technique in understanding a) disease dispersal b) the build-up of genetic diversity in the pathogen c) the effectiveness of silvicultural management was clearly demonstrated.
- The risk assessment tool developed by the project indicates that management choices can affect the level of mortality due to root rot at the end of rotation (particularly as age is a major factor in the level of mortality). The following factors interact in making a site high or low risk:
  - Second rotation sites are more prone than first
  - Sites older than around 6 years have higher risk
  - Spacing < 2.5 x 3 m (or 3 x 2.5 m) have a greater risk
  - Soil types vary in risk (listed in order of increasing risk below) and, when ranked, risk increases more or less linearly for the set of soil types examined (R2 = 0.97).
  - Compartments with a slope value of 5 have a slightly higher risk
  - Root-rot risk generally decreases as one travels east but is highest closest to the equator as one travels north to south. (Rainfall / temperature information was not included as it is only collected at the estate level. It does not appear that average rainfall is related to measured changes in the rate of infection over the life of the project)

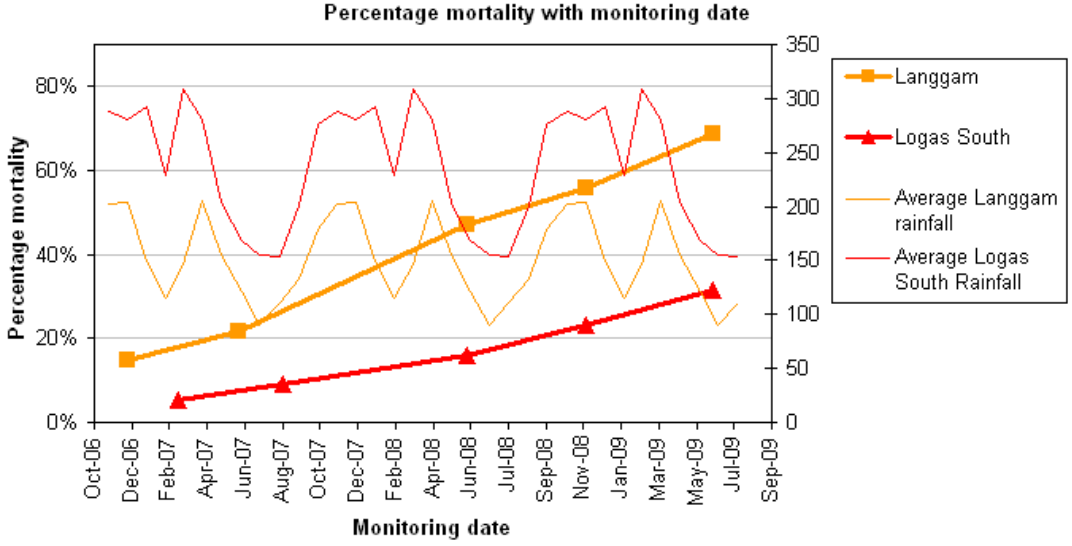


**Figure 7: Mortality data for Langgam and Logas South (left-hand axis, percentage mortality) and Average monthly rainfall (right-hand axis, mm) plotted against monitoring date. Please note the rainfall figures are long-term averages over the history of the plantations in these areas and not the volumes recorded for the actual months in question – this is why the patterns repeat.**

- Pathogenicity tests have conclusively re-isolated *G. philippii* from infected *A. mangium* and shown latent infections are possible in *E. pellita* and *Alstonia solaris*.

**Figure 8: Latent *Ganoderma philippii* infection on *Alstonia solaris* root collar.**

- Molecular results
  - **Species-specific primers.** Several primers were designed for specific detection of *G. philippii*, *G. mastoporum* and *G. steyaertanum*. BLAST searches using the primer sequences revealed a high degree of specificity, though several of the potential primers had 100% sequence similarity to non-target species. Primer pairs were assessed against a small group of fungi and those with the desired specificity were assessed against a larger group. Amplification of a fragment from a non-target species, *Fomitopsis feei*, was observed at an annealing temperature of 60°C but optimisation of the thermocycler program prevented this. The optimised temperature profile consisted of: 95°C for 2 min, 35 cycles of 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec, followed by a final extension at 72°C for 7 minutes.
  - **DNA sequencing.** An additional 37 Operational Taxonomic Units (OTUs) (where an OTU represents a collection of sequences not more than 3% distant from each other) were distinguished by sequencing of the rDNA ITS. These represent fungi found as sporocarps in *A. mangium* plantations or isolated from *A. mangium* roots. Sequencing of DNA from herbarium specimens and isolates from herbarium specimens increased confidence in species designation of OTUs.

## 7.1 Objective 1: Identify the main causal agent(s) of root-rot disease and characterise their field biology

### 7.1.1 Activity 1.1: Identification of fungi associated with root rot disease

Table 4 indicates the molecular identity of fungi found during the project. These isolates and sporocarps were collected from root rot affected pulpwood plantations in Riau, South Sumatra and East Kalimantan provinces of Indonesia. The species isolated include demonstrated pathogenic types including *Ganoderma philippii* and *Phellinus noxius*, putative biological control agents including *Cerrena sp.* 1 and *Phlebiopsis* sp. 1 and a range of other fungi associated with root rot disease either by colonising rotten roots or by spatial association with diseased areas and surrounding trees. The occurrence of fungi in this list however does not automatically indicate pathogenicity to pulpwood tree species. Pathogenicity tests such as those performed under Activity 1.3 (section 7.1.3) are required

to demonstrate pathogenicity. The vast majority of cultures obtained come from root sampling on the project semi-permanent plots explaining the preponderance of *Ganoderma philippii* isolates. It should also be noted that the designation of a species with a number does not indicate that the identity of this species is in question (except where the sequence obtained was not of sufficiently good quality) but that no sequences from conclusively identified members of this species (or closely related congeners) have been included in the public databases used for identification. The sequences produced in this project can be used to link these isolates and sporocarps to names should conclusively identified sequences be lodged in the public databases. The vouchered sporocarp collections are to be lodged at Herbarium Bogoriense (BO) to allow these currently unnamed species to be named in the future.

After the Mt Merapi activity in November 2010 the mycological laboratory was left for a period of 3 weeks without air-conditioning and cleaning of the laboratory. It was feared that the isolates in the culture collection at CFBTI, Jogjakarta would have been killed through high temperatures or lost through contamination. ACIAR provided additional funding for Dr Morag Glen to travel to Indonesia in April 2011 and to assist with the restoration of the laboratory. All isolates were examined and a large majority were sub-cultured to test if they were still living. An assessment of the number of isolates that survived is still in progress but all indications are that attrition is relatively small. Approximately 10,000 AUD was provided by ACIAR for the replacement of consumables lost in the incident such as those essential for the mycology laboratory such as agar and petri dishes and molecular consumables required for the identification of isolates. As well as the work with restoring cultures Dr Glen provided mycological and molecular training.

**Table 4: List of active cultures and sporocarp collections that have been DNA fingerprinted. Note: the number of active cultures includes duplicates of some isolates. Species with ** have been confirmed as pathogenic via pathogenicity tests (see section 7.1.3).**

| Identification | Number of Active cultures | Number of sporocarp collections |
|---|---|---|
| *Amauroderma rugosum* | 5 | 5 |
| *Amauroderma sp. M5* | 17 | 11 |
| *Amauroderma sp. M7* | 5 | |
| *Antrodia sp. 1* | 2 | 1 |
| *Cerrena sp. 1* | 2 | |
| *Cordyceps aff. sobolifera* | 1 | |
| *Fomes sp. 1* | 6 | 5 |
| *Fomes sp. 2* | 5 | 2 |
| *Fomes sp. 3* | 12 | 3 |
| *Fomitiporella caryophylli* | 6 | |
| *Fomitopsis feei* | 6 | 4 |
| *Ganoderma aff. australe (sp. M1A.)*** | 16 | 6 |
| *Ganoderma mastoporum* | 44 | 11 |
| *Ganoderma philippii*** | 2009 | 28 |
| *Ganoderma subresinosum* | 24 | 3 |
| *Ganoderma weberianum* | 1 | 1 |
| *Ganoderma sp. M1B* | 15 | 6 |
| *Ganoderma sp. M3* | 1 | 1 |
| *Ganoderma sp. M4* | 1 | 1 |
| *Ganoderma sp. M6* | 5 | 2 |
| *Ganoderma sp M8* | 6 | 1 |
| *Ganoderma sp. M9* | 20 | 3 |
| *Gymnopilus sp. 1* | 1 | 1 |
| *Gymnopilus sp. 2* | 1 | |

| | | |
|---|---|---|
| *Gymnopilus sp. 3* | 2 | |
| *Hypochnicium sp. 1* | 7 | |
| *Inonotus pachyphloeus* | 8 | 4 |
| *Inonotus sp.* | 5 | |
| *Aff. Irpex sp.* | 4 | 2 |
| *Lentinus sp. 1* | 3 | 1 |
| *Neonothopanus aff. nambi* | 4 | |
| *Phanerochaete sp. 1* | 5 | 2 |
| *Phellinus noxius*** | 10 | 5 |
| *Phellinus sp. 1* | 3 | 1 |
| *Phlebia sp. 1* | 10 | 4 |
| *Phlebia sp. 2* | 10 | 1 |
| *Phlebia sp. 3* | 1 | 1 |
| *Phlebiopsis sp. 1* | 11 | |
| *Pycnoporus sp. 1* | 4 | 1 |
| *Trametes sp. 1* | 6 | 1 |
| *Trametes sp. 2* | 5 | 1 |
| *Trametes sp. 3* | 3 | 1 |
| *Trametes sp. 4* | 9 | 1 |
| *Tinctoporellus sp. 1* | 8 | |
| *Xerula sp 1* | 1 | |
| *Xylaria sp. 1* | 2 | 1 |
| Polyporaceae sp. 1 | 5 | |
| Hypocreales sp. | 1 | |
| Basidiomycete sp. 1 | 4 | 1 |
| Basidiomycete sp. 2 | 5 | 1 |
| Basidiomycete sp. 3 | 1 | |
| **TOTAL** | **2348** | **124** |

### 7.1.2 Activity 1.2: Determination of mode of dispersal for root-rots pathogens

The macro-morphology of the pairing tests was described and provides a solid basis for further research (see Figures 8, 9 and 10).

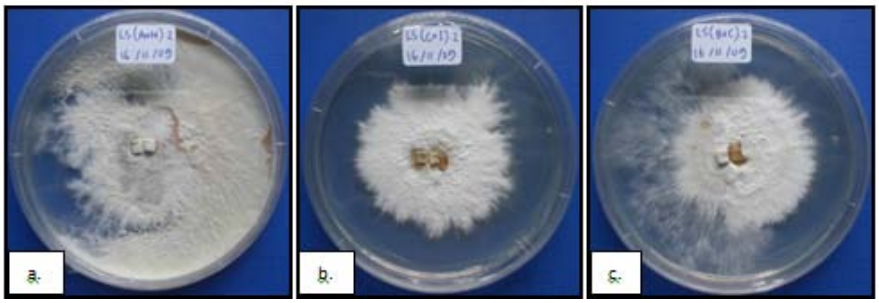



**Figure 8. Single colonies formed in the reaction between compatible isolates.**

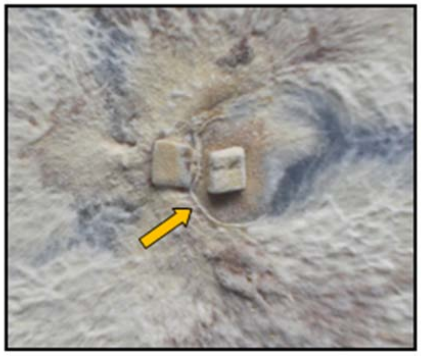


**Figure 9. Formation of dam wall sclerotia (arrow) which is indicative of somatic incompatibility between two isolates.**

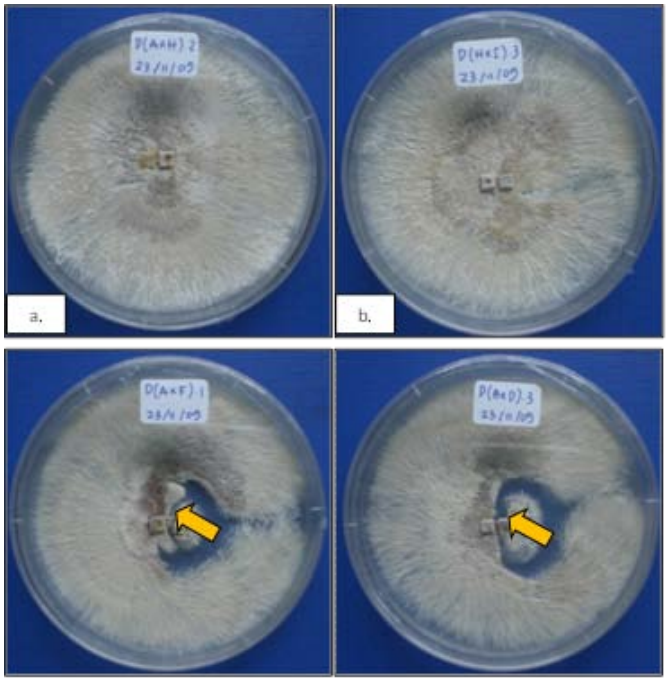



**Figure 10. Comparison of compatible (top) and incompatible pairings (bottom) - arrows clear barrier zone indicative of somatic incompatibility between two isolates**

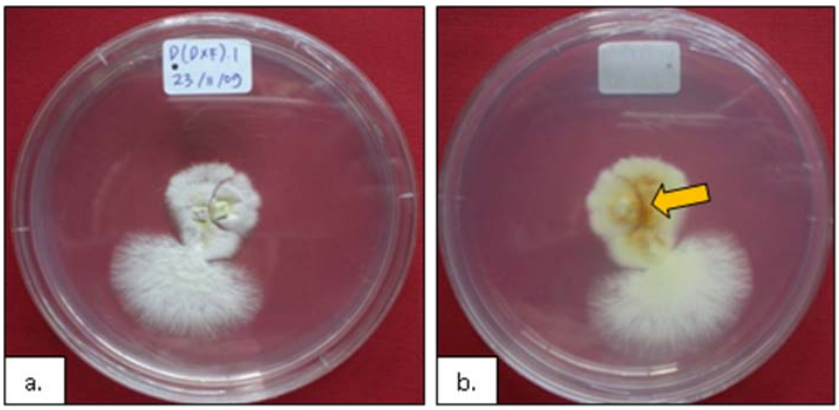



**Figure 11. Reddish-orange-brown pigmentation (arrow) is formed in the medium along the barrier zone (and seen from the bottom of the petri dish) in some incompatible pairings.**

Somatic compatibility and incompatibility reactions at the micro-morphology level were more difficult to observe but anastomoses and the formation of clamp cells were observed in compatible reactions and the degeneration of hyphae in the barrier zone of incompatible reactions.

All 9 isolates (paired in every possible combination) from 1st rotation *Acacia mangium* crop at the Logas site were compatible. The pairings between isolates from Deras (Table 5) showed that there was an increasing level of genetic diversity within the pathogen population within both the second and third rotation crops.

**Table 5: Results of somatic incompatibility pairings between *Ganoderma philippii* isolates A-I originating from 2nd (isolates A-E) and 3rd rotation (isolates F-I) *A. mangium* trees with root-rot disease at Deras (South Sumatra)**

| Plates paired | D-A | D-B | D-C | D-D | D-E | D-F | D-G | D-H | D-I |
|---|---|---|---|---|---|---|---|---|---|
| D-A | 0 | 0 | 0 | 0? | 0 | 2 | 0 | 0 | 0 |
| D-B | | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| D-C | | | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| D-D | | | | 0 | 2 | 2 | 2 | 1 | 2 |
| D-E | | | | | 0 | 2 | 0 | 0 | 0 |
| D-F | | | | | | 0 | 2 | 1 | 1 |
| D-G | | | | | | | 0 | 1? | 0 |
| D-H | | | | | | | | 0 | 0 |
| D-I | | | | | | | | | 0 |

Key: 0 = compatible, 1 = incompatible without pigmentation, 2 = incompatible with pigmentation. ? indicates that the outcome is unexpected i.e. A is compatible with D which is incompatible in all other pairings yet A is compatible with all other pairings except F. However compatibility reactions for *Ganoderma philippi* require more investigation; there may be the influence of medium on the reactions or the involvement of semi-incompatible reactions.

Somatic compatibility testing indicates the following:

- Isolates obtained from infections in a first rotation crop (Logas 1st rotation) are compatible indicating no or low genetic diversity for the pathogen at this sites. This indicates that there a single source of inoculum from the native forest that was cleared and that the pathogen in the first rotation spread by root-to-root contact. As the rotation number increases the level of diversity at a site increases (as shown by the Deras somatic incompatibility pairings) and is most likely the result of spore infection originating from sporocarps formed at the end of the 1st and 2nd rotations. Spores most likely colonise debris which remains at the site between infections.

- When the compatibility groups were overlaid over the maps of disease gaps at Deras there appeared to be no or low isolate diversity within disease gaps at the site but higher diversity between gaps in the second rotation. The level of diversity within gaps appeared to increase with the third rotation crop. This indicates that the distance of spore dispersal may be limited in distance. It also suggests that the experimental object of interest is the disease gap and it is necessary to understand the progression of the gap and what genetic diversity contributes to this progression. Future research should utilise ground- or remote sensing- (e.g. LIDAR) based assessments of the number of disease gaps rather than focussing on individual trees (although these will ultimately form gaps) and the number and size of these gaps in a previous rotation at different locations in conjunction with somatic incompatibility testing could be used to inform site risk. Ground-based observations by project staff suggest that not all sites with root-rot infections are equally prone to have the sexual fruiting stage (sporocarps). It must be established whether sexual fruiting is dependent on site conditions and if genetic diversity enhances gap formation.
- Isolates present before and after the implemented harvest and burning trial were compatible indicating that the burn had not prevented isolates present in one rotation from infecting the next rotation.

### 7.1.3 Activity 1.3: Assessment of fungal pathogenicity and host susceptibility

*Acacia mangium* trees were killed very quickly by the root-rot pathogen. Symptoms of infection (yellowing of leaves followed by wilting) were observed 2-months after inoculation and the smaller trees died soon after symptoms were expressed. Control treatments remained healthy; a total of 10 trees died out of the 18 inoculated but all *A. mangium* trees at harvested were infected by the root-rot pathogen. The pathogen was re-isolated from 15 trees proving that the pathogen that had been inoculated caused the disease. All three isolates of *Ganoderma philippi* appeared equally aggressive to *A. mangium.*

Three out of the 18 *Eucalyptus pellita* seedlings inoculated showed symptoms of root rot infection and *G. philippi* was re-isolated from one tree. This indicates that *E. pellita* can be infected by *G. philippi* but perhaps infection is slower. This explains observations in the field that *E. pellita* is more tolerant to root-rot infection. However it does not mean that given conditions of high inoculum load in the field and a rotation length of 5-6 years that this species will not succumb to root-rot disease as indeed has already been observed in the field.

*Alstonia scholaris which* has been reported by company tests to be immune to root-rot disease did not show symptoms of infection. However its roots did harbour the pathogen and the typical rhizomorphic skin of *G. philippi* was present on this species' roots at harvest.

It is now important to investigate the mechanisms of pathogen infection and host defence to better understand the factors contributing to tolerance or longer periods required for infection. It will also be important to test these hosts with other root-rot pathogens such as common such as *Phellinus noxius*.

## 7.2 Objective 2: Identification of site factors influencing the development of disease symptoms and root-disease distribution

### 7.2.1 Activity 2.1: Ground based surveys of root- rot disease incidence and severity

Data collected by companies in Indonesia and Malaysia were made available in respect to the incidence and severity of root-rot disease and where possible were used for the development of the root-rot risk assessment tool. Data from the ground-based surveys carried out by project staff were also incorporated into the development of the root rot risk assessment tool.

There are many possible biotic causes of mortality in *Acacia mangium* plantations apart from root-rot such i.e. damage from termites, elephants, squirrels and monkeys, other fungal diseases such as *Ceratocystis.* Losses that cannot be attributed to biotic agents are also numerous and caused by the seedling establishment process, water logging, compaction. Across an estate the percentage of losses from root-rot will vary and as for any other disease be enhanced by poor silvicultural conditions. Root-rot is a disease which appears as gaps which are not equally spread across and estate.

While companies do not wish for the details of survey data to be released, data from the different surveys can be presented as case studies without reference to specific locations.

- Out of approximately 800 Permanent Sample Plots (PSPs), one for each compartment of *Acacia mangium*, a total of 104 PSPs had root-rot disease recorded as being present; 16% of the diseased PSPs had an incidence of root-rot >20% and 18% had a mortality rate of 10-20% i.e. over a third of those plots with root-rot disease had an incidence of root rot greater than 10%. Data from PSPs were useful to develop correlations with site parameters but since root-rot is a disease that has a patchy distribution it did not provide a reliable indication of overall disease incidence and severity. A single PSP representing a compartment means that root-rot disease could be missed entirely or grossly overestimated as the small size of a PSP meant that a single disease gap in a compartment could cause a high level of mortality if the disease gap overlapped with the PSP. Other surveys were carried out by scoring transects or blocks of trees across compartments.
- Thirty-five blocks of *Acacia mangium* trees from four estates managed were surveyed. Trees (including stumps) were assessed for their health status, and root symptoms were classified and recorded. The red root-rot symptom caused by *Ganoderma philippi* was clearly observed in 2.4% of the trees (including stumps). However a black root-rot symptom was present in another 8.3% of the trees (including stumps). This symptom is often associated with an advanced or resting red root rot stage or another type of root rot pathogen. Stumps numbered 4850 (11.4%) of the 42,600 trees and stumps surveyed, with 59.5% of the stumps recorded as having root-rot symptoms. There were 37,750 trees not classified as stumps. Of these, 4.7% were recorded as having root rot symptoms. In summary, 10.7% of the total trees and stumps surveyed were observed with indications of root-rot disease with half of the stumps surveyed appearing to have died from root-rot.
- A survey was carried out for 11 estates (109 compartments) of *Acacia mangium*: 71 were in rotation 1 (65.1 %) and 38 in rotation 2 (34.9 %), covering 81 compartments with mineral (74.3 %) and 28 lowland compartments (25.7 %) with wetland (peat soils). The total rate of mortality was over a third of the trees and root-rot represented 8% of these losses which, although significant, falls behind the combined total for mortality at establishment and other abiotic or natural causes (= 17%).

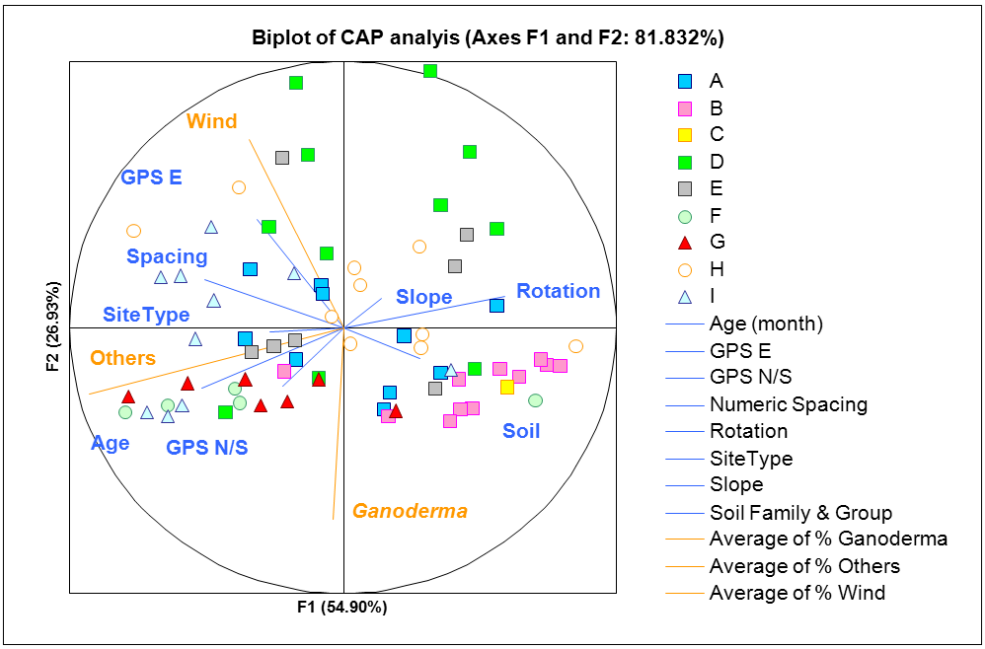
- While 8-11% mortality due to root-rot disease may not be the highest category of mortality, the incidence of root-rot increases exponentially over rotations i.e. set of data for *A. mangium* showed a clear increase in the incidence of root-rot estate wide at harvest across rotations; R1: 5%, R2: 15%, R3: 35%. This type of data has driven the shift to growing eucalypts which appear less susceptible to root-rot. It also has to be remembered that 8% mortality estate wide is representative of trees of all ages and that any particular site will take a trajectory of increasing mortality as it reaches harvest (Figure 6) and an estate wide value grossly underestimates the impact of root-rot on productivity.

### 7.2.2 Activity 2.2: Monitoring the symptoms of root rot disease development

Draft field guides have been produced but require additional funding before they can be published (a few high quality photos need to be taken and the costs of publication covered). However they have been made available to all project participants in electronic format. These guides have also been made more widely available through an AusAID project which provided training in forest disease management strategies. Field guides give instructions on how to monitor for root rot and photos of signs and symptoms of root rot.

### 7.2.3 Activity 2.3: Development of site-risk rating systems

There were several survey data sets available for the development of a root-rot assessment tool. Multivariate analyses of various data sets explored the different parameters that could be contributing to the risk of root-rot were carried out e.g. sites at different locations (A-I) in the analysis below have different risks of root-rot. Locations B and G have a lower risk that others and sites at D have a variation in this risk.




However significant problems were encountered because the data sets had not been collected in a standard fashion and it was difficult to match the different spatial scales for which data was available. One problem is the lack of data from sites with no root-rot disease and therefore obtaining site information associated with no root-rot disease status. For example, one data set indicates that plots with root-rot mortality exceeding 20% were associated with steep (>20°), older (8.3-10.4 years) sites with mixtures of certain types (soil texture was important), within a particular region. However this analysis was based on plots which all had root-rot disease.

A simple site-risk rating system was however developed (Figures 12 and 13). It is acknowledged that this is a first step towards developing such a tool and that further investigation is required.

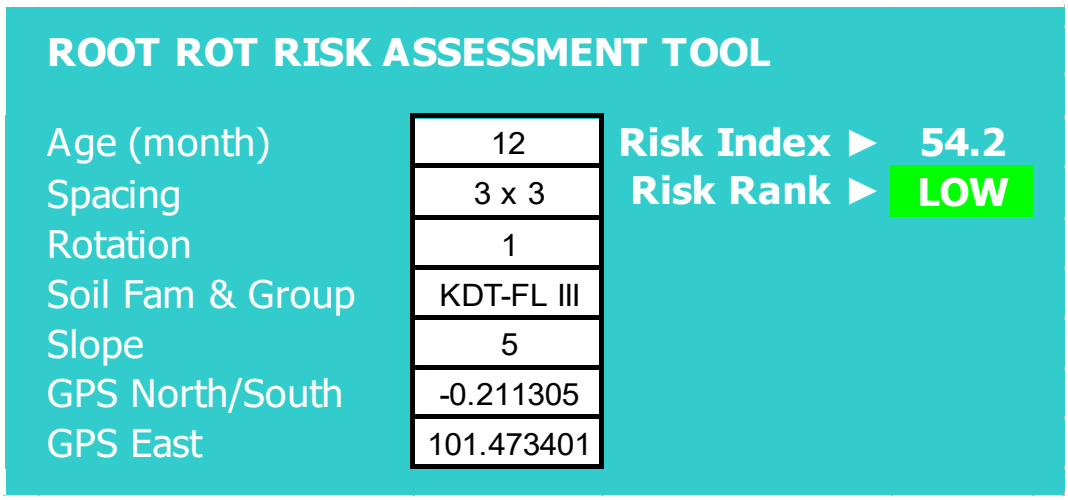



**Figure 12: Screen shot of project root-rot "risk assessment tool". By entering only the site factors shown, an estimate of the level of risk can be allocated.**

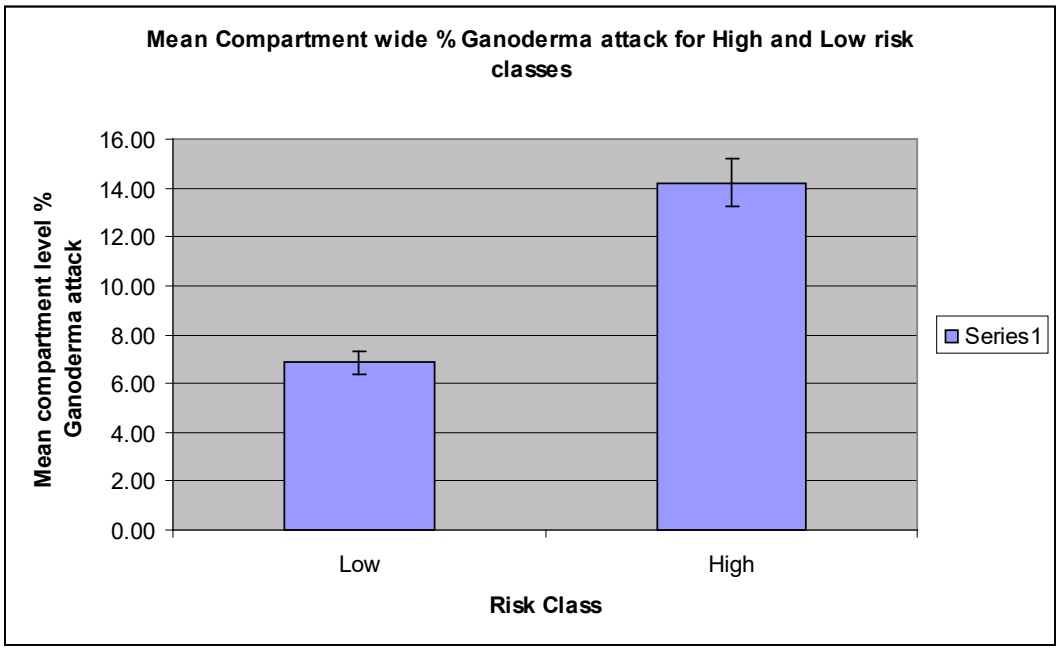



**Figure 13: Figure showing the significant difference between levels of root rot on sites assessed as being high or low risk. Error bars are ± standard error of the mean.**

## 7.3 Objective 3: Development of simple and cost-effective root rot reduction strategies

The combined output of all activities in 3 is the field testing of interventions that can be applied simply in an integrated management plan for root rot reduction. This field testing was carried out by project partners but stringent controls were difficult.

### 7.3.1 Activity 3.1: Evaluation of existing and new biocontrol and chemical agents

As outlined as a risk in the final proposal for this project it was difficult to sources a sufficient number of promising biocontrol control agents. However those already available to the companies such as *Trichoderma* were tested with little success. The potential agent discovered by surveys of eucalypt plantations was obtained too late in the project for field trials. However PT Arara Abadi towards the end of the project has started field trials to test a potential agent discovered in *Acacia* with indications of success. These results were presented at the final review and will require further assessment.

### 7.3.2 Activity 3.2: Evaluation of stump application technologies for the delivery of biocontrol agents

PT Arara Abadi has developed a semi-commercial method of application based on the inclusion of woody blocks infested with the biocontrol agent into the planting hole.

### 7.3.3 Activity 3.3: Field testing of control options

None of the field options tested by the companies apart from one biocontrol system showed any real indications of success. While this is a predictable result given the literature on any basidiomycete root rot disease it does mean that it is now understood that there is no "quick-fix" to root rot and that future control will depend on root rot monitoring, understanding risk, the employment of resistance and/or biocontrol agents. Treatments could be applied to stumps after a first rotation at sites with little or no root rot disease.

## 7.4 Objective 4 Training, information packaging, dissemination and adoption of outcomes

### 7.4.1 Activity 4.1: Training

The following table indicates the training outputs from the project and other project activities enabled by each.

**Table 6: Indicating training outputs for project FST2003/048. NOTE: Indonesian Forestry Research and Development Agency (**FORDA**) centres referred to in the table:** CFBTI **= Centre for Forest Biotechnology and Tree improvement,** FNCRDC **= Forest and Nature Conservation Research and Development Centre**

| Name | Institution | Level of training | Title of the work, duration, comments etc. |
|---|---|---|---|
| **Hobart – Academic, intensive and collaborative training in molecular methods & field & laboratory pathology, disease detection and control method development** | | | |
| Istiana Prihatini | FORDA, CFBTI | PhD (Hobart) | 2007-2010. Molecular detection and identification of fungi associated with Spring Needle Cast in plantations of *Pinus radiata*. Istiana is currently completing her studies in Hobart. |
| Luci Agustini | FORDA, FNCRDC | MSc Research (Hobart, Sumatra) | 2007-2009. Signs and symptoms of root rot in *Eucalyptus pellita* plantations in Indonesia<br>- Luci's new skills in mycology and mycological experimentation have enabled her to begin testing isolates found through the project for biocontrol efficacy see section 0 (2010). |
| Purnamila Sulistiawati | FORDA, CFBTI | MSc Research (Hobart) | 2007-2009. Does FTA card technology improve sampling efficiency of DNA from fungal tissue associated with forest tree diseases?<br>- Purnamila's new familiarity with fungal DNA extraction and PCR amplification has enabled her to carry out molecular SI-group investigations see section 7.1.2 (2010). |
| Antonius Widyatmoko | FORDA, CFBTI | Training (Hobart) | January-February 2007. 4 weeks training in fungal molecular techniques necessary for processing plant pathogen samples in Indonesia.<br>- Following his training Antonius was able to carry out molecular identification of fungi in the Jogjakarta laboratory marking the beginning of the FORDA CFBTI laboratory's capacity for fungal molecular diagnostics & facilitating activities under objectives one and 3 see sections 7.1.1 & 0. |
| Heru Indrayadi<br><br>Abdul Gafur | P.T. Arara Abadi<br><br>P.T. Riau Andalan Pulp & Paper | Collaborative research & discussions (Hobart, Melbourne, Brisbane) | February-March 2009. Opportunity to collaborate intensively on issues of root-rot risk assessment & biological control with discussions with experts on biological control development and industrial production, remote sensing for disease detection, industrial forest health surveillance programs, Australian experience with forestry biocontrol R&D & tree-pathogens in urban/native vegetation situations<br>- This collaborative research lead to the development of the project's risk assessment tool see section 7.2.3 and discussions have contributed to project partners incorporating RR surveys into their PSP monitoring schemes. |

| | | | |
|---|---|---|---|
| **Jogjakarta – On-site training in laboratory methods in mycology and molecular biology** | | | |
| Desy Puspitasari | FORDA, CFBTI | Training (Jogjakarta) | 2007-2010. Training in mycology throughout the project by CM & AF during trips to Jogjakarta; Desy's background was in tissue culture. Training included sample processing, culturing, culture/lab maintenance, morphological macroscopic & microscopic examination & description of fungal structures including assessment of antagonistic reactions.<br>- Desy's training in Jogja facilitated all activities under objective one (section 7.1), activity 3.1 (see section 0) and her skills and the lab developed for the project has substantially increased FORDA's capacity to deal with fungal pathogens being on the same site as FORDA's molecular facility. |
| Vivi Yuskianti | FORDA, CFBTI | Training (Jogjakarta) | February 2008. (During 3 week visit by Morag Glen to CFBTI, Jogja) Training in fungal molecular techniques necessary for processing plant pathogen samples in Indonesia.<br>- To meet the work-load from the project Vivi was trained in the same molecular techniques as Antonius, boosting the fungal diagnostics capacity of the CFBTI laboratory and facilitating work under under objectives one and 3 see sections 7.1.1 & 0. |
| **Field pathology training – Disease recognition, pathogen description & monitoring scheme design** | | | |
| Ragil Irianto<br><br>Nur (Inung) Hidayati & Desy Puspitasari | FORDA, FNCRDC<br>FORDA, CFBTI | Training (Sumatra, East Kalimantan) | Fieldtrips in 2007 & 2008. Training in morphological & field mycology.<br>- The field pathology skills imparted enabled and supported activities under objectives one to three (sections 7.1.1, 7.1.2, 7.2.1, 7.2.2 & 0) and provided skills for these FORDA staff members to carry out similar work in the future including disease/fungus description. |
| **Jogjakarta - Training at workshop on Disease management in tropical plantation tree crops** | | | |
| Jogjakarta workshop participants – see list in PSLP book | various Indonesian & regional government & industrial agencies | Practical Workshops (Jogjakarta) | May 2009. Practical workshop sessions on techniques in morphological / field mycology & fungal diagnostics, processes for molecular disease diagnosis, and forest health surveillance including comparisons of different methods.<br>- A wide selection of industry (pulpwood, oilpalm & rubber), university and government (FORDA, Ministry of Forestry, LIPI - Herbarium Bogoriense, Indonesian Quarantine) participants were able to hone their skills in the practical sessions run including hands on activities and demonstrations of methods and tools from the project (e.g. disease / pathogen identification guides, survey methods). |

### 7.4.2 Activity 4.1: Delivery of root-rot management strategies to outgrowers

Because of the early stage of the research in the project (Category one), the project has relied on the systems in place linking the research and development arms of our industry partners with the small-holders within their respective outgrower schemes.

Demonstration trials were the management trials set up by the companies but were only shown to Indonesian researchers and managers.

Guides have been produced for both the identification of common pathogenic and non-pathogenic species.

- Field guides to the identification of root rots in tropical plantations
  - Part I – *Acacia mangium*

  - Part II – *Eucalyptus pellita*
  - Part III – *Elais guineensis*
  - Part IV – *Hevea brasiliensis*
- Morphological identification of fungal fruiting bodies: A guide to the process of identifying fungal fruiting bodies - the link between taxonomy and function.

# 8 Impacts

## 8.1 Scientific impacts – now and in 5 years

### 8.1.1 Now

- Confirmation that both *Eucalyptus pellita* and *Acacia mangium* are susceptible to root rot diseases in Indonesia
- Developing fungal recognition skills in the scientific staff of the Indonesian plantation industry by information transfer and direct hands-on training.
- Developing experimental design skills through discussion of field trials and pathogenicity testing. This has been a significant contribution in terms of showing the value of controls in experiments.
- Introduction of Ground Penetrating Radar (GPR) technology to the Indonesian pulpwood industry for the assessment of root architecture and making an assessment of the capacity of this technology for determining the location of diseased roots.
- Understanding pathogen dispersal and the implications for management such as the need to combat not only spore dispersal but also root-to-root contact and contact with buried inoculum.
- Biological control - Increasing knowledge of potential biological control species (as each will have strengths and weaknesses) such as *Phlebiopsis sp.* recently isolated from areas with low root-rot incidence.
- Differences in the species associated with root rot in different host species studied indicating that there are many pathogens already present on land planted to *Acacia mangium*, that their distribution is not uniform and that it is the susceptibility of the plantation species selected that causes any particular pathogen to become dominant - this is very important in terms of risk management and matching species selection to particular sites
- Invitation to prepare a special issue on root rot project for "Forest Pathology" (citation index 1.25)

### 8.1.2 In 5 years

- Use of tropical pulpwood plantations as a model system for research into woody root rots in plantation systems.
- Movement towards use of analysis of the size and number of gaps as a means of estimating loss at the end of rotation and realistic wood volumes.
- Movement towards competitive basidiomycetes being more frequently investigated as targets for root-rot biocontrol in tropical systems along with the currently common myco-parasitic hyphomycetes such as *Trichoderma* to facilitate a multi-pronged attack on root-rot organisms.

## 8.2 Capacity impacts – now and in 5 years

### 8.2.1 Capacity impacts – now and in 5 years

***Now***

- Indonesian scientists from project partners and the Indonesian Forestry Research and Development Agency (FORDA) have received training in Hobart in fungal DNA diagnostic methods and the FORDA laboratory in Jogjakarta now has the capacity to molecularly identify fungal isolates, eliminating the necessity to send cultures to Australia.
  - FORDA staff are now capable of fungal molecular analysis and routine molecular processing of fungal samples is now underway
  - Through training and infrastructure development in fungal laboratory and culture management, the FORDA laboratory in Jogja has a hitherto unrealised capacity to deal with a wide range of fungal pathogens that has the potential to expand their field of activities.
- Linking the laboratories of the industry with herbaria and culture collections run by the government to provide culture and specimen preservation facilities for companies that otherwise would lose this valuable material.
- Demonstrating the diversity and potential severity of forest pathogens to Indonesian Quarantine staff (who at the time of our discussions had only three listed forest pathogens when, for example, Australian Quarantine lists more than 20 just in the short publication Mireku & Roach (2000) *Forests and timber: a field guide to exotic pests and diseases*.
- Through participation in the AusAID PSLP workshop we have built capacity in disease management. This includes practical skills in mycology, molecular biology and disease detection methodologies. The workshop also promoted the possibility of integrating different strategies, and, despite the fact there is no quick fix, several tools used in concert can reduce disease incidence.
- The laboratory at Yogyakarta has been enabled to expand the provision of diagnostic services to the oil palm industry where until now, species identification was dependant on the unverified appearance of cultures on plates.
- The FORDA laboratory, through capacity developed by the project has been approached by an industry partner for the identification of an unknown pathogen. Sample handling, culturing, DNA extraction from fungal material, PCR for fungal & bacterial targets and interpretation of sequence information are all skills provided by project and associated John Allwright Fellowship training which FORDA staff can now employ to identify fungal pathogens attacking industrial, community and native forests in Indonesia.

***In 5 years***

- Increasing demand leading to an expansion of FORDA forest pathology diagnostic services supported by within-FORDA knowledge/skills transfer.
- Industry molecular infrastructure able to be used for 'in-house' mycological / pathology investigations.

## 8.3 Community impacts – now and in 5 years

### 8.3.1 Economic impacts

***Now***

- Due to the drop in price of oil palm and rubber with the 2008/09 economic crisis our research for the company out-growers has taken on a fresh importance as there is now more economic dependence on getting a profitable result from planted *Acacia mangium* and less ability to derive money from alternative crops. This concern is reflected in the fact that we have been pledged substantial in-kind work for continued research from our industry partners.
- Mortality rates for root-rot infected areas increase as a plantation ages and can be 30-60% between ages 5 and 7 years on certain sites. With a MAI 35 m3/ha/y for a site, landowners would harvest 133 m3 with a mortality rate of 30% at age 6. The yield foregone due to root-rot is 57 m3 with a value of US$ 2280 if the price of pulpwood is US$ 40/t. Harvesting at 5 years when the mortality rate with a mortality rate of 25% would mean that landowners would harvest 131 m3, only slightly less that harvesting at age 6. Depending on the rate of disease progress after 5 years at a particular site it could be more profitable to harvest earlier rather than later.

***In 5 years***

- Current trials indicate treatment with *Cerrena* can reduce mortality by 40 to 75%. If even 5% of this can be achieved this will be a substantially greater saving to the industry and small holder farmers than that represented by the savings of 4.2M USD$ annually suggested in the background section. It is hoped that other candidate biological control agents would be developed and allow easy and cost effective production and application (i.e. allowing the spraying of debris and stumps).
- If the site-risk rating system can be validated sufficiently the potential to reduce the rate of pathogen spread on high risk sites through management decisions such as spacing.

### 8.3.2 Social impacts

***Now***

- Policy - The Indonesian Ministry of Forestry (MOF) does not have specific policies concerning the assessment and maintenance of forest health by forestry concession holders. This lack of policy was explored during the PSLP workshop and proposals made to redress this fact.
- Quarantine - Forestry Pests have not received significant attention to date and the PSLP workshop highlighted this fact. The quarantine service is Indonesia is seriously understaffed.

***In 5 years***

- Forest Health beginning to be incorporated into assessments of plantation management.
- Forest pathogens incorporated in Indonesian Quarantine surveillance

### 8.3.3 Environmental impacts

***Now***

- Maintaining adequate production from plantations is essential to alleviate reliance of mills on native timbers.

***In 5 years***

- Reduction in mortality due to biocontrol application enabling companies to better meet their wood supply needs and reducing pressure on native forests.
- If un-granted areas are assessed as being unprofitably susceptible to root rot using validated project assessment tools, these areas may be left as reserves.

## 8.4 Communication, dissemination & training activities

### 8.4.1 Training activities

- Postgraduate training of three John Allwright Postgraduates, Luci Agustini, Purnamila Sulistyawati (MSc) and Istiana Prihatini (PhD) is in the final phase of completion
- Organising and participation in PSLP AusAID sponsored Workshop and running practical sessions in:
  - Disease monitoring techniques and strategies
  - Morphological mycology
  - Molecular mycology
- Visit by Dr. Anton Widyatmoko to Hobart for training in fungal molecular methods
- Collaborative visit by Dr. A. Gafur & Mr. H. Indrayadi (risk analysis, biological control and remote sensing for forest health)
  - Meetings / training / information sessions on
  - Risk and distribution modelling using CLIMEX and similar programs (CSIRO CSE)
  - Remote sensing using a variety of technologies owned or trialled by our industry partners (CSIRO CSE)
  - Development, application and industrial scale-up of biological control production (Biocontrol Australia)
  - Disease monitoring and mapping (Forestry Tasmania)
  - Integration of GIS and edaphic data with disease mapping (DPIQ)
  - Collaborative work and training on data preparation, model selection, model development and data assessment for risk prediction

### 8.4.2 Communication & dissemination activities

- Participation in the AUSAID PSLP workshop; a position paper "Disease-management strategies for the rural sector that help deliver sustainable wood production from exotic plantations" and a number of papers based on this project given by project staff. Indonesian and Australian project staff were a very effective way of delivering outcomes to a wider audience that contained interested parties from the rubber and oil palm industries involved in the management of root rot in their crops (the ACIAR Project has been very effective at promoting cooperation between pulpwood companies dealing with root rot that had been previously absent) . This has included the production of a DVD with all presentations including those based on research from this ACIAR project.

- A series of meetings, presentations and seminars were given as part of the tour of duty by our Indonesian collaborators during the biological control and risk modelling visit. These outlined the current state of play in the Indonesian pulpwood industry and facilitated communication between Indonesian project partners and a wider variety of Australian pathologists, entomologists, physiologists and modellers from Tasmania, Victoria and Queensland.
- Chris Beadle participated in the FST/2006/058 Mid-term review in August 2008 and presented a talk on linkages and synergies between this project and the root-rot project
- Through the review for the current project in December 2008 all project partners were brought up to date with the current state of play in the project and brought together for discussion and comment.
- Project staff are actively involved in network building through contacts made at the Yogyakarta AusAID PSLP workshop and through contacts with university staff. Project staff proposed and are taking responsibility for developing an Indonesian consortium across the root-rot community in different crops to work with root rot and other diseases.
- Through contacts made through the project, Dr Wiwik Widyastuti of Gadjah Mada University submitted a successful proposal to travel to Hobart in early 2010 to scope a book on basidiomycete root rot fungi and their management in Indonesia. An agreed outline has been completed and a potential authorship is now being sought.

# 9 Conclusions and recommendations

The conclusions and recommendations of this project are qualified on two bases, 1) that we are dealing with forestry crops where the minimum operational rotation length is at least four years and normally longer (thus none of the experiments instituted during this project have been able to span even a single rotation) and 2) that the project was proposed as a category 2 (impact in 5 to 10 years) and, as such, " A follow-on or extension of the project is likely to advance the project outputs to outcomes and/or extend information to a wider audience." - ACIAR Project Development Guidelines Appendix 1, Category 2 Projects.

## 9.1 Conclusions

- There are multiple pathogenic species found in *Acacia mangium* plantations that have the capacity to kill not just this species, but also the most commonly planted alternative tree species, *Eucalyptus pellita*.
- *Eucalyptus pellita* is susceptible to root-rot caused by both *Ganoderma philippii* and *Phellinus noxius* and can suffer disease gaps as large as those seen *in A. mangium*. It was previously thought that *E. pellita* and *Alstonia scholaris* could provide break crops for *A. mangium* thus reducing disease incidence. However *Eucalyptus pellita* has been shown to be susceptible to root-rot at certain sites and although *A. scholaris* does not show symptoms of root-rot disease the roots of this species will still harbour root-rot pathogens lethal to *A. mangium*.
- Losses from root-rot at disease sites increase across rotations, smaller tree spacing incurs higher risk, soil types vary in risk, soil texture may be important in determining risk, compartments with a slope value of 5 have a slightly higher risk, plantations older that 5-6 years have higher risk and delayed harvesting could mean significant economic loss.
- Spore dispersal could be responsible for new infections in a plantation, though within a first rotation most trees appeared killed by infection from existing below-ground inoculum. Control solutions should therefore be found for *both* stump protection (above ground, preventive) and root protection / inoculum reduction (below ground, curative/protective).
- The possibility of developing a successful biocontrol agent (BCA) has been demonstrated by the PT Arara Abadi's pragmatic approach to the application of *Cerrena* infested blocks in pot trials and in the field. However this BCA cannot be effectively applied in any other medium as yet and therefore could not be sprayed onto stumps. More research is required into the production and application of inoculum and testing of other potential candidates such as *Phlebiopsis* which could produce a spore type during its life cycle which is suited for its production and dispersal as a BCA.
- It is unlikely that root-rot disease can be managed by the application of a BCA alone or that there is any one solution to disease management. However a sound understanding of the pathogen, its biology and the environmental risk factors contributing to the occurrence of severe damage suggest that a suite of strategies can be applied in concert i.e. early harvesting and the application of a BCA at high risk sites, the use of RS technologies to detect disease gaps and assist in the refinement of site-risk rating tools. More research needs to be carried out to understand resistance or tolerance to the different root-rot pathogens but this is still a pathway that offers considerable promise in terms of disease management.

## 9.2 Recommendations

### 9.2.1 Specific recommendations from project research

- That estimates of losses due to root rot be adjusted to account for disease progression through to harvest and incorporate aspects of the spatial distribution of root rot.
- That several methods of biological control be sought to both 1) prevent stumps being infected generating new infection centres (e.g. with *Phlebiopsis*) , and 2) to remediate already infected sites / protect planted trees from below-ground inoculum (e.g. with *Cerrena*).
- Understanding pathogen infection and resistance or tolerance is the next crucial step to root-rot disease management.

### 9.2.2 General recommendations from project reviews and workshop

- An increase in levels of disease incidence is the norm in pulpwood, rubber and oil palm plantations; rates of disease spread are highest in pulpwood plantations established to *Acacia mangium*; alternative pulpwood species, for example *Eucalyptus pellita* are planted, but none to date are resistant to root-rot disease. No one approach to disease management has worked to contain the spread of root-rot disease. A multi-pronged approach or integrated disease management (IDM) is recommended. Plantation managers need to consider the complexities of their individual plantation systems to develop an IDM strategy that best meets their needs.
- Reviews of control strategies indicate that the use of genetically resistant trees is the most cost-effective means for managing disease in plantation crops. Conventional breeding programs should be supported by molecular research which can provide a better understanding of the interactions between trees and pathogens. Cultural practices in the nursery and field that encourage vigorous growth and minimise or eliminate sources of pathogen inoculum should be adopted. Chemical control, while not a preferred approach, and one that has had mixed success in pulpwood plantations, may be greatly improved if treatment is based on a good understanding of pathogen biology so that weak points in the pathogen cycle can be targeted.
- There has been only one effective biological control agent (BCA) developed. This is used against a single basidiomycete root-rot pathogen *Heterobasidion annosum* of conifers at high latitudes in the northern hemisphere. In Indonesia, the main BCA adopted has been *Trichoderma* but this provides only short-term control of root-rot diseases. As for chemical control, the most important information in developing effective biological control is the timing of the weak points in the pathogen life cycle. Screening for BCAs should be carried out in the relevant niche; in the case of basidiomycete root-rot pathogens, this is the rhizosphere and debris such as stumps. The production of a compost, biofungicide and biofertiliser from waste material, using microorganisms selected for antagonistic and decomposer fungi, is a highly desirable strategy for Indonesia's plantation industries although product efficacy as a BCA is not yet proven.
- Close integration between the molecular and non-molecular aspects of research is critical to a full understanding of pathogen biology, host response and the effectiveness of control measures. This includes use of non-molecular skills in the morphological recognition of fungal fruit bodies, the use of herbaria to build reference collections of pathogens, and the maintenance of traditional breeding programmes for disease resistance.

- Forestry development in Indonesia is increasingly dependent on plantations; most are monocultures which can beat high risk of disease outbreaks. Root and stem rots caused by basidiomycete pathogens are the biggest disease issue confronted by the pulpwood, rubber and oil palm industries. A coherent policy for strengthening both capacity building and proper use of this capacity for disease management is crucial to the long-term sustainability of these plantation industries.
- The Ministry of Forestry does not have a policy for measures that must be taken in response to a disease outbreak, yet the pulpwood plantation estate is planned to triple in the next ten years. This and the lack of capacity within companies to respond to disease outbreaks are factors contributing to the lack of uptake of trained graduates in pathology. Plantation companies need to understand the benefits from implementing systematic forest health measures and be encouraged to form dedicated forest protection units with trained pathologists. Outbreaks of gall rust disease affecting most albizia stands in Java are an example of what happens in the absence of policy and action by government for dealing with tree diseases.
- Indonesian expertise in forest pathology is dispersed and pathologists working with plantation crops have little contact with each other. While this project enabled new contacts to develop and common interests to be discussed, a formal system across the pulpwood (and rubber and oil palm) industries for exchanging new ideas to be developed in the area of disease management, particularly for root and stems rots, should be nurtured. This requires a willingness by plantation companies in the private sector to share information.
- Biological invasions represent one of the most serious contemporary threats to ecosystem and economic stability. Strict government inspections and quarantine of imported plants, plant products, and soil can be effective ways of excluding disease pathogens. A pre-emptive strategy that develops biosecurity systems to manage pathways of pathogen movement should be adopted including penalties for breaches of biosecurity. Research to support biosecurity, capability training to recognise alien diseases, and government and public awareness of plant biosecurity, are critically important considerations for Indonesia.

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## 10.2 List of publications produced by project

### 10.2.1 Published works and public presentations

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# 11 Appendixes

## 11.1 Appendix 1: List of isolates and sporocarp collections molecularly identified by ACIAR project FST2003/048

### *Amauroderma rugosum*

Another five collections (19, 32, 56, 66 and 82) had highest similarity (>99%) to Amauroderma rugosum (E7366). Note that BLAST searches do not pick up A. rugosum, best match is 93% to G. carnosum, A/G subresinosum, G. lipsiense and G. philippii. The collections were T63, T57, E8852, E8845 and E8806. T57 and E8852 differed from T63 and E8845 at 5-6 nts (T63 and E8845 differ at one nt). E8806 differed from T57 and E8852 at 4-5 nts and from T63 and E8845 at 2 nts. Morph = Amauroderma rugosum. Isolates T72B1 and E8852B1 are in this group, T57A1 has a different sequence, see Fomes group.

E8852-B, T72-B,

### *Amauroderma sp. M5*

Twelve cultures had 99% similarity to an unknown basidiomycete (AY605709) and 93% similarity to G. subresinosum - E8838B, E8838C, T5A, T6B, T6C, (not in excel file but I assumed that these should have been T66B and T66C which were in the excel file) E8818A, E8818B, E8818C, E8818D, T66A, E8812A1, E8823A2. These sequences are 93% similar to those of A. rugosum, which are also 93% similar to G. subresinosum. E8878C2 produced only ~150bp of readable sequence (latter half of ITS2) but this was identical to E8838B etc. No fruitbody sequences match this group. A sp. M5 Nine cultures sequenced in November 08 matched this group –080228-11, 080327-17, 080411-06, 080411-07, 080506-04 (E8818B1), 080506-05 (E8818C1), 080506-06 (E8818D1), 080506-11, 080506-25. 090331-28 belongs to this group.

2-LS-1-A-39-A.1, 2-LS-1-C-12-B.2, 3-L-2-C-31(R, MAT)-A.2, 3-L-2-C-31(R, MAT)-A.2.1, 3-L-2-C-31(R, MAT)-A.2.2, E8818-B.1, E8818-C.1, E8818-D.1, E8838-C, E8838-C.1, T66-A, T66-A.2

### *Amauroderma sp. M7*

080226-05 is also closest to this sequence (AY607509) but similarity is 95% rather than 99%, designated A sp. M7.

3-AA-7(R, BMAT)-A.1, 3-AA-7(R, BMAT)-A.1.1, 3-AA-7(R, BMAT)-A.1.2

### *Antrodia sp. 1*

TFB1-B.2

### *Cerrena sp. 1*

Co227Bogor had almost 100% similarity to an un-named basidiomycete (supposedly an endophyte from Taxus mairei in Taiwan - GenBank AY456192). The closest sequence match to a named isolate was 99% similarity to a Cerrena sp., except for a high match (222/223) to a Ganoderma lucidum? ITS1 sequence. With the exception of the Cerrena sp., none of the above sequences are linked to published papers.

090325-16 has 99.7% similarity to FJ010208, Cerrena sp., compare with Co227.

Cordyceps aff. sobolifera

D47RA is most similar (>99%) to Cordyceps sobolifera, designated Cordyceps aff. sobolifera.

### *Fomes sp. 1*

T193 and T163 (46 and 67), had highest sequence similarity to Fomes fasciatus and F. fomentarius, respectively. Three cultures had 92% sequence similarity to F. fomentarius and >99% similarity to T163 - T42B1, T57A1 and T75A2.080505-03 is very close to T193 (4nt variations at the beginning that may be due to poor sequence quality), Fomes sp. 1

BS-FB7-A, BS-FB7-A.1, BS-FB7-A.1.1, BS-FB7-A.1.2

### *Fomes sp. 2*

T193 was 90% similar to F. fasciatus and T163 was 90% similar to F. fomentarius. They are 91% similar to each other. Probably two different species of Fomes? and 080506-28 is identical the other group, Fomes sp. 2.

T75-B, T75-B.2, T75-B.2.1, T75-B.2.3

### *Fomes sp. 3*

T42-B, T42-B.1, T57-A, T57-A.1, T75-A, T75-A.2, T75-A.2.1, T75-A.2.2, T75-A.2.3

### *Fomitiporella caryophylli*

080319-11 is 98% similar to AY558611 (Fomitiporella caryophylli). Other matches are predominantly in the 5.8S region (Inocutis, Inonotus and Phellinus).

4-LS-2-C-76(W)-A.2, 4-LS-2-C-76(W)-A.2.1, 4-LS-2-C-76(W)-A.2.2, 4-LS-2-C-76(W)-A.2.3

### *Fomitopsis feei*

E8828A, E8828B, E8828C, E8828D, E8816B and T1A had 100% sequence similarity to E7395 - Fomitopsis feei. 080506-10 (E8828D1) has 1 nt different from this group.

E8828-C, E8828-C.1, E8828-D.1

### *Ganoderma aff. australe (sp. M1A.)*

Six collections had highest similarity (99.6%) to GenBank accession AF255147, Ganoderma sp., and 93% similarity to Ganoderma philippii. These were 13, 30, 62, 78, 84 and 90 (T171, E8827, T191, T170, T190 and E8851). There were 9 variable nucleotides in this group with the greatest individual variation being 3 nt (0.5%). All of the variable sites were polymorphic within an individual. Designated Ganoderma sp. M1A. Morph = G. aff. australe. Approximately 8% sequence variation from previous Indo G. australe E7101.

4-D-2-A-50(M)-A, 4-D-2-A-50(M)-A.1, 4-D-2-A-50(M)-B, 4-D-2-A-50(M)-B.2, E8851-A, E8851-A.1, L-T170-A.2

### *Ganoderma mastoporum*

Thirteen collections had ITS sequences with highest similarity to Ganoderma mastoporum/cupreum. These were numbers 8, 10, 12, 22, 23, 35, 44, 48, 54, 59, 76 and 77, corresponding to collections E8858, T154, T177, T204, T2, T203, E8867, E8859, T169, T166 and T195. There were 22 variable nucleotides in this group with the greatest individual variation being 6 nt (1%).

Seven cultures had >99% sequence similarity to G. mastoporum/cupreum - E8858A, FB2A, T169A, T168A, T168B, T7B and T7C. Also three company isolates - 1A1RAPP, 2B2RAPP and 3C2RAPP.

Several isolates were selected on the basis of PCR-RFLP results as likely to be G. mastoporum/cupreum. These will be tested with specific primers when I go to Yogya in December. Five others were identified as G. mastoporum/cupreum by sequencing – 080319-07, 080506-20 (T7B1), 080506-21 (T7C1), 080506-23 (E8884B2), 080708-01 and 080708-04.

4-LS-2-A-53N(FB)-A.1, 4-LS-2-A-53N(FB)-A.1.1, 4-LS-2-A-53N(FB)-A.1.2, 5-D-3-F-22(M), 5-D-3-F-22(M).1, 5-D-3-F-22(M).2, 6-L-3-B-4-A, 6-L-3-B-4-A.1, 6-L-3-B-4-A.2, 6-L-3-B-4-A.3, 7-LS-4-C-90(M)-A, 7-LS-4-C-90(M)-A.1, 7-LS-4-C-90(M)-A.2, 7-LS-4-C-90(M)-A.3, AA-C0367, BS-FB2-A.2, BS-FB2-A.2.1, BS-FB2-A.2.2, BS-FB2-A.2.3, E8884-B.2, E8884-B.2.1, E8884-B.2.2, L-T168-A, L-T168-A.2, L-T168-A.2.1, L-T169-A, L-T169-A.2, L-T169-A.2.3, L-T169-A.3, T7-B.1, T7-B.1.1, T7-B.1.2, T7-C, T7-C.1

### *Ganoderma philippii*

Twenty-eight collections had ITS sequences with highest similarity to Ganoderma philippii. These were numbers 14, 17, 25, 29, 31, 38, 42, 50, 55, 60, 63, 65, 69, 72, 74, 79, 81, 83, 85, 87, 88, 89, 91, 92, 93, 94, 95 and 96, which correspond to collections T180, E8826, T207, T176, E8831, T209, T181, T208, E8821, T206, T200, E8823, T182, T173, E8832, T201, E8835, T185, T172, E8842, T183, T178, T174, E8841, T184, T179, T175 and T202. There were 16 variable nucleotides in the 564 bp ITS1/5.8S/ITS2 region within this group, but the greatest individual variation was 7 nt (1.2%). Many of the variable sites were polymorphic within an individual. T83 (15) may also be Ganoderma philippii, though both forward and reverse sequences produced only short readable fragments. Both of these fragments had highest sequence similarity to G. philippii (96/100 and 148/164 nt similarity).

None of the first batch of cultures that were sequenced matched G philippii - the Gps were all growing too slowly. In the second batch of sequencing, E8832A1 and C9MB matched G. philippii.

No sequenced isolates from this latest batch were Ganoderma philippii, all were picked up by specific PCR.

3-AA-11(M, BMAT)-A.1, 3-AA-11(M, BMAT)-A.1.1, 3-AA-11(M, BMAT)-A.1.3, 3-AA-8(R, BMAT)-A.1, 3-AA-8(R, BMAT)-A.1.1, 3-AA-8(R, BMAT)-A.1.2, 3-AA-8(R, BMAT)-A.1.2.1, 3-AA-8(R, BMAT)-A.1.2.2, 3-AA-8(R, MAT)-B.1, 3-AA-8(R, MAT)-B.1.1, 3-AA-8(R, MAT)-B.1.2, 3-BS-10(M, MAT)-B.2, 3-BS-10(M, MAT)-B.2.1, 3-BS-10(M, MAT)-B.2.2, 3-BS-10(M, MAT)-B.2.3, 3-BS-15(M, BMAT)-A.1.1, 3-BS-15(M, BMAT)-A.1.1.2, 3-BS-20(M, BMAT)-A2.1, 3-BS-20(M, BMAT)-A2.1.1, 3-BS-20(M, BMAT)-A2.1.2, 3-BS-20(M, BMAT)-A2.1.3, 3-BS-20(M, BMAT)-A2.2, 3-BS-20(M, BMAT)-A2.2.1, 3-BS-20(M, BMAT)-A2.2.2, 3-BS-20(M, MAT)-A.1.1, 3-BS-5(M, BMAT)-A.2, 3-BS-5(M, BMAT)-A.2.1, 3-BS-5(M, BMAT)-A.2.2, 3-BS-5(M, BMAT)-A.2.3, 3-BS-5(M, MAT)-A1.2, 3-BS-5(M, MAT)-A1.2.2, 3-BS-5(M, MAT)-A1.2.3, 3-L-10(M, MAT)-A.2, 3-L-10(M, MAT)-A.2.1, 3-L-10(M, MAT)-A.2.2, 3-L-10(M, MAT)-B.1, 3-L-10(M, MAT)-B.1.1, 3-L-10(M, MAT)-B.1.2, 3-L-10(M, MAT)-B.1.3, 3-L-2-A-21(M,BMAT)-A.1, 3-L-2-A-21(M,BMAT)-A.1.1, 3-L-2-A-21(M,BMAT)-A.1.2,

3-L-2-A-21(M,BMAT)-A.1.3, 3-L-2-A-21(R,MAT)-A.1, 3-L-2-A-21(R,MAT)-A.1.1, 3-L-2-A-21(R,MAT)-A.1.2, 3-L-2-A-21(R,MAT)-A.1.3, 3-L-2-A-23(M, BMAT)-A.1, 3-L-2-A-23(M, BMAT)-A.1.1, 3-L-2-A-23(M, BMAT)-A.1.2, 3-L-2-A-23(M, BMAT)-B.1, 3-L-2-A-23(M, BMAT)-B.1.1, 3-L-2-A-23(M, BMAT)-B.1.2, 3-L-2-A-23(M,MAT)-B.1, 3-L-2-A-23(M,MAT)-B.1.1, 3-L-2-A-23(M,MAT)-B.1.2, 3-L-2-A-23(M,MAT)-B.1.3, 3-L-2-A-24(M, BMAT)-A.1, 3-L-2-A-24(M, BMAT)-A.1.1, 3-L-2-A-24(M, BMAT)-A.1.2, 3-L-2-A-24(M, BMAT)-A.1.3, 3-L-2-A-24(M, BMAT)-B.1, 3-L-2-A-24(M, BMAT)-B.1.1, 3-L-2-A-24(M, BMAT)-B.1.2, 3-L-2-A-24(M, MAT)-A.1, 3-L-2-A-24(M, MAT)-A.1.2, 3-L-2-A-24(M, MAT)-A.1.3, 3-L-2-A-24(M, MAT)-B.1, 3-L-2-A-24(M, MAT)-B.1.1, 3-L-2-A-24(M, MAT)-B.1.2, 3-L-2-A-26(M, BMAT)-A.1, 3-L-2-A-26(M, BMAT)-A.1.1, 3-L-2-A-26(M, BMAT)-A.1.2, 3-L-2-A-26(M, BMAT)-A.1.3, 3-L-2-A-26(M, BMAT)-B.1, 3-L-2-A-26(M, BMAT)-B.1.1, 3-L-2-A-26(M, BMAT)-B.1.2, 3-L-2-A-26(M, BMAT)-B.1.3, 3-L-2-A-26(M, MAT)-A.1, 3-L-2-A-26(M, MAT)-A.1.1, 3-L-2-A-26(M, MAT)-A.1.2, 3-L-2-A-26(M, MAT)-A.1.3, 3-L-2-A-26(M, MAT)-B.1, 3-L-2-A-26(M, MAT)-B.1.1, 3-L-2-A-26(M, MAT)-B.1.2, 3-L-2-A-26(M, MAT)-B.1.3, 3-L-2-A-28(M, BMAT)-A.1, 3-L-2-A-28(M, BMAT)-A.1.1, 3-L-2-A-28(M, BMAT)-A.1.2, 3-L-2-A-28(M, BMAT)-B.1, 3-L-2-A-28(M, BMAT)-B.1.1, 3-L-2-A-28(M, BMAT)-C.1, 3-L-2-A-28(M, BMAT)-C.1.1, 3-L-2-A-28(M, BMAT)-C.1.2, 3-L-2-A-28(M, BMAT)-C.1.3, 3-L-2-A-28(M, MAT)-A.1, 3-L-2-A-28(M, MAT)-A.1.1, 3-L-2-A-28(M, MAT)-A.1.2, 3-L-2-A-28(M, MAT)-A.1.3, 3-L-2-A-29(M, BMAT)-A.1, 3-L-2-A-29(M, BMAT)-A.1.2, 3-L-2-A-29(M, BMAT)-A.1.2.1, 3-L-2-A-29(M, BMAT)-A.1.2.2, 3-L-2-A-29(M, BMAT)-A.1.2.3, 3-L-2-A-29(M, MAT)-A.1, 3-L-2-A-29(M, MAT)-A.1.1, 3-L-2-A-29(M, MAT)-A.1.2, 3-L-2-A-29(M, MAT)-A.1.3, 3-L-2-A-29(M, MAT)-B.1, 3-L-2-A-29(M, MAT)-B.1.1, 3-L-2-A-31(M, BMAT)-A.1, 3-L-2-A-31(M, MAT)-A.1, 3-L-2-A-31(M, MAT)-A.1.1, 3-L-2-A-31(M, MAT)-A.1.2, 3-L-2-A-31(M, MAT)-A.1.3, 3-L-2-A-31(M, MAT)-B.1, 3-L-2-A-31(M, MAT)-B.1.1, 3-L-2-A-31(M, MAT)-B.1.2, 3-L-2-A-31(M, MAT)-B.1.3, 3-L-2-A-32(M, MAT)-A.1, 3-L-2-A-32(M, MAT)-A.1.1, 3-L-2-A-32(M, MAT)-A.1.2, 3-L-2-A-32(M, MAT)-A.1.3, 3-L-2-A-45(M, MAT)-A.2, 3-L-2-A-45(M, MAT)-A.2.2, 3-L-2-A-45(M, MAT)-A.2.2.1, 3-L-2-A-45(M, MAT)-A.2.2.2, 3-L-2-A-45(M, MAT)-B.2, 3-L-2-A-45(M, MAT)-B.2.1, 3-L-2-A-45(M, MAT)-B.2.2, 3-L-2-A-45(M, MAT)-B.2.2.1, 3-L-2-A-45(M, MAT)-B.2.2.2, 3-L-2-A-48(M, BMAT)-A.1, 3-L-2-A-48(M, BMAT)-A.1.2, 3-L-2-A-48(M, BMAT)-A.1.2.1, 3-L-2-A-48(M, BMAT)-A.1.2.2, 3-L-2-A-48(M, BMAT)-B.1, 3-L-2-A-48(M, MAT)-A.2, 3-L-2-A-48(M, MAT)-A.2.1, 3-L-2-A-48(M, MAT)-A.2.2, 3-L-2-A-48(M, MAT)-A.2.3, 3-L-2-A-56(M, BMAT)-A.1, 3-L-2-A-56(M, BMAT)-A.1.1, 3-L-2-A-56(M, BMAT)-A.1.2, 3-L-2-A-56(M, BMAT)-A.1.3, 3-L-2-A-56(M, BMAT)-B.1, 3-L-2-A-56(M, BMAT)-B.1.1, 3-L-2-A-56(M, BMAT)-B.1.2, 3-L-2-A-56(M, BMAT)-B.1.3, 3-L-2-A-56(M, MAT)-A.2, 3-L-2-A-56(M, MAT)-A.2.1, 3-L-2-A-56(M, MAT)-A.2.3, 3-L-2-A-61(M, MAT)-A.2, 3-L-2-A-61(M, MAT)-A.2.1, 3-L-2-A-61(M, MAT)-A.2.2, 3-L-2-A-61(M, MAT)-A.2.3, 3-L-2-A-61(M, MAT)-B.2, 3-L-2-A-61(M, MAT)-B.2.1, 3-L-2-A-61(M, MAT)-B.2.2, 3-L-2-A-61(M, MAT)-B.2.3, 3-L-2-A-65(M, BMAT)-A.1, 3-L-2-A-65(M, BMAT)-A.1.1, 3-L-2-A-65(M, BMAT)-A.1.1.2, 3-L-2-A-65(M, BMAT)-A.1.2, 3-L-2-A-65(M, BMAT)-A.1.2.1, 3-L-2-A-65(M, BMAT)-A.1.2.2, 3-L-2-A-65(M, BMAT)-A.1.2.3, 3-L-2-A-65(M, BMAT)-A.1.3, 3-L-2-A-75(M, BMAT)-A.1, 3-L-2-A-75(M, BMAT)-A.1.1, 3-L-2-A-75(M, BMAT)-A.1.2, 3-L-2-A-75(M, BMAT)-A.1.3, 3-L-2-A-75(M, MAT)-A.2, 3-L-2-A-75(M, MAT)-A.2.1, 3-L-2-A-75(M, MAT)-A.2.2, 3-L-2-A-75(M, MAT)-A.2.3, 4-D-(D)-A.1, 4-D-(D)-A.1.1, 4-D-(D)-A.1.2, 4-D-2-A-42(M)-A, 4-D-2-A-42(M)-A.2, 4-D-2-A-42(M)-A.2.1, 4-D-2-A-42(M)-A.2.2, 4-D-2-A-63(M)-A, 4-D-2-A-63(M)-A.1, 4-D-2-A-63(M)-A.1.1, 4-D-2-A-63(M)-B, 4-D-2-A-63(M)-B.1, 4-D-2-A-63(M)-B.1.2, 4-D-2-A-63(M)-C.1, 4-D-2-A-63(M)-C.1.1, 4-D-2-A-63(M)-C.1.1.2, 4-D-2-A-63(M)-C.1.1.3, 4-D-2-A-64(M)-A, 4-D-2-A-64(M)-A.1, 4-D-2-A-64(M)-A.1.1, 4-D-2-A-64(M)-A.1.2, 4-D-2-A-64(M)-B, 4-D-2-A-64(M)-B.1, 4-D-2-A-64(M)-B.1.1, 4-D-2-A-64(M)-B.1.2.2, 4-D-2-A-64(M)-B.1.2.3, 4-D-2-A-81(R)-A, 4-D-2-A-81(R)-A.2, 4-D-2-A-81(R)-A.2.1, 4-D-2-A-81(R)-A.2.2, 4-D-2-A-82(M)-B, 4-D-2-A-82(M)-B.2, 4-D-2-B-22(M)-A, 4-D-2-B-22(M)-B, 4-D-2-B-22(M)-B.1, 4-D-2-B-22(M)-B.1.1, 4-D-2-B-22(M)-B.1.2, 4-D-2-B-22(M)-B.1.3, 4-D-2-BLANK.1, 4-D-2-BLANK.2, 4-D-2-BLANK.3, 4-D-2-C-10(M)-A.1, 4-D-2-C-10(M)-A.1.1, 4-D-2-C-11(M)-A, 4-D-2-C-11(M)-A.1, 4-D-2-C-11(M)-A.1.1, 4-D-2-C-11(M)-B.2.1, 4-D-2-C-11(M)-B.2.2, 4-D-2-C-11(M)-B.2.3, 4-D-2-C-115(M)-A, 4-D-2-C-115(M)-A.2, 4-D-2-C-115(M)-A.2.1, 4-D-2-C-115(M)-A.2.2, 4-D-2-C-12(M)-A, 4-D-2-C-12(M)-A.2, 4-D-2-C-12(M)-A.2.2, 4-D-2-C-13(2)(M)-A, 4-D-2-C-13(2)(M)-A.2, 4-D-2-C-13(2)(M)-B, 4-D-2-C-13(2)(M)-B.2.1, 4-D-2-C-13(2)(M)-B.2.2, 4-D-

2-C-16(M)-A, 4-D-2-C-16(M)-A.1, 4-D-2-C-16(M)-A.1.2, 4-D-2-C-25(M)-A.2.1, 4-D-2-C-26(M)-A.2.1, 4-D-2-C-26(M)-A.2.1.1, 4-D-2-C-26(M)-A.2.2, 4-D-2-C-26(M)-A.2.2.1, 4-D-2-C-26(M)-A.2.2.3, 4-D-2-C-36(M)-A, 4-D-2-C-36(M)-A.2.1, 4-D-2-C-36(M)-A.2.2, 4-D-2-C-36(M)-B, 4-D-2-C-36(M)-B.1, 4-D-2-C-36(M)-B.1.2, 4-D-2-C-36(M)-C, 4-D-2-C-36(M)-C.1, 4-D-2-C-36(M)-C.1.2, 4-D-2-C-37(M)-A.1, 4-D-2-C-37(M)-A.1.1, 4-D-2-C-37(M)-A.1.2, 4-D-2-C-40(R)-A, 4-D-2-C-40(R)-A.2, 4-D-2-C-40(R)-A.2.1, 4-D-2-C-40(R)-A.2.2, 4-D-2-C-40(R)-A.2.3, 4-D-2-C-44(M)-A, 4-D-2-C-44(M)-A.1, 4-D-2-C-44(M)-A.1.1, 4-D-2-C-44(M)-A.1.2, 4-D-2-C-50(M)-A, 4-D-2-C-50(M)-A.1, 4-D-2-C-50(M)-A.1.1, 4-D-2-C-50(M)-B, 4-D-2-C-50(M)-B.1, 4-D-2-C-50(M)-C, 4-D-2-C-50(M)-C.1, 4-D-2-C-50(M)-C.1.1, 4-D-2-C-50(M)-C.1.2, 4-D-2-C-51(M)-A, 4-D-2-C-51(M)-A.2, 4-D-2-C-51(M)-A.2.1, 4-D-2-C-51(M)-A.2.2, 4-D-2-C-9(M)-A, 4-D-2-C-9(M)-A.2.1, 4-D-2-C-9(M)-A.2.2, 4-D-2-C-9(M)-B, 4-D-2-C-9(M)-B.2, 4-D-2-C-9(M)-B.2.1, 4-D-2-C-9(M)-B.2.2, 4-D-2-C-9(M)-C, 4-D-2-C-9(M)-C.1, 4-D-2-C-9(M)-C.1.2, 4-D-2-C-9(M)-C.1.3, 4-D-2-D-16(M)-A, 4-D-2-D-16(M)-A.1, 4-D-2-D-16(M)-A.1.1, 4-D-2-D-16(M)-B, 4-D-2-D-16(M)-B.1, 4-D-2-D-16(M)-B.1.1, 4-D-2-D-16(M)-B.1.2, 4-D-2-D-19(M)-A, 4-D-2-D-19(M)-A.2, 4-D-2-D-19(M)-A.2.1, 4-D-2-D-19(M)-B, 4-D-2-D-19(M)-B.2, 4-D-2-D-19(M)-B.2.1, 4-D-2-D-19(M)-B.2.2, 4-D-2-D-22(M)-A.1.2, 4-D-2-D-22(M)-A.1.3, 4-D-2-D-58(M)-A, 4-D-2-D-58(M)-A.2, 4-D-2-D-58(M)-A.2.1, 4-D-2-D-58(M)-B, 4-D-2-D-58(M)-B.1, 4-D-2-D-58(M)-B.1.1, 4-D-2-D-58(M)-B.1.2, 4-D-2-D-58(M)-B.1.3, 4-D-2-D-58(M)-C.1.1, 4-D-2-D-58(M)-C.1.2, 4-D-2-D-58(M)-C.1.3, 4-D-2-D-62(M)-A, 4-D-2-D-62(M)-A.1, 4-D-2-D-62(M)-A.1.1, 4-D-2-D-62(M)-A.1.2, 4-D-2-D-62(M)-B, 4-D-2-D-62(M)-B.1, 4-D-2-D-62(M)-B.1.1, 4-D-2-D-62(M)-B.1.2, 4-D-2-D-62(M)-B.1.3, 4-D-2-D-65(M)-A.1, 4-D-2-D-65(M)-A.1.1, 4-D-2-D-65(M)-A.1.2, 4-D-2-D-65(M)-A.1.3, 4-D-2-D-65(M)-B, 4-D-2-D-65(M)-B.2, 4-D-2-D-65(M)-B.2.1, 4-D-2-D-65(M)-B.2.2, 4-D-2-D-74/75-A.2, 4-D-2-D-74/75-A.2.2, 4-D-2-D-74/87-A.1, 4-D-2-D-74/87-A.1.1, 4-D-2-D-74/87-A.1.2, 4-D-2-Z-14-A.2, 4-D-2-Z-14-A.2.1, 4-D-2-Z-14-A.2.2, 4-D-2-Z-14-A.2.2.2, 4-D-2-Z-14-A.2.2.3, 4-D-2-Z-14-B.1, 4-D-2-Z-16-A.2, 4-DP74(2)-(D)-A.2, 4-DP74(2)-(D)-A.2.1, 4-DP74(2)-(D)-A.2.2, 4-LS-2-A-57(R)-B.2, 4-LS-2-A-57(R)-B.2.2, 4-LS-2-A-57(R)-B.2.3, 4-LS-2-A-65(M)-A.1, 4-LS-2-A-65(M)-A.1.1, 4-LS-2-A-65(M)-A.1.2, 4-LS-2-A-65(M)-A.1.2.1, 4-LS-2-A-65(M)-A.1.2.3, 4-LS-2-A-65(R)-A.1, 4-LS-2-A-65(R)-A.1.1, 4-LS-2-A-65(R)-A.1.1.1, 4-LS-2-A-65(R)-A.1.1.3, 4-LS-2-A-65(R)-B.1, 4-LS-2-A-65(R)-B.1.1, 4-LS-2-A-65(R)-B.1.2, 4-LS-2-B-38(M)-A.1, 4-LS-2-B-38(M)-A.1.1, 4-LS-2-B-38(M)-A.1.2, 4-LS-2-B-38(R)-A.1, 4-LS-2-B-38(R)-A.1.1, 4-LS-2-B-38(R)-A.1.2, 4-LS-2-B-38(R)-B.1, 4-LS-2-B-38(R)-B.1.1, 4-LS-2-B-38(R)-B.1.1.1, 4-LS-2-B-38(R)-B.1.1.2, 4-LS-2-B-94(R)-A.1, 4-LS-2-B-94(R)-A.1.1, 4-LS-2-B-94(R)-A.1.2, 4-LS-2-B-94(R)-A.1.3, 4-LS-2-D-51(R)-A.2, 4-LS-2-D-51(R)-A.2.1, 4-LS-2-D-51(R)-A.2.2, 4-LS-2-D-56(R)-A.2, 4-LS-2-D-56(R)-A.2.1, 4-LS-2-D-56(R)-A.2.2, 4-LS-2-D-56(R)-B.2, 4-LS-2-D-56(R)-B.2.1, 4-LS-2-D-56(R)-B.2.2, 4-LS-2-D-65(R)-A1.1, 4-LS-2-D-65(R)-A1.1.1, 4-LS-2-D-65(R)-A1.1.2, 4-LS-2-D-65(R)-A2.2, 4-LS-2-D-65(R)-A2.2.1, 4-LS-2-D-65(R)-A2.2.2, 4-S-2-A-2(M)-A.2.1, 4-S-2-A-2(M)-A.2.3, 4-S-2-A-2(M)-B.1, 4-S-2-A-2(M)-B.1.1.1, 4-S-2-A-2(M)-B.1.1.2, 4-S-2-A-2(M)-B.1.2, 4-S-2-B-53(M)-A.2, 4-S-2-B-53(M)-A.2.1, 4-S-2-B-53(M)-A.2.2, 4-S-2-B-67(M)-A.1, 4-S-2-B-67(M)-A.1.1, 4-S-2-B-67(M)-A.1.2, 4-S-2-B-68(M)-A.1, 4-S-2-B-68(M)-A.1.2, 4-S-2-B-75(M)-A.1, 4-S-2-B-75(M)-A.1.2, 4-S-2-B-75(M)-B.1, 4-S-2-B-75(M)-B.1.2, 4-S-2-B-75(M)-B.1.2.1, 4-S-2-B-75(M)-B.1.2.2, 4-SU-1-A-24(M)-A.1, 4-Su-1-A-24(M)-B.1, 4-Su-1-A-24(M)-B.1.1, 4-Su-1-A-24(M)-B.1.1.2, 4-Su-1-A-24(M)-C.1, 4-Su-1-A-24(M)-C.1.2, 4-Su-1-A-25(R)-A.1, 4-Su-1-A-25(R)-A.1.1, 4-Su-1-A-25(R)-A.1.2, 4-SU-1-A-30(M)-A.2, 4-SU-1-A-30(M)-A.2.1, 4-SU-1-A-30(M)-A.2.2, 4-Su-1-A-31(M)-A.1, 4-Su-1-A-31(M)-A.1.1, 4-Su-1-A-31(M)-A.1.2, 4-Su-1-A-36(M)-A.1, 4-Su-1-A-36(M)-A.1.1, 4-Su-1-A-36(M)-A.1.1.1, 4-Su-1-A-36(M)-A.1.1.2, 4-Su-1-A-48(FB)-A.1, 4-Su-1-A-48(FB)-B.2, 4-Su-1-A-48(FB)-B.2.1, 4-Su-1-A-48(FB)-B.2.2, 4-Su-1-A-48(M)-C.2, 4-Su-1-A-48(M)-C.2.2, 4-Su-1-A-56(M)-A.1, 4-Su-1-A-56(M)-A.1.1, 4-Su-1-A-56(M)-A.1.2, 4-SU-1-A-56(M)-B.2, 4-Su-1-A-80(M)-A, 4-Su-1-A-80(M)-A.1, 4-Su-1-A-80(M)-A.2, 4-Su-1-A-81(FB)-A.2, 4-Su-1-A-81(FB)-A.2.2, 4-Su-1-A-81(FB)-B.1, 4-Su-1-A-81(FB)-B.1.1, 4-Su-1-A-81(FB)-B.1.2, 4-Su-1-A-81(M)-A.2, 4-Su-1-A-81(M)-A.2.2, 4-Su-1-A-86(M)-A.2, 4-Su-1-A-86(M)-A.2.2, 4-SU-1-A-96(FB)-A.1, 4-SU-1-A-96(FB)-A.1.1, 4-SU-1-A-96(FB)-A.1.2, 4-SU-1-A-96(FB)-A.1.3, 4-Su-1-A-96(M)-A.1, 4-Su-1-A-96(M)-A.1.1, 4-Su-1-A-96(M)-A.1.2, 4-Su-1-A-96(M)-A.1.3, 4-Su-1-B-19(M)-A.2, 4-Su-1-B-19(M)-A.2.1, 4-Su-1-B-19(M)-A.2.2, 4-SU-1-B-22(M)-A.1.1, 4-SU-1-B-22(M)-A.1.1.1, 4-SU-1-B-22(M)-A.1.1.2, 4-SU-1-B-22(M)-A.1.1.3, 4-SU-1-B-

22(R)-A.2, 4-SU-1-B-22(R)-A.2.1, 4-Su-1-B-29(M)-A.2, 4-Su-1-B-29(M)-A.2.1, 4-Su-1-B-29(M)-A.2.1.1, 4-Su-1-B-3(M)-A.2, 4-SU-1-B-36(M)-A.1, 4-SU-1-B-36(M)-A.1.1, 4-SU-1-B-36(M)-A.1.2, 4-SU-1-B-36(M)-A.2, 4-SU-1-B-36(M)-A.2.1, 4-SU-1-B-36(M)-A.2.2, 4-SU-1-B-36(M)-B.2, 4-SU-1-B-36(M)-B.2.1, 4-SU-1-B-36(M)-B.2.2, 4-Su-1-B-40(M)-A.2, 4-Su-1-B-45(M)-A.1, 4-Su-1-B-45(M)-A.1.1, 4-Su-1-B-45(M)-A.1.2, 4-SU-1-B-45(R)-A.1, 4-SU-1-B-45(R)-A.1.1, 4-SU-1-B-45(R)-A.1.1.2, 4-SU-1-B-45(R)-A.1.1.3, 4-Su-1-B-46(M)-A.2, 4-Su-1-B-46(M)-A.2.2, 4-SU-1-B-56(FB)-A.2, 4-SU-1-B-56(FB)-A.2.1, 4-SU-1-B-56(FB)-A.2.2, 4-Su-1-B-56(FB)-B.2, 4-Su-1-B-56(FB)-B.2.1, 4-Su-1-B-56(FB)-B.2.2, 4-Su-1-B-56(FB)-B.2.3, 4-Su-1-B-56(FB)-C.2, 4-Su-1-B-56(FB)-C.2.1, 4-Su-1-B-56(FB)-C.2.2, 4-Su-1-B-56(FB)-C.2.3, 4-Su-1-C-36(FB)-A.2, 4-Su-1-C-36(FB)-A.2.1, 4-Su-1-C-36(FB)-A.2.2, 4-Su-1-C-36(M)-A.1, 4-Su-1-C-36(M)-A.1.1, 4-Su-1-C-36(M)-A.1.2, 4-Su-1-C-44(M)-A.1, 4-Su-1-C-44(M)-A.1.1, 4-Su-1-C-44(M)-A.1.2, 4-Su-1-C-44(M)-B.2, 4-Su-1-C-44(M)-B.2.1, 4-Su-1-D-45(R1)-A.2, 4-Su-1-D-45(R1)-A.2.2, 4-Su-1-D-73(M)-A.2, 4-Su-1-D-73(M)-A.2.2, 4-Su-1-D-73(M)-A.2.2.1, 4-Su-1-D-73(M)-A.2.2.2, 4-Su-1-D-73(M)-B.2, 4-Su-1-D-73(M)-B.2.1, 4-SU-1-D-94(R)-A.1, 4-SU-1-D-94(R)-A.1.1, 4-SU-1-D-94(R)-A.1.1.1, 4-SU-1-D-94(R)-A.1.2, 5-D-3-A-48, 5-D-3-A-48.1, 5-D-3-A-48.2, 5-D-3-A-72, 5-D-3-A-72.1, 5-D-3-A-72.2, 5-D-3-A-72.3, 5-D-3-A-80, 5-D-3-A-80.1, 5-D-3-A-80.2, 5-D-3-A-80.3, 5-D-3-C-12(M), 5-D-3-C-12(M).1, 5-D-3-C-12(M).2, 5-D-3-C-12(M).3, 5-D-3-C-15(X), 5-D-3-C-15(X).1, 5-D-3-C-15(X).2, 5-D-3-C-15(X).3, 5-D-3-C-4, 5-D-3-C-4.1, 5-D-3-C-4.2, 5-D-3-C-4.3, 5-D-3-C-41, 5-D-3-C-41.1, 5-D-3-C-41.2, 5-D-3-C-41.3, 5-D-3-C-46(M), 5-D-3-C-46(M).1, 5-D-3-C-46(M).2, 5-D-3-C-46(M).3, 5-D-3-C-51(M), 5-D-3-C-51(M).1, 5-D-3-C-51(M).1.1, 5-D-3-C-51(M).1.2, 5-D-3-C-53(M), 5-D-3-C-53(M).2, 5-D-3-C-53(M).3, 5-D-3-C-63, 5-D-3-C-63.1, 5-D-3-C-75(M), 5-D-3-C-75(M).1.1, 5-D-3-C-75(M).1.1.1, 5-D-3-C-75(M).2, 5-D-3-C-75(M).3, 5-D-3-D-19(M).1, 5-D-3-D-19(M).2, 5-D-3-D-19(M).2.1, 5-D-3-D-19(M).2.2, 5-D-3-D-40(M, BMAT), 5-D-3-D-40(M, BMAT).1, 5-D-3-D-40(M, BMAT).2, 5-D-3-D-40(M, BMAT).2.2, 5-D-3-D-40(M, BMAT).3, 5-D-3-D-48(M, BMAT), 5-D-3-D-48(M, BMAT).1.1, 5-D-3-D-48(M, BMAT).1.2, 5-D-3-D-48(M, BMAT).3, 5-D-3-D-57(M, BMAT), 5-D-3-D-57(M, BMAT).1.1, 5-D-3-D-57(M, BMAT).1.2, 5-D-3-D-57(M, MAT), 5-D-3-D-57(M, MAT).1, 5-D-3-D-57(M, MAT).2, 5-D-3-D-57(M, MAT).3, 5-D-3-D-75(M), 5-D-3-D-75(M).1, 5-D-3-D-75(M).2, 5-D-3-D-75(M).3, 5-D-3-H(br)-2, 5-D-3-H(br)-2.1, 5-D-3-H(br)-2.2, 5-D-3-H(br)-2.3, 5-D-3-H(br)-21(M), 5-D-3-H(br)-21(M).1, 5-D-3-H(br)-21(M).2, 5-D-3-H(br)-21(M).3, 5-D-3-H(br)-22(X), 5-D-3-H(br)-22(X).1, 5-D-3-H(br)-22(X).2, 5-D-3-H(br)-22(X).3, 5-D-3-H(br)-3(M), 5-D-3-H(br)-3(M).1, 5-D-3-H(br)-3(M).2, 5-D-3-H(br)-3(M).3, 5-D-3-H(br)-4, 5-D-3-H(br)-4.1, 5-D-3-H(br)-4.3, 5-D-3-H-25(M), 5-D-3-H-25(M).1, 5-D-3-H-25(M).2, 5-D-3-H-25(M).3, 5-D-3-H-43(M).1.1, 5-D-3-H-43(M).1.2, 5-D-3-H-43(M).1.3, 5-D-3-H-43(M).3, 5-D-3-H-45(M), 5-D-3-H-45(M).1, 5-D-3-H-45(M).2, 5-D-3-H-45(M).3, 5-D-3-H-47, 5-D-3-H-47.1, 5-D-3-H-47.2, 5-D-3-H-47.3, 5-D-3-H-50(M), 5-D-3-H-50(M).1, 5-D-3-H-50(M).2, 5-D-3-H-50(M).3, 5-D-3-H-51(M), 5-D-3-H-51(M).1, 5-D-3-H-51(M).2, 5-D-3-H-51(M).3, 5-D-3-H-64, 5-D-3-H-64.1, 5-D-3-H-64.2, 5-D-3-H-65(M), 5-D-3-H-65(M).1, 5-D-3-H-65(M).2, 5-D-3-H-65(M).2.2, 5-D-3-H-65(M).3, 5-D-3-H-72, 5-D-3-H-72.1, 5-D-3-H-72.2, 5-D-3-H-72.3, 6-D-4-A(br)-16-B, 6-D-4-A(br)-16-B.1, 6-D-4-A(br)-18-A, 6-D-4-A(br)-18-A.1, 6-D-4-A(br)-18-A.2, 6-D-4-A(br)-18-A.3, 6-D-4-A(br)-18-B, 6-D-4-A(br)-18-B.1, 6-D-4-A(br)-18-B.3, 6-D-4-A(br)-18-B.3.1, 6-D-4-A(br)-18-B.3.2, 6-D-4-H(br)-14-A, 6-D-4-H(br)-14-A.1, 6-D-4-H(br)-14-A.2, 6-D-4-H(br)-14-A.3, 6-D-4-H(br)-21-A, 6-D-4-H(br)-21-A.1, 6-D-4-H(br)-21-A.2, 6-D-4-H(br)-21-A.3, 6-D-4-H(br)-21-B, 6-D-4-H(br)-21-B.1, 6-D-4-H(br)-21-B.3, 6-D-4-H(br)-8-A, 6-D-4-H(br)-8-A.2, 6-D-4-H(br)-8-A.3, 6-L-3-A-15, 6-L-3-A-15.1, 6-L-3-A-15.2, 6-L-3-A-15.3, 6-L-3-A-19(M)-A, 6-L-3-A-19(M)-A.1.1, 6-L-3-A-19(M)-A.1.2, 6-L-3-A-19(M)-A.2, 6-L-3-A-19(M)-B, 6-L-3-A-19(M)-B.1, 6-L-3-A-19(M)-B.2, 6-L-3-A-26, 6-L-3-A-26.1, 6-L-3-A-26.2, 6-L-3-A-26.3, 6-L-3-A-40-B, 6-L-3-A-40-B.1, 6-L-3-A-40-B.2, 6-L-3-A-40-B.3, 6-L-3-A-41, 6-L-3-A-41.1, 6-L-3-A-41.2, 6-L-3-A-41.3, 6-L-3-A-45, 6-L-3-A-45.1, 6-L-3-A-45.2, 6-L-3-A-45.3, 6-L-3-A-47, 6-L-3-A-47.1, 6-L-3-A-47.2, 6-L-3-A-75(M), 6-L-3-A-75(M).1, 6-L-3-A-75(M).2, 6-L-3-A-75(M).3, 6-L-3-B-35(M), 6-L-3-B-54(M), 6-L-3-B-54(M).1, 6-L-3-B-54(M).2, 6-L-3-B-54(M).3, 6-L-3-C-25(M), 6-L-3-C-25(M).2, 6-L-3-C-25(M).3.1, 6-L-3-C-25(M).3.2, 6-L-3-C-25(M).3.3, 6-L-3-C-26(M), 6-L-3-C-26(M).1, 6-L-3-C-26(M).3, 6-L-3-C-35(M), 6-L-3-C-35(M).2, 6-L-3-C-35(M).3, 6-L-3-C-4, 6-L-3-C-4.1, 6-L-3-C-4.2, 6-L-3-C-4.3, 6-L-3-C-5, 6-L-3-C-5.1, 6-L-3-C-5.3, 6-L-3-C-66(M), 6-L-3-C-66(M).1, 6-L-3-C-66(M).2, 6-L-3-C-66(M).3, 6-L-3-C-67(W), 6-L-3-C-67(W).3.2, 6-L-3-C-

67(W).3.2.1, 6-L-3-C-67(W).3.2.2, 6-L-3-C-72(M), 6-L-3-C-72(M).2, 6-L-3-C-72(M).3, 6-LS-3-A-36(M), 6-LS-3-A-36(M).1, 6-LS-3-A-36(M).3, 6-LS-3-A-44(M), 6-LS-3-A-44(M).1, 6-LS-3-A-44(M).3, 6-LS-3-A-45(M), 6-LS-3-A-45(M).1, 6-LS-3-A-45(M).3.1, 6-LS-3-A-45(M).3.2, 6-LS-3-A-54(M), 6-LS-3-A-54(M).2, 6-LS-3-A-54(M).3.1, 6-LS-3-A-54(M).3.2, 6-LS-3-A-55(M), 6-LS-3-A-55(M).1, 6-LS-3-A-55(M).2, 6-LS-3-A-64(M), 6-LS-3-A-66(M), 6-LS-3-A-66(M).3, 6-LS-3-A-76(M), 6-LS-3-A-76(M).2, 6-LS-3-A-76(M).3, 6-LS-3-A-77(M), 6-LS-3-A-77(M).1, 6-LS-3-A-77(M).2, 6-LS-3-A-78(M), 6-LS-3-A-78(M).1, 6-LS-3-A-78(M).2, 6-LS-3-A-78(M).3.1, 6-LS-3-A-78(M).3.2, 6-LS-3-B-14(M), 6-LS-3-B-14(M).1, 6-LS-3-B-14(M).2, 6-LS-3-B-14(M).3, 6-LS-3-B-54(M), 6-LS-3-B-54(M).2, 6-LS-3-B-58(M).2, 6-LS-3-B-58(M).3, 6-LS-3-B-80(M).3, 6-LS-3-B-88(M), 6-LS-3-B-88(M).1, 6-LS-3-B-94(M), 6-LS-3-B-94(M).1, 6-LS-3-B-94(M).3, 6-LS-3-C-37(M), 6-LS-3-C-37(M).1, 6-LS-3-C-37(M).3, 6-LS-3-C-4(M), 6-LS-3-C-4(M).1, 6-LS-3-C-4(M).2, 6-LS-3-C-4(M).3, 6-LS-3-C-5(M), 6-LS-3-C-5(M).1, 6-LS-3-C-5(M).2, 6-LS-3-C-5(M).3, 6-LS-3-C-65(M), 6-LS-3-C-65(M).1, 6-LS-3-C-65(M).2, 6-LS-3-C-65(M).3, 6-LS-3-C-7(M), 6-LS-3-C-7(M).1, 6-LS-3-C-7(M).2, 6-LS-3-C-7(M).3, 6-LS-3-D-100(M)-A, 6-LS-3-D-100(M)-B, 6-LS-3-D-100(M)-B.1, 6-LS-3-D-100(M)-B.2, 6-LS-3-D-38(M), 6-LS-3-D-38(M).1, 6-LS-3-D-38(M).2, 6-LS-3-D-38(M).3, 6-LS-3-D-55(M)-A, 6-LS-3-D-55(M)-A.1, 6-LS-3-D-55(M)-A.2, 6-LS-3-D-55(M)-A.3, 6-LS-3-D-55(M)-B, 6-LS-3-D-55(M)-B.1, 6-LS-3-D-55(M)-B.2, 6-LS-3-D-55(M)-B.3, 6-LS-3-D-56(M), 6-LS-3-D-56(M).1, 6-LS-3-D-56(M).2, 6-LS-3-D-56(M).3, 6-LS-3-D-57(M).1, 6-LS-3-D-57(M).2, 6-LS-3-D-65(M), 6-LS-3-D-65(M).1, 6-LS-3-D-65(M).2, 6-LS-3-D-65(M).3, 6-LS-3-D-66(M), 6-LS-3-D-66(M).1, 6-LS-3-D-66(M).2, 6-LS-3-D-66(M).3, 6-LS-3-D-68(M), 6-LS-3-D-68(M).1, 6-LS-3-D-68(M).2, 6-LS-3-D-68(M).3, 6-LS-3-D-71(M), 6-LS-3-D-71(M).1, 6-LS-3-D-71(M).3, 6-LS-3-D-75(M), 6-LS-3-D-75(M).1, 6-LS-3-D-75(M).3, 6-LS-3-D-76(M), 6-LS-3-D-76(M).1, 6-LS-3-D-76(M).2, 6-LS-3-D-76(M).3, 6-LS-3-D-81(M), 6-LS-3-D-81(M).1, 6-LS-3-D-81(M).2, 6-LS-3-Mikoriza, 6-LS-3-Mikoriza.1, 6-LS-3-Mikoriza.2, 6-S-3-B-53, 6-S-3-B-53.2, 6-S-3-D-70, 6-S-3-D-70.1, 6-S-3-D-70.2, 6-S-3-D-70.3, 6-SU-2-A-11(R)-A, 6-SU-2-A-11(R)-A.1.1, 6-SU-2-A-11(R)-A.1.3, 6-SU-2-A-11(R)-A.3, 6-SU-2-A-15(M)-A, 6-SU-2-A-15(M)-A.2, 6-SU-2-A-15(M)-A.3, 6-SU-2-A-24(M)-A, 6-SU-2-A-24(M)-A.1, 6-SU-2-A-24(M)-A.3, 6-SU-2-A-30(M)-B, 6-SU-2-A-30(M)-B.1, 6-SU-2-A-31(M)-A, 6-SU-2-A-31(M)-A.1, 6-SU-2-A-31(M)-A.2, 6-SU-2-A-33(M)-A, 6-SU-2-A-33(M)-A.3, 6-SU-2-A-34(M)-A, 6-SU-2-A-34(M)-A.1, 6-SU-2-A-34(M)-A.2, 6-SU-2-A-34(M)-A.3, 6-SU-2-A-34(M)-B, 6-SU-2-A-34(M)-B.2, 6-SU-2-A-34(M)-B.3, 6-SU-2-A-35(M)-A, 6-SU-2-A-35(M)-A.2, 6-SU-2-A-35(M)-A.3, 6-SU-2-A-45(M)-A, 6-SU-2-A-45(M)-A.1, 6-SU-2-A-45(M)-A.2, 6-SU-2-A-45(M)-A.3, 6-SU-2-A-48(M)-A, 6-SU-2-A-48(M)-A.1, 6-SU-2-A-48(M)-A.2, 6-SU-2-A-48(M)-A.3.3, 6-SU-2-A-48(M)-B.1, 6-SU-2-A-48(M)-B.2, 6-SU-2-A-48(M)-B.3, 6-SU-2-A-48FB(M)-A, 6-SU-2-A-48FB(M)-A.1, 6-SU-2-A-48FB(M)-A.1.1, 6-SU-2-A-48FB(M)-A.1.2, 6-SU-2-A-55(M)-A, 6-SU-2-A-55(M)-A.2, 6-SU-2-A-55(M)-A.3, 6-SU-2-A-55(M)-B.2, 6-SU-2-A-55(M)-B.2.2, 6-SU-2-A-55(M)-B.2.3, 6-SU-2-A-56(M)-B.1, 6-SU-2-A-56(M)-B.2, 6-SU-2-A-56(M)-B.3, 6-SU-2-A-58(M)-A, 6-SU-2-A-58(M)-A.1, 6-SU-2-A-58(M)-A.3, 6-SU-2-A-58(M)-B.1, 6-SU-2-A-58(M)-B.2, 6-SU-2-A-58(M)-B.3, 6-SU-2-A-59(M)-A, 6-SU-2-A-59(M)-A.1, 6-SU-2-A-59(M)-A.3, 6-SU-2-A-59(M)-B.2, 6-SU-2-A-59(M)-B.3, 6-SU-2-A-60(M)-A, 6-SU-2-A-60(M)-A.1, 6-SU-2-A-60(M)-B.2, 6-SU-2-A-60(M)-B.3, 6-SU-2-A-61(M)-A.1, 6-SU-2-A-61(M)-A.1.1, 6-SU-2-A-61(M)-A.3, 6-SU-2-A-68(M)-A, 6-SU-2-A-68(M)-A.1, 6-SU-2-A-68(M)-A.2, 6-SU-2-A-68(M)-A.3, 6-SU-2-A-68(M)-B.1.1, 6-SU-2-A-68(M)-B.1.2, 6-SU-2-A-68(M)-B.2.3, 6-SU-2-A-68(M)-B.3, 6-SU-2-A-69(M)-A, 6-SU-2-A-69(M)-A.1, 6-SU-2-A-69(M)-A.2, 6-SU-2-A-69(M)-A.3, 6-SU-2-A-71(M)-A, 6-SU-2-A-71(M)-A.2, 6-SU-2-A-71(M)-B.1, 6-SU-2-A-71(M)-B.3, 6-SU-2-A-75(M)-A, 6-SU-2-A-75(M)-A.1, 6-SU-2-A-75(M)-A.2, 6-SU-2-A-76(M)-A.2.1, 6-SU-2-A-76(M)-A.3, 6-SU-2-A-76(M)-A.3.2, 6-SU-2-A-76(M)-B, 6-SU-2-A-76(M)-B.1, 6-SU-2-A-76(M)-B.3.2, 6-SU-2-A-79(M)-A, 6-SU-2-A-79(M)-A.1.1, 6-SU-2-A-79(M)-A.1.2, 6-SU-2-A-79(M)-A.1.3, 6-SU-2-A-80(M)-A.1.1, 6-SU-2-A-80(M)-A.1.2, 6-SU-2-A-82(M)-B, 6-SU-2-A-82(M)-B.1, 6-SU-2-A-82(M)-B.2, 6-SU-2-A-82(M)-B.3, 6-SU-2-A-95(M)-A, 6-SU-2-A-95(M)-A.1, 6-SU-2-A-95(M)-A.2, 6-SU-2-A-95(M)-B.1, 6-SU-2-A-95(M)-B.2, 6-SU-2-A-95(M)-B.2.3, 6-SU-2-A-95(M)-B.3, 6-SU-2-A-97(M)-A, 6-SU-2-A-97(M)-A.1, 6-SU-2-A-97(M)-B.1, 6-SU-2-A-97(M)-B.2, 6-SU-2-A-98(M)-A.3, 6-SU-2-A-98(M)-B, 6-SU-2-A-98(M)-B.2, 6-SU-2-A-98(M)-B.3, 6-SU-2-B-18(M)-A, 6-SU-2-B-18(M)-A.1, 6-SU-2-B-18(M)-A.2, 6-SU-2-B-18(M)-A.3, 6-SU-2-B-18(M)-B, 6-SU-2-B-18(M)-B.1,

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38(M)-A.5, 7-L-4-B-44(M)-A, 7-L-4-B-44(M)-A.1, 7-L-4-B-44(M)-A.2, 7-L-4-B-44(M)-B.1, 7-L-4-B-44(M)-B.2, 7-L-4-B-44(M)-B.3, 7-L-4-B-44(M)-C, 7-L-4-B-44(M)-C.2, 7-L-4-B-44(M)-C.3, 7-L-4-B-45(M)-A.1, 7-L-4-B-45(M)-A.2, 7-L-4-B-45(M)-A.3.2, 7-L-4-B-45(M)-B, 7-L-4-B-45(M)-B.1, 7-L-4-B-45(M)-B.2, 7-L-4-B-48(M)-A.1, 7-L-4-B-48(M)-A.2, 7-L-4-B-48(M)-A.3, 7-L-4-B-54(M)-A, 7-L-4-B-54(M)-A.1, 7-L-4-B-54(M)-A.2, 7-L-4-B-54(M)-A.3, 7-L-4-B-55(M)-A, 7-L-4-B-55(M)-A.1, 7-L-4-B-55(M)-A.2, 7-L-4-B-55(M)-A.3, 7-L-4-B-55(M)-B, 7-L-4-B-55(M)-B.1, 7-L-4-B-55(M)-B.2, 7-L-4-B-57(M)-A.1, 7-L-4-B-57(M)-A.2, 7-L-4-B-57(M)-A.3, 7-L-4-C-33(M)-A, 7-L-4-C-33(M)-A.3, 7-L-4-C-33(M)-B, 7-L-4-C-33(M)-B.1, 7-L-4-C-33(M)-B.2, 7-L-4-C-33(M)-C, 7-L-4-C-33(M)-C.1, 7-L-4-C-33(M)-C.2, 7-L-4-C-33(M)-C.3, 7-L-4-C-37(BL)-A.1, 7-L-4-C-37(BL)-A.2, 7-L-4-C-37(BL)-A.3, 7-L-4-C-37(BL)-B.1, 7-L-4-C-37(BL)-B.2, 7-L-4-C-37(M)-A, 7-L-4-C-37(M)-A.1, 7-L-4-C-37(M)-A.2, 7-L-4-C-37(M)-B.1, 7-L-4-C-37(M)-B.2, 7-L-4-C-37(M)-B.3, 7-L-4-C-47(M)-A.1, 7-L-4-C-47(M)-A.3, 7-L-4-C-47(M)-A.4, 7-L-4-C-47(M)-B, 7-L-4-C-47(M)-B.1, 7-L-4-C-47(M)-B.3, 7-L-4-C-50(M)-A, 7-L-4-C-50(M)-A.1, 7-L-4-C-50(M)-A.2, 7-L-4-C-50(M)-A.3, 7-L-4-C-50(M)-B, 7-L-4-C-50(M)-B.1, 7-L-4-C-50(M)-B.2, 7-L-4-C-50(M)-B.3, 7-L-4-C-68(M)-A, 7-L-4-C-68(M)-A.1, 7-L-4-C-68(M)-A.2, 7-L-4-C-68(M)-B, 7-L-4-C-68(M)-B.1, 7-L-4-C-68(M)-B.2, 7-L-4-C-68(M)-B.3, 7-L-4-C-72(M)-A.1, 7-L-4-C-72(M)-A.2, 7-L-4-C-72(M)-A.3, 7-L-4-C-72(M)-C, 7-L-4-C-72(M)-C.1, 7-L-4-C-72(M)-C.1.1, 7-L-4-C-72(M)-C.1.2, 7-LS-4-A-25(M)-A, 7-LS-4-A-25(M)-A.2, 7-LS-4-A-25(M)-A.3, 7-LS-4-A-25(M)-B, 7-LS-4-A-25(M)-B.1, 7-LS-4-A-25(M)-B.2, 7-LS-4-A-25(M)-B.3, 7-LS-4-A-35(M)-A, 7-LS-4-A-35(M)-A.1, 7-LS-4-A-35(M)-A.2, 7-LS-4-A-35(M)-B, 7-LS-4-A-35(M)-B.1, 7-LS-4-A-35(M)-B.2, 7-LS-4-A-35(M)-B.3, 7-LS-4-A-37(M)-A, 7-LS-4-A-37(M)-A.1, 7-LS-4-A-37(M)-A.2, 7-LS-4-A-37(M)-A.3, 7-LS-4-A-37(M)-B, 7-LS-4-A-37(M)-B.1, 7-LS-4-A-37(M)-B.2, 7-LS-4-A-37(M)-B.3, 7-LS-4-A-44(M)-A, 7-LS-4-A-44(M)-A.1, 7-LS-4-A-44(M)-A.2, 7-LS-4-A-44(M)-A.3, 7-LS-4-A-44(M)-B, 7-LS-4-A-44(M)-B.1, 7-LS-4-A-44(M)-B.2, 7-LS-4-A-44(M)-B.3, 7-LS-4-A-65(M)-A, 7-LS-4-A-65(M)-A.1, 7-LS-4-A-65(M)-A.2, 7-LS-4-A-65(M)-A.3, 7-LS-4-A-75(W)-A, 7-LS-4-A-75(W)-A.2, 7-LS-4-A-75(W)-A.3, 7-LS-4-A-75(W)-B, 7-LS-4-A-75(W)-B.1, 7-LS-4-A-75(W)-B.2, 7-LS-4-A-75(W)-B.3, 7-LS-4-A-77(M)-A, 7-LS-4-A-77(M)-A.1, 7-LS-4-A-77(M)-A.2, 7-LS-4-A-77(M)-A.3, 7-LS-4-A-78(M)-A, 7-LS-4-A-78(M)-A.2, 7-LS-4-A-78(M)-A.3, 7-LS-4-A-78(M)-B, 7-LS-4-A-78(M)-B.1, 7-LS-4-A-78(M)-B.2, 7-LS-4-A-78(M)-B.3, 7-LS-4-B-14(M)-B, 7-LS-4-B-14(M)-B.1, 7-LS-4-B-14(M)-B.2, 7-LS-4-B-14(M)-B.3, 7-LS-4-B-15(M)-B, 7-LS-4-B-15(M)-B.1, 7-LS-4-B-15(M)-B.2, 7-LS-4-B-15(M)-B.2.1, 7-LS-4-B-15(M)-B.2.2, 7-LS-4-B-15(W)-B, 7-LS-4-B-15(W)-B.2, 7-LS-4-B-15(W)-B.3, 7-LS-4-B-27(M)-A, 7-LS-4-B-27(M)-A.1, 7-LS-4-B-27(M)-B, 7-LS-4-B-27(M)-B.1, 7-LS-4-B-27(M)-B.2, 7-LS-4-B-27(M)-B.3, 7-LS-4-B-36(M)-B, 7-LS-4-B-36(M)-B.1, 7-LS-4-B-36(M)-B.2, 7-LS-4-B-38(M)-B, 7-LS-4-B-38(M)-B.1, 7-LS-4-B-38(M)-B.2, 7-LS-4-B-38(M)-B.3, 7-LS-4-B-44(M)-A, 7-LS-4-B-44(M)-A.1, 7-LS-4-B-44(M)-A.2, 7-LS-4-B-44(M)-B, 7-LS-4-B-55(M)-B, 7-LS-4-B-55(M)-B.2, 7-LS-4-B-55(M)-B.3, 7-LS-4-B-61(M)-A, 7-LS-4-B-61(M)-A.1, 7-LS-4-B-61(M)-A.2, 7-LS-4-B-61(M)-A.3, 7-LS-4-B-80(M)-B, 7-LS-4-B-80(M)-B.1, 7-LS-4-B-80(M)-B.2, 7-LS-4-B-80(M)-B.3, 7-LS-4-B-86(M)-A, 7-LS-4-B-86(M)-A.2, 7-LS-4-B-86(M)-A.2.1, 7-LS-4-B-86(M)-A.2.2, 7-LS-4-B-86(M)-B, 7-LS-4-B-86(M)-B.1, 7-LS-4-B-86(M)-B.1.1, 7-LS-4-B-88(M)-A, 7-LS-4-B-88(M)-A.2, 7-LS-4-B-88(M)-A.4, 7-LS-4-B-88(M)-A.5, 7-LS-4-B-94(M)-A, 7-LS-4-B-94(M)-A.2, 7-LS-4-B-94(M)-B.1, 7-LS-4-B-94(M)-B.1.1, 7-LS-4-B-94(M)-B.1.2, 7-LS-4-C-15(M)-A, 7-LS-4-C-15(M)-A.2, 7-LS-4-C-15(M)-A.3, 7-LS-4-C-3(M)-A, 7-LS-4-C-3(M)-A.2, 7-LS-4-C-3(M)-A.3, 7-LS-4-C-3(M)-B, 7-LS-4-C-3(M)-B.1, 7-LS-4-C-3(M)-B.3, 7-LS-4-C-37(M)-A, 7-LS-4-C-37(M)-A.1, 7-LS-4-C-37(M)-A.2, 7-LS-4-C-37(M)-A.3, 7-LS-4-C-4(M)-A, 7-LS-4-C-4(M)-A.2, 7-LS-4-C-4(M)-B, 7-LS-4-C-4(M)-B.1, 7-LS-4-C-4(M)-B.3, 7-LS-4-C-5(M)-A, 7-LS-4-C-5(M)-A.1, 7-LS-4-C-5(M)-A.2, 7-LS-4-C-5(M)-A.3, 7-LS-4-C-64(M)-B, 7-LS-4-C-64(M)-B.1, 7-LS-4-C-64(M)-B.2, 7-LS-4-C-64(M)-B.3, 7-LS-4-C-64(M)-C, 7-LS-4-C-64(M)-C.2, 7-LS-4-C-64(M)-C.3, 7-LS-4-C-65(M)-A, 7-LS-4-C-65(M)-A.1, 7-LS-4-C-65(M)-A.3, 7-LS-4-D-100(M)-A, 7-LS-4-D-100(M)-A.1, 7-LS-4-D-100(M)-A.2, 7-LS-4-D-100(M)-B, 7-LS-4-D-100(M)-B.3, 7-LS-4-D-100(M)-B.3.1, 7-LS-4-D-100(M)-B.3.2, 7-LS-4-D-38(M)-A, 7-LS-4-D-38(M)-A.1, 7-LS-4-D-38(M)-A.2, 7-LS-4-D-38(M)-A.3, 7-LS-4-D-55(M)-A, 7-LS-4-D-55(M)-A.1, 7-LS-4-D-55(M)-A.2, 7-LS-4-D-55(M)-A.3, 7-LS-4-D-57(M)-B, 7-LS-4-D-57(M)-B.1, 7-LS-4-D-57(M)-B.2, 7-LS-4-D-57(M)-B.3, 7-LS-4-D-66(M)-A, 7-LS-4-D-66(M)-A.1, 7-LS-4-D-66(M)-A.2, 7-LS-4-D-66(M)-A.3, 7-LS-4-D-67(M)-A, 7-LS-4-D-67(M)-A.1, 7-LS-4-D-67(M)-A.2, 7-LS-4-D-

67(M)-A.3, 7-LS-4-D-71(M)-A, 7-LS-4-D-71(M)-A.1, 7-LS-4-D-71(M)-A.3, 7-S-4-A-30-A, 7-S-4-A-30-A.1, 7-S-4-A-30-A.2, 7-S-4-A-30-B, 7-S-4-A-30-B.1, 7-S-4-A-30-B.2, 7-S-4-A-41(M)-A, 7-S-4-A-41(M)-A.1.2, 7-S-4-A-41(M)-A.1.3, 7-S-4-A-41(M)-A.2.1, 7-S-4-A-41(M)-A.2.2, 7-S-4-A-41(M)-A.2.3, 7-S-4-A-41(M)-B, 7-S-4-A-41(M)-B.3, 7-S-4-A-50(M)-B, 7-S-4-A-50(M)-B.1, 7-S-4-A-50(M)-B.2, 7-S-4-A-50(M)-B.3, 7-S-4-B-53(M)-B, 7-S-4-B-53(M)-B.3, 7-S-4-D-47(M)-B, 7-S-4-D-51(M)-A, 7-S-4-D-51(M)-A.1, 7-S-4-D-51(M)-A.2, 7-S-4-D-52(M)-A, 7-S-4-D-52(M)-A.1, 7-S-4-D-52(M)-A.2, 7-S-4-D-52(M)-A.3, 7-S-4-D-65(M)-A, 7-S-4-D-65(M)-A.1, 7-S-4-D-65(M)-A.2, 7-S-4-D-65(M)-A.3, 7-S-4-D-68(M)-A, 7-S-4-D-68(M)-A.1, 7-S-4-D-68(M)-A.2, 7-S-4-D-68(W)-A, 7-S-4-D-68(W)-A.1, 7-S-4-D-68(W)-A.1.1, 7-S-4-D-68(W)-A.1.2, 7-S-4-D-68(W)-A.1.3, 7-S-4-D-69(M)-A, 7-S-4-D-69(M)-A.1, 7-S-4-D-69(M)-A.2, 7-S-4-D-69(M)-A.2.1, 7-S-4-D-69(M)-A.2.2, 7-S-4-D-69(M)-B, 7-S-4-D-69(M)-B.1, 7-S-4-D-69(M)-B.2, 7-S-4-D-70(M)-A, 7-S-4-D-70(M)-A.1, 7-S-4-D-70(M)-A.1.1, 7-S-4-D-70(M)-A.1.2, 7-S-4-D-70(M)-A.2, 7-SU-3-A-11(M)-B.1, 7-SU-3-A-11(M)-B.2, 7-SU-3-A-11(M)-B.3, 7-SU-3-A-24(M)-C.1, 7-SU-3-A-24(M)-C.2, 7-SU-3-A-29(M)-A, 7-SU-3-A-29(M)-A.1, 7-SU-3-A-29(M)-A.2, 7-SU-3-A-31(M)-B.1, 7-SU-3-A-31(M)-B.2, 7-SU-3-A-31(M)-B.3, 7-SU-3-A-33(M)-A.1, 7-SU-3-A-33(M)-A.2, 7-SU-3-A-33(M)-C, 7-SU-3-A-33(M)-C.2, 7-SU-3-A-33(M)-C.3, 7-SU-3-A-34(M)-A.2, 7-SU-3-A-34(M)-A.3, 7-SU-3-A-35(M)-B.1, 7-SU-3-A-35(M)-B.3, 7-SU-3-A-45(M)-A, 7-SU-3-A-45(M)-A.1, 7-SU-3-A-45(M)-A.2, 7-SU-3-A-45(M)-A.3, 7-SU-3-A-58(M)-A, 7-SU-3-A-58(M)-A.1, 7-SU-3-A-58(M)-A.3, 7-SU-3-A-58(M)-B, 7-SU-3-A-58(M)-B.1, 7-SU-3-A-58(M)-B.2, 7-SU-3-A-58(M)-B.3, 7-SU-3-A-59(M)-B.1, 7-SU-3-A-59(M)-B.2, 7-SU-3-A-59(M)-B.3, 7-SU-3-A-63(M)-A.1, 7-SU-3-A-63(M)-A.2, 7-SU-3-A-63(M)-A.3, 7-SU-3-A-63(M)-B, 7-SU-3-A-63(M)-B.1, 7-SU-3-A-91(M)-B, 7-SU-3-A-91(M)-B.1, 7-SU-3-A-97(M)-B, 7-SU-3-A-97(M)-B.1, 7-SU-3-A-97(M)-B.2, 7-SU-3-A-97FB(M)-A.1, 7-SU-3-A-97FB(M)-A.2, 7-SU-3-A-99(M)-C.1, 7-SU-3-A-99(M)-C.2, 7-SU-3-B-13(M)-A, 7-SU-3-B-13(M)-A.1, 7-SU-3-B-13(M)-A.2, 7-SU-3-B-13(M)-B.1, 7-SU-3-B-13(M)-B.2, 7-SU-3-B-14(M)-A, 7-SU-3-B-14(M)-A.1, 7-SU-3-B-14(M)-A.2, 7-SU-3-B-14(M)-B.1, 7-SU-3-B-14(M)-B.2, 7-SU-3-B-14(M)-B.3.2, 7-SU-3-B-17(M)-A, 7-SU-3-B-17(M)-A.1, 7-SU-3-B-17(M)-A.2, 7-SU-3-B-17(M)-B, 7-SU-3-B-17(M)-B.1, 7-SU-3-B-17(M)-B.2, 7-SU-3-B-17(M)-B.3, 7-SU-3-B-21(M)-A.1, 7-SU-3-B-21(M)-A.2, 7-SU-3-B-21(M)-A.3, 7-SU-3-B-21(M)-B, 7-SU-3-B-21(M)-B.2, 7-SU-3-B-21(M)-B.3, 7-SU-3-B-28(M)-A, 7-SU-3-B-28(M)-A.1, 7-SU-3-B-28(M)-A.2, 7-SU-3-B-28(M)-B, 7-SU-3-B-28(M)-B.2, 7-SU-3-B-28(M)-B.3, 7-SU-3-B-35(M)-A, 7-SU-3-B-35(M)-A.1, 7-SU-3-B-35(M)-A.3, 7-SU-3-B-35(M)-B, 7-SU-3-B-35(M)-B.1, 7-SU-3-B-35(M)-B.2, 7-SU-3-B-36(M)-A, 7-SU-3-B-36(M)-A.1, 7-SU-3-B-36(M)-A.3, 7-SU-3-B-38(M)-A, 7-SU-3-B-38(M)-A.1, 7-SU-3-B-38(M)-A.2, 7-SU-3-B-38(M)-A.3, 7-SU-3-B-38(M)-B, 7-SU-3-B-38(M)-B.1, 7-SU-3-B-41(M)-A, 7-SU-3-B-41(M)-A.1, 7-SU-3-B-41(M)-A.2, 7-SU-3-B-41(M)-A.3, 7-SU-3-B-41(M)-B, 7-SU-3-B-41(M)-B.1, 7-SU-3-B-41(M)-B.2, 7-SU-3-B-41(M)-B.3, 7-SU-3-B-42(M)-A, 7-SU-3-B-42(M)-A.1, 7-SU-3-B-42(M)-A.2, 7-SU-3-B-42(M)-A.3, 7-SU-3-B-42(M)-B, 7-SU-3-B-42(M)-B.1, 7-SU-3-B-42(M)-B.2, 7-SU-3-B-42(M)-B.3, 7-SU-3-B-56(M)-A, 7-SU-3-B-56(M)-A.1, 7-SU-3-B-56(M)-A.2, 7-SU-3-B-56(M)-A.3, 7-SU-3-B-8(M)-A, 7-SU-3-B-8(M)-A.1, 7-SU-3-B-8(M)-A.2, 7-SU-3-B-8(M)-A.3, 7-SU-3-B-89(M)-B.1, 7-SU-3-B-89(M)-B.2.1, 7-SU-3-B-BLANK, 7-SU-3-B-BLANK.1, 7-SU-3-B-BLANK.2, 7-SU-3-C-25(M)-A.1, 7-SU-3-C-25(M)-A.2, 7-SU-3-C-25(M)-B.1, 7-SU-3-C-25(M)-B.2, 7-SU-3-C-25(M)-B.3, 7-SU-3-C-25(W)-A.2, 7-SU-3-C-25(W)-A.3, 7-SU-3-C-26(M)-A, 7-SU-3-C-26(M)-A.1, 7-SU-3-C-26(M)-A.2, 7-SU-3-C-26(M)-A.3, 7-SU-3-C-26(M)-B, 7-SU-3-C-26(M)-B.1, 7-SU-3-C-26(M)-B.2, 7-SU-3-C-26(M)-B.3, 7-SU-3-C-35(M)-B.1, 7-SU-3-C-35(M)-B.2, 7-SU-3-C-35(M)-B.3, 7-SU-3-C-49(FB)-A.1, 7-SU-3-C-49(FB)-A.2, 7-SU-3-C-49(FB)-A.3, 7-SU-3-C-49(FB)-B, 7-SU-3-C-49(FB)-B.1, 7-SU-3-C-49(FB)-B.2, 7-SU-3-C-49(M)-A.1, 7-SU-3-C-49(M)-A.2, 7-SU-3-C-49(M)-B.1, 7-SU-3-C-49(M)-B.2, 7-SU-3-C-49(M)-B.3, 7-SU-3-C-77(M)-B.1, 7-SU-3-C-77(M)-B.2, 7-SU-3-C-77(M)-B.3, 7-SU-3-D-10(M)-A.1, 7-SU-3-D-10(M)-A.2, 7-SU-3-D-15(M)-A, 7-SU-3-D-15(M)-A.2, 7-SU-3-D-46(M)-A, 7-SU-3-D-46(M)-A.1.1, 7-SU-3-D-46(M)-A.1.2, 7-SU-3-D-46(M)-A.2, 7-SU-3-D-46(M)-B, 7-SU-3-D-46(M)-B.1, 7-SU-3-D-46(M)-B.2, 7-SU-3-D-46(M)-B.3, 7-SU-3-D-72(M)-A, 7-SU-3-D-72(M)-A.2, 7-SU-3-D-72(M)-B, 7-SU-3-D-72(M)-B.1, 7-SU-3-D-72(M)-B.4, 7-SU-3-D-72(M)-B.5, 7-SU-3-D-73(M)-B, 7-SU-3-D-73(M)-B.1, 7-SU-3-D-89(M)-A.1, 7-SU-3-D-89(M)-A.2, 7-SU-3-D-9(M)-A, 7-SU-3-D-9(M)-A.1, 7-SU-3-D-9(M)-A.2, 8-ND-6-A-2(M)-B, 8-ND-6-A-2(M)-B.2, 8-ND-

6-A-2(M)-B.3, 8-ND-6-A-56(M)-A, 8-ND-6-A-56(M)-A.1, 8-ND-6-A-56(M)-A.2, 8-ND-6-A-56(M)-A.3, 8-ND-6-A-67(M)-C, 8-ND-6-A-67(M)-C.1, 8-ND-6-A-67(M)-C.2, 8-ND-6-A-67(M)-C.3, 8-ND-6-A-68(M)-A, 8-ND-6-A-68(M)-A.1, 8-ND-6-A-68(M)-A.2, 8-ND-6-A-87(M)-A, 8-ND-6-A-87(M)-A.1, 8-ND-6-A-87(M)-A.2, 8-ND-6-A-87(M)-A.3, 8-ND-6-A-90(M)-A, 8-ND-6-A-90(M)-A.2, 8-ND-6-A-90(M)-A.3, AA-C0196-1, AA-C0365, AA-GA 4, AA-GA 4 Field.1, AA-GA 4 Field.2, AA-GA Pot.1, AA-GA Pot.2, AA-GA(1) Pot.1, AA-GA(1) Pot.2, AA-Ganoderma(18/3..), AA-Ganoderma(18/3..).1, AA-Ganoderma(18/3..).2, AA-Sttm 1, AA-Sttm 2 Field, AA-Sttm 2 Field.2, AA-Sttm 3, AA-T201-A, AA-T201-A.1, AA-T201-B, AA-T201-B.1, BS-FB16-A, BS-FB16-A.2, BS-FB16-A.2.1, BS-FB16-B, BS-FB16-B.1, BS-FB16-B.1.1, BS-FB16-B.1.2, BS-FB16-B.1.3, BS-FB4-A, BS-FB4-A.1, E8831-A, E8831-A.1, E8832-A, E8832-A.1, E8832-B.1, E8832-B.1.1, E8832-B.1.2, E8832-B.1.3, E8842-C, E8842-C.1

### *Ganoderma subresinosum*

Three collections (7, 21 and 57 = T159, T162, and T153) had 100% similarity to G. subresinosum. Two collections each had a single polymorphism. Five cultures had >99% sequence similarity to G. subresinosum - FB17A1, B86MB1-1, B86MB1-2, B94MA1-1 and B94MA1-2. 080226-12, 080228-01 and 080327-16 matched this group. 090331-09 and 090331-11 have 99.5% similarity to AY627583, Ganoderma subresinosum.

3-BS-17(M, MAT)-A.2, 3-BS-17(M, MAT)-A.2.1, 3-BS-17(M, MAT)-A.2.2, 4-D-2-B-86(M)-A.1, 4-D-2-B-86(M)-A.1.1, 4-D-2-B-86(M)-A.1.2, 4-D-2-B-86(M)-C.1, 4-D-2-B-86(M)-C.1.2, 4-D-2-B-86(M)-C.1.2.1, 4-D-2-B-86(M)-C.1.2.2, 4-D-2-B-94(M)-A1.1, 4-D-2-B-94(M)-A1.1.1, 4-D-2-B-94(M)-A1.1.2, BS-FB17-A, BS-FB17-A.1, BS-FB17-A.1.2

### *Ganoderma weberianum*

T139 (20) had highest similarity (99%) to AY569451, Ganoderma weberianum.

### *Ganoderma sp. M1B*

Ten cultures matched this group - T170A, T170B, E8851A, E8851B, T171B, E8857C, E8853, E8861C, A50MA and A50MB.

090324-22, 090324-24, 090324-26 and 090324-29 are 99% similar to AF255144 (G. sp.) and to EU239389 (1 of the clones from E7101). Designated Ganoderma sp M1B. 090323-14 is close to this group.

### *Ganoderma sp. M3*

A partial sequence only was obtained from T88 (6), but this fragment was identical to T165 (24), for which only ITS1 and 5.8S rDNA sequence was obtained. This was 99% similar to AF255144, Ganoderma sp. Designated Ganoderma sp. M3.

### *Ganoderma sp. M4*

The 5.8S region of T205 (53) had 98-99% similarity to a range of Ganoderma spp., with only up to 90% similarity from fragments of the ITS1 and ITS2 regions. Designated Ganoderma sp. M4.

### *Ganoderma sp. M6*

Apart from a ~40bp region in which the sequence of T155 was not reliably determined T155 (9) and T160 (49) differed at 4 nucleotides. These two collections were most similar (99%) to Ganoderma sp. (AF255122) and G. gibbosum. Designated Ganoderma sp. M6. T160A1, T160A2, 5T160A2 all matched this group. As did 080318-01

4-S-2-C-36(FB)-B.1, 4-S-2-C-36(FB)-B.1.1, 4-S-2-C-36(FB)-B.1.2

### *Ganoderma sp M8*

080506-22 (T15C) matched most closely (~ 95%) to EU735845, Ganoderma sp. Second best match was Coriolopsis caperata, but several other Ganodermas, including G. philippii were about 90% similar.

Designated Ganoderma sp M8.

T15-C, T15-C.1, T15-C.2

### *Ganoderma sp. M9*

E8812-A.1, E8823-A, E8823-A.2, E8861-C, E8861-C.2, MHP-T160-A.2, MHP-T160-A.2.1, MHP-T160-A.2.1.1, MHP-T160-A.2.1.2, AA-T205-B, AA-T205-B.1, AA-T205-B.1.1, AA-T205-B.1.2

### *Gymnopilus sp. 1*

E8831B1 had >99% sequence similarity to several Gymnopilus spp. including G. lepidotus, G. dilepis, G. cerasinus, G. medius, G. norfolkensis and G. subearlei. 080409-02 matches this group but has 4 nt different to E8831B1.

090325-25 and 090331-19 have ~98.5% similarity to AY280979, Gymnopilus purpureosquamulosus, AY280992 G. luteofolius and AY280974 G. aeruginosus – designated Gymnopilus sp. 1.

### *Gymnopilus sp. 2*

090331-04 has ~99% similarity to AY280980 Gymnopilus dilepis, AY280991 G. lepidotus and EU401709 G. ochraceus – designated Gymnopilus sp. 2. Compare with above isolates.

### *Gymnopilus sp. 3*

4-SU-1-D-89(R2)-B.2, E8831-B, E8831-B.1

### *Hypochnicium sp. 1*

080318-09, 080327-15 and 080506-08 have almost identical sequences (080327-15 varies a bit at the end, but this is likely to be poor sequencing) that are 98% similar to DQ658163 H. cystidiatum, with lower similarity to other Hypochnicium and Fomitopsis spp. Designated Hypochnicium sp. 1.

2-S-1-A-54-B.2, E8824-C, E8824-C.1, 4-D-2-Z-3-B.1.1, 4-D-2-Z-3-B.1.2, 4-D-2-Z-3-B.1.3

### *Inonotus pachyphloeus*

Three collections, T61, T157 and T158 (16, 18 and 39), had highest sequence similarity to Inonotus pachyphloeus, but these three are not identical. T61 produced an excellent quality full-length sequence in both directions, that is >99% similar to I. pachyphloeus. T157 produced a full-length sequence after joining the F and R sequences, T158 has only an ITS1 sequence. PPA, Mal-15, E8809A1 and C105MA also fall into this group. I have fiddled with alignments from all these sequences and apart from a highly variable region in the middle of ITS1, they are very similar. Several are incomplete and need sequencing from the other direction. 080708-02 and 080708-06 fit into this group, but sequencing is very poor and needs to be repeated to determine closest affinities.

090323-34 is closest to Inonotus pachyphloeus, with Phellinus noxius the next best match.

### *Inonotus sp. 1*

4-D-2-C-105(M)-A, 4-D-2-C-105(M)-A.2, 4-D-2-C-105(M)-A.2.1, 4-D-2-C-105(M)-A.2.2

### *Aff. Irpex sp. 1*

Three cultures had highest similarity (93%) to Irpex lacteus and Ceriporia lacerata - E8815B, E8864A and E8864C. E8815B differed from E8864A and E8864C at 3 nts. The read for E8815B was interrupted near the beginning of ITS1, but as it was sequenced with ITS4, most of the sequence is readable. E8815C was also sequenced (using ITS1-F) but only the first ~30 bp were readable - these seemed to match E8815B. Designated Aff. Irpex.

### *Lentinus sp. 1*

E8822C1 produced only ~100bp of readable sequence that was >98% similar to various Lentinus and Ganoderma spp. Needs repeat or reverse sequencing. 080506-07 and 080708-14 were identical apart from a few differences in the first 100bp that may have been due to poor quality sequencing. The best match for these two was Lentinus tigrinus (AF516519, and 5-6 others), ~94%. They appear different to E8822C1 but better sequencing is necessary to confirm this. Designated Lentinus sp. 1.

E8822-B, E8822-B.1

### *Neonothopanus aff. nambi*

B71MA had only a short readable sequence that was >99% similar to Neonothopanus nambi (Tricholomataceae). Designated Neonothopanus aff. nambi. 090324-36, 090324-39 and 090324-41 are also in this group.

### *Phanerochaete sp. 1*

T172A and E8854C had > 99% similarity to Phanerochaete sordida and P. australis. Also FB19AB5, 3A2RAPP from batch 2. 080327-21 has 99% sequence identity with this group.

L-T172-A, 4-Su-1-A-68(FB)-A.2, 4-Su-1-A-68(FB)-A.2.1, 4-Su-1-A-68(FB)-A.2.2

### *Phellinus noxius*

Some P. noxius sequences have recently been added to the database and all the above sequences need to be compared to them, though many still need cleaner sequence results. The following isolates all come up with the P. noxius sequences as best match and Inonotus pachyphloeus as next best:

090323-17, 090323-25, 090323-26, 090323-30, 090323-35, 090323-38, 090323-41, 090331-03, 090331-21, 090331-30

### *Phellinus sp. 1*

E8809-A, E8809-A.1

### *Phlebia sp. 1*

Four isolates, E8898A, 12T175B1, FB1A2 and 6T200A2 had 91-93% sequence similarity to various Phlebia spp. 12T175B1 and FB1A2 differ by 1 bp and are closest to P. acanthocystis and P. subochracea designated Phlebia sp. 1,

4-LS-2-B-38(M)-B.1, 4-LS-2-B-38(M)-B.1.1, 4-LS-2-B-38(M)-B.1.2, E8898-A.1, L-T173-A, L-T173-A.2, L-T173-A.2.1, L-T173-A.2.2, BS-FB1-A, BS-FB1-A.2, BS-FB1-A.2.3, L-T175-B, L-T175-B.1, L-T175-B.1.1, L-T175-B.1.2, L-T175-B.1.3, MHP-T200-A.2

### *Phlebia sp. 2*

E8898A is closest to P. brevispora designated Phlebia sp. 2,

### *Phlebia sp. 3*

and 6T200A2 to P. chrysocreas designated Phlebia sp. 3. Four cultures are closest to Phlebia spp., 080327-19, 080505-05, 080505-18 and 080506-24. 080327-19 and 080505-05 are almost identical to 12T175B1, 080505-18 matches 6T200A2 and 080506-24 matches E8898A.

### *Phlebiopsis sp. 1*

090325-02, 090325-03, 090325-06, 090325-07, 090325-08, 090325-10, 090325-11, 090325-17, 090325-24, 090325-26 and 090325-27 are all 95% similar to EU118662 (Phlebiopsis flavidoalba) and EF174437 (P. gigantea) plus about 16 other P. gigantea sequences. Designated Phlebiopsis sp. 1.

### *Pycnoporus sp. 1*

Four isolates were >99% similar to Pycnoporus sanguineus and P. cinnabarinus, and 98% similar to P. coccineus. E8892A, D15MA, D15MB, FB20A2. Designated Pycnoporus sp. 1.

BS-FB20-A, BS-FB20-A.2.1, BS-FB20-A.2.2, BS-FB20-A.2.3

### *Trametes sp. 1*

T19A1 was 98% similar to Trametes elegans. E8887 (culture) was 93% similar to Trametes hirsuta and 92% similar to T. maxima. Four isolates matched Trametes spp. 080318-06 and 080409-04 were very close to T19A1, designated Trametes group 1

090325-14, 090325-15, 090331-12 and 090331-13 were 98% similar to EU661879 (Trametes elegans) and AY684178 (T. palisotii), both = Lenzites elegans. Approx. 95% similarity to assorted Pycnoporus spp. Compare with Trametes group 1, above.

4-LS-2-A-55(FBA)-A.1, 4-LS-2-A-55(FBA)-A.1.1, 4-LS-2-A-55(FBA)-A.1.2, 4-LS-2-A-55(FBW)-A.2

### *Trametes sp. 2*

while 080409-11 was almost identical to E8887, Trametes group 2 .

4-SU-1-A-59(M)-A.2, 4-SU-1-A-59(M)-A.2.1, 4-SU-1-A-59(M)-A.2.2, E8872-A, E8872-A.2

### *Trametes sp. 3*

080506-19 (E8872A2) has similarities to both groups but is also different to both so is designated Trametes group 3. The sequence from 080318-07 was too poor and needs to be repeated, but the short fragment obtained indicates a Trametes affiliation.

4-DP74/75-(B,D)-A.2, 4-DP74/75-(B,D)-A.2.1, 4-DP74/75-(B,D)-A.2.2, 4-DP74/75-(B,D)-A.2.3

### *Trametes sp. 4*

T19-A, T19-A.1

### *Tinctoporellus sp. 1*

090325-01, 090325-04, 090325-09, 090325-13, 090325-18, 090325-19, 090325-20 and 090325-21 are all from Luci's site 11 (Block 5A). There is a low level <1% of sequence variation and these are 99% similar to AY216475 (Marasmius cladophyllus), FJ711050 and FJ711051 (both Tinctoporellus epimiltinus). I suspect that the M. cladophyllus is misidentified and consider these isolates to be closely related to Tinctoporellis epimiltinus. FJ711051 is from CBS isolate 389.61, isolated from Liquidamber in Georgia, USA. There are only 2 species as yet described in the genus but there are no sequences for T. isabellinus available. Antrodia  is considered likely to be the closest relative.

### *Xerula sp 1*

080228-09 has ~90% sequence similarity to Xerula (Oudemansiella) radicata followed by several other species of this genus. Designated Xerula sp 1.

### *Xylaria sp. 1*

4-LS-2-B-72N(FB)-B.2, 4-LS-2-B-72N(FB)-B.2.2

### *Polyporaceae sp. 1*

Three isolates, 080226-22, 080327-01 and 080327-23 had identical sequences, with the best match being AY089736, Poria subvermispora (94%), followed closely by Veluticeps fimbriata and Diplomitoporus lindbladii. Designated Polyporaceae sp. 1.

3-D2MHP-6(M, BMAT)-A.2, 3-D2MHP-6(M, MAT)-B.2, 3-D2MHP-6(M, MAT)-B.2.2, 3-L-2-C-26(R, MAT)-A.1

### *Hypocreales sp. 1*

090324-37 is 93% similar to FJ554224, a Hypocreales sp.

### *Basidiomycete sp. 1*

080226-22 only found matches in the 5.8S region, to assorted basidiomycete species. Designated Basidiomycete sp. 2. Sequencing was poor quality and stopped before the end of the 5.8S region.

### *Basidiomycete sp. 2*

E8822-C, E8822-C.1, Arara BC, Arara BC 1 (ABC 1)

### *Basidiomycete sp. 3*

090325-12 has 90% similarity to AY593868 (Rigidoporus ulmarius). No other Rigidoporus sequences for comparison. Designated Basidiomycete sp. 3.