Heart rot and root rot in tropical *Acacia* plantations

Proceedings of a workshop held in Yogyakarta, Indonesia, 7–9 February 2006

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Foreword

Fast-growing hardwood plantations are increasingly important to the economies of many countries around the Pacific rim, including Australia, Indonesia and the Philippines. While the initial emphasis in most countries has been on the market for fibre, pulp and paper, there is now increasing interest in using the species for the production of solid-wood products.

Indonesia has been at the forefront of the domestication of Australian acacias, especially *Acacia mangium,* and there is now an impressive estate of over one million hectares of acacias in the country. This resource is progressively replacing natural forest as a source of fibre for Indonesia's large paper-producing industry. *Acacia mangium* and a number of other acacias produce good-quality timbers which are also well suited to the production of furniture and other higher-value products. A factor limiting this application is the occurrence of fungal heart rot in the central core of the stem at an early age. This defect does not seriously affect pulping properties if the crop is harvested early but it does significantly reduce the recovery of sawn wood and veneer from older logs grown for higher-value products. Identification of the factors causing the condition, and potential management strategies, have been the subjects of a major ACIAR project, 'Heart rots in plantation hardwoods in Indonesia and southeast Australia'.

Indonesian acacia plantations are also potentially threatened by root rot, another fungal disease. Root-rot diseases of *A. mangium* can cause crown dieback, reduced growth and tree death. In some areas, over 25 per cent of trees are affected. If not managed, this problem could seriously threaten Indonesia's acacia plantation industry. ACIAR is now initiating a project to investigate it.

A workshop with participants from Indonesia's Forest Research and Development Agency, forestry companies and local universities, and research agencies in Australia and New Zealand, was conducted in Yogyakarta in February 2006 to review the results of the heart-rot project and define a strategy for root-rot research.

These workshop proceedings provide valuable guidance on minimising the impacts of these two serious diseases in hardwood plantations.

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Preface

About 1000 species of *Acacia* are native to Australia and neighbouring Indonesia, Papua New Guinea (PNG) and some Pacific island nations. Several are now used in commercial plantations in Southeast Asia and are the basis of substantial plantation-based industries. Within this region, plantations of tropical acacias have been established in China, Indonesia, Malaysia, Papua New Guinea, the Philippines, Thailand, Vietnam, and on Melville Island in Australia. The total area planted is now approaching 2 million ha and the largest of these estates (about 1.2 million ha) is located in Indonesia where the major species planted is *Acacia mangium* Willd. The majority of acacia plantations are used as feedstock for kraft pulp mills. However, substantial quantities of wood are now finding their way into markets based on high-value solid timber.

Heart rot is caused by white-rot fungi which preferentially remove lignin. They are thought to enter trees through injuries and branch stubs. In Indonesia, this disease is a threat to solid-wood production and prevents the management of plantations for solid-wood values on some sites. Root rot is caused by basidiomycete fungi that can survive saprophytically on dead woody material or debris. The fungi attack the conducting tissues of living trees, rapidly causing tree death. The saprophytic nature of the fungi, together with the short rotations used by the pulpwood industry (6–7 years), have led to a rapid build-up of inoculum at some sites in Indonesia. *Acacia mangium* is particularly susceptible to root-rot disease on mineral soils in Sumatra and Kalimantan, where it is grown extensively.

This volume brings together the current state of knowledge about these diseases. The more recent advances in knowledge have been made possible by funding through ACIAR project FST/2000/123 that supported a substantial body of research on heart rot. A project extension further encouraged the development of a knowledge base on root rot in Indonesia that will now be carried forward in a new ACIAR project, FST/2003/048. The research has been undertaken to date in close cooperation with the Indonesian Ministry of Forestry through its Centre of Plantation Forestry and Development, Gadjah Mada University's Faculty of Forestry and PT. Musi Hutan Persada. The contributions of other Indonesian-based companies and the Forest Research Institute of Malaysia (FRIM) to the workshop 'Heart rot and root rot in tropical *Acacia* plantations: a synthesis of research progress' held in Yogyakarta on 7–9 February 2006, from which these proceedings are drawn, ensured a comprehensive coverage of the available information. The cooperating partners in Australia were the University of Tasmania and Ensis (the joint forces of CSIRO and Scion). The interest and expertise of the chapter authors is gratefully acknowledged.

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Summary

The total area planted to tropical acacias is now approaching 2 million ha. The largest of these estates (about 1.2 million ha) is in Indonesia where the major species planted is *Acacia mangium* Willd. *Acacia mangium* is generally grown as an exotic. It was first introduced into Malaysia in 1966 and into Indonesia in 1979 where it was used in South Sumatra as a fire break, for land rehabilitation and for reforestation of alang-alang grassland. Its excellent adaptability to degraded sites, rapid growth and wood properties quickly led to its commercial exploitation. In Indonesia, pulpwood production from *A. mangium* plantations is currently more than 9 million m^3/year, while the potential for solid-wood production is around 165,000 m^3/year. Thus, domestication of the species has progressed rapidly with extensive research on its genetics, silviculture and wood utilisation. These proceedings add a new focus by providing an insight into two diseases that threaten the sustainability of pulp and solid-wood industries based on tropical acacias.

The sustainability of planted forests in the tropics is threatened by their improper management, fire, and the illegal logging of native forests. Combating these threats is a challenge that should be met through effective forest management and a multi-stakeholder approach: the latter, in particular, is essential in the drive against illegal logging. Sustainable management therefore embraces the interests of the *planet* – conserving biodiversity and preventing environmental degradation; the *people* – providing opportunities for social development and poverty alleviation; and *profit* – ensuring a steady supply of renewable, high-quality, internationally cost-effective fibre. These interests are all threatened if monocultures are not managed to minimise the effects of diseases on sustained production across rotations.

Heart rot is a major disease problem in *A. mangium*, particularly where trees are grown for solid-wood products. Heart-rot fungi are wound parasites that enter through broken branches and branch stubs after self pruning, singling and manual pruning. The fungi attack cellulose and lignin, causing a typical white rot that is associated with changes in colour, texture and quality of the wood. These changes have been used to rapidly assess the incidence and severity of heart rot on harvested log-ends in the field. An assessment of two trials within different commercial growing areas in Indonesia (Riau and South Sumatra) showed that provenance could influence the incidence of heart rot. The basis for this observation remains unclear, as heart-rot incidence was not correlated with the content of polyphenolic wood extractives which are a known antifungal defence.

Root-rot diseases of *A. mangium* are associated with crown dieback, reduced growth and tree death. When first detected, infected trees or disease foci tend to be randomly distributed but then enlarge and may aggregate. The rate of disease progress appears to be positively correlated with current levels of root rot. Accurate surveys to investigate spread are required and should record above-ground symptoms, inspect the extent of root infection, and observe patterns of disease infection. It is recognised that such surveys are operationally laborious and costly. Hence, survey options based on remote sensing should be considered. Root-rot diseases are by no means isolated to acacias, and surveys to identify the disease organisms are being conducted in forest plantations of *Azadirachta excelsa, Tectona grandis* and *Khaya ivorensis* throughout Peninsular Malaysia.

Several significant root diseases that affect plantations in tropical Asia are caused by certain species of basidiomycetes. This group of fungi produces sexual spores on the outside of microscopic structures called basidia which are held on macroscopic fruit bodies such as mushrooms, toadstools, puff balls, earthstars, and bracket, shelf, crust and coral fungi. Basidiomycetes occupy many niches, e.g. decomposing litter, decaying wood and soil organic matter, in a variety of habitats that includes forests. Some species form beneficial mycorrhizal relationships with the roots of host trees, but others are pathogens of the foliage, stems or root systems of many tree species, including acacias. Basidiomycete species are traditionally identified by the form and microscopic structure of their fruitbodies, and by their appearance when isolated in laboratory culture. Important root diseases of trees in Indonesia are caused by the basidiomycete fungi *Rigidoporus microporus*, *Junghuhnia vincta*, *Phellinus noxius*, and certain species of *Ganoderma*. In Peninsular Malaysia, *Rigidoporus lignosus* and *P. noxius* are the two major root diseases of *A. excelsa, T. grandis* and *K. ivorensis*. Root rots are

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characterised according to the colour of the infected fungal tissues/roots. DNA-based molecular methods have identified *Ganoderma philippii* as the causative agent of red root rot in *A. mangium*. This disease is considered a major threat to the efficiency of wood production by the pulpwood industry in Indonesia.

Traditional taxonomic methods for describing fungi causing heart rot and root rot continue to play an important role in identifying the causal agents of these diseases. However, molecular techniques for fungal identification are gaining in popularity, value and effectiveness. DNA provides an abundance of taxonomic characters for the identification of organisms that have inadequate morphological characters, or possess distinguishing features only during particular stages of their life cycle. At present, because of their speed, sensitivity and high throughput, variations of PCR-based techniques are popular for assessing these DNA characteristics. Nevertheless, the exact method selected for a particular application will depend on several factors, including the number of samples and number of candidate species. For heart rot and root rot, large numbers of fungal species are implicated, so a technique that can simultaneously identify many species is more efficient than one based on species-specific probes or primers. In addition, as no comprehensive database of root- and heart-rot fungi from *A. mangium* exists, techniques that require an exact match to a known species will provide only limited information. All DNA-based techniques depend on an adequate herbarium source of carefully prepared specimens with detailed morphological descriptions to verify the DNA-based protocol.

Although a new challenge for growers of tropical acacia plantations, root-rot diseases have a long history of association with forest monocultures. This has inevitably attracted research initiatives to understand the dynamics of the diseases involved and options for disease control. In Peninsular Malaysia, poor land preparation and areas with a previous history of root disease were strongly associated with the incidence of *R. lignosus* and *P. noxius* in forest plantations. Based on experience gained from the management of root-rot disease in rubber plantations, good land management, the construction of isolation trenches and the application of fungicides are considered valuable tools in the control of root-rot disease in forest tree plantations. To date, cost-effective management tools based on biological and chemical treatments to soil and stumps have yet to be developed for controlling root rot in *A. mangium* plantations, though *Trichoderma* spp. have been shown to act as biological control agents of *Ganoderma*.

Biological control of plant pathogens aims to reduce dependence on chemical treatments that may cause environmental pollution and the development of resistant strains. Filamentous fungi such as *Trichoderma* are mycoparasites of plant pathogens and thus have potential for the biocontrol of plant disease: species of *Trichoderma* are among the most widely tested agents. Although the mechanism of mycoparasitism is not fully understood, expression of extracellular cell-wall degrading enzymes is assumed to be involved, including the action of chitinolytic and glucanolytic enzymes. As reported for other chitinolytic systems, endochitinase (EC 3.2.1.14) is among the most effective for both antifungal and lytic activities in comparison with other types of chitinolytic enzymes. Recently, a 32-kDa endochitinolytic enzyme has been purified from *Trichoderma reesei*. These *Trichoderma* isolates have an antagonistic ability against some plant pathogenic fungi, such as *Ganoderma* spp., *R. microporus*, *Rhizoctonia* spp., *Fusarium* spp., and *Sclerotium rolfsii* and can effectively suppress the development of these fungal pathogens *in vitro* and in glasshouse experiments.

Disease management is not just an issue out in the plantations. The foliar diseases *Pestalotiopsis* leaf spot, *Phaeotrichoconis* leaf spot, bacterial leaf blight caused by *Xanthomonas*, phyllode rust disease caused by *Atelocauda digitata*, and anthracnose disease and tip necrosis caused by *Colletotrichum* sp. are all associated with tropical *Acacia* seedlings being raised in nurseries, as are *Pythium, Rhizoctonia,* and *Fusarium* fungi that commonly cause damping-off. Disease control requires integrated mnagement strategies based on a detailed knowledge of these pathogens and their interactions with the seedlings and their environment, including the wider context of the nursery operation.

Acacia mangium wood has proved to be not only suitable for producing high-quality pulp and paper, but also an excellent material for solid-wood products. In Indonesia, there has been growing interest in utilising wood of *A. mangium* for solid wood, corresponding with the declining availability of logs from native forests. When managed for pulpwood, *A. mangium* plantations are established at around 1000 stems/ha and are clear-felled at age 6–7 years. Silvicultural techniques that incorporate thinning and pruning from below are of crucial importance in growing plantations for solid wood, as there is poten-

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tial for large and persistent branches to develop in plantations: both are associated with the development of heart rot. Thinning systems must ensure that final-crop trees retain green branches until pruning is completed as well as maintaining acceptable rates of growth of the retained trees. Form-pruning — the selective removal of large branches or those competing with the leader — ahead of lift-pruning, has been shown to increase the straightness of trees in a silvicultural system based on a final crop of about 300 stems/ha pruned to 4.5 m. *Acacia mangium* can be grown for solid-wood products with a rotation of around 10 years, which is expected to produce a total stem volume of more than 200 m^3 per ha: about 30% of it will be for solid wood. The minimum tree diameter at breast height will be 30 cm. Heart rot is exacerbated by pruning when the plant material is susceptible and a sufficient source of fungal inoculum is present to invade pruning wounds.

The workshop from which these proceedings are derived was billed as 'a synthesis of research progress': what have we learnt about heart rot and root rot to date and what are the challenges that must be confronted?

The incidence of heart rot will probably exclude the sustainable production of *A. mangium* for its solid-wood values on some sites. While we now have a quick way of assessing the incidence and severity of heart rot that will help determine disease risk, what defines a high-risk site remains unknown. As with other tree crops, good silviculture that includes pruning prescriptions based on live-branch pruning should reduce disease incidence. Use of selected lines and the best seed source — one producing straight-growing and small-branched trees, may also contribute to managing disease incidence. However, in the absence of better information, *A. mangium* is a species that appears very susceptible to heart rot and, if acacia wood is to be grown on high-risk sites, it may be necessary to consider alternative species or hybrids that demonstrate an inherent resistance to heart rot. This need to focus on the host rather than the pathogen to manage the disease makes even more sense now that it has been established that a suite of fungi causes heart rot.

We did not need a workshop to come to the conclusion that root rot is a more intractable problem than heart rot. Not only does root rot kill *A. mangium*, other organisms that fall into the same basket of diseases kill trees across a range of species in the temperate as well as the tropical zone. Solutions have been difficult to find: to date, biological control has been shown to work effectively for only one root-rot disease, that caused by *Heterobasidion annosum*. If there is a challenge here, it is to integrate the breadth of skills and resources that are available in both the private and public sectors in the region to at least understand disease behaviour and then do the right things on the ground to contain the disease and, if possible, begin moving towards disease management based on biological control. The heart-rot project demonstrated it was possible through research to make progress by embracing a multi-stakeholder approach on how to manage a more tractable disease. Are we now ready to meet the more difficult challenge?

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Acacia mangium — a historical perspective on its cultivation

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Abstract

The paper describes the cultivation of *Acacia mangium* from a historical perspective. The first introduction of the species to Sabah (Malaysia) in 1966 and South Sumatra in 1979 was for use as a fire break, land rehabilitation and reforestation of alang-alang (*Imperata cylindrica*) grassland. Eventually, *A. mangium* was planted in commercial plantations due to its excellent adaptability to degraded sites, rapid growth and good wood properties. In Indonesia the species has now become very important, with over 1 million ha planted in industrial forest plantations. Pulpwood production from *A. mangium* plantations is currently more than 9 million m^3/year, while the potential for solid-wood production is around 165,000 m^3/year. The domestication of the species has progressed rapidly, with extensive research in a range of aspects including genetics, silviculture and wood utilisation.

Acacia mangium Willd. has emerged as a key species grown for industrial forest plantations in Indonesia. From a total of around 2.5 million ha of industrial plantations, over 1 million ha have been established to *A. mangium*. The current use of *A. mangium* wood is primarily for pulp and paper. Other uses include medium-density fibreboard, furniture, plywood, flooring and light construction. This paper presents a historical perspective of *A. mangium* cultivation.

Domestication

Acacia mangium is native to northern Queensland, Australia, mostly in the coastal tropical lowlands. The species also occurs naturally in the Western Province of Papua New Guinea and the Indonesian provinces of Papua (Manokwari, Merauke) and Maluku (Seram, Aru).

The first introduction of *A. mangium* for use as a plantation species was in Sabah, Malaysia in 1966 by D.I. Nicholson, an Australian forester. The seed was originally collected from a single tree at Mission Beach (Queensland). The initial seedlings were planted at Ulu Kukut as a firebreak. The species grew remarkably well and was subsequently used for reforestation of grasslands of alang-alang (the shade intolerant grass, *Imperata cylindrica*) and planted for commercial plantations in 1976 (Udarbe and Hepburn 1986; Pinyopusarerk et al. 1993).

Following its successful introduction to Sabah, *A. mangium* was planted, and is now grown extensively, in many countries, including Indonesia, Thailand, Vietnam and the Philippines, transforming what was once an unknown species with limited use to a popular tropical tree species (Pinyopusarerk et al. 1993).

In Indonesia, the early introduction of *A. mangium* as an exotic species was undertaken in November 1979 into South Sumatra. Seeds consisting of three

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seedlots were brought by Mr R. Darmono (a former director of the Seed Division, Directorate General of Forestry) from Sabah (Malaysia) and given to Hardjono Arisman for use in a field trial in Subanjeriji, South Sumatra. The object of the trial was to examine the potential for using the species for the rehabilitation and reforestation of large areas of alang-alang grassland existing in the region. The plots of the initial seedlots from Sabah still exist today and remain in fairly good condition (Figure 1).

In South Sumatra, *A. mangium* grows reasonably well on former alang-alang grassland. The species has a rapid initial growth rate and starts closing its canopy early, suppressing the growth of alang-alang quite effectively. In a species trial conducted in Subanjeriji by the Directorate of Reforestation and Land Rehabilitation and a Japan International Cooperation Agency project in the early 1980s, *A. mangium* emerged as the most promising in terms of adaptability and growth. The site was dominated by red–yellow podsolic soil (Ultisol or Oxisol) that is inherently acid and poor in nutrient reserves. *Acacia mangium*, a leguminous species, thus appears to adapt well to poor soils.

Following the successful species trial in Subanjeriji, seed from four provenances of Queensland's Cairns region, namely Cassowary, Jullaten, Mossman and Daintree, were imported in 1980. Provenance resource stands were established in Subanjeriji (300 ha) and Benakat (100 ha) using these new Australian provenances and are referred to as the Subanjeriji local landrace. Each provenance consisted of 10 individual parent trees. In 1982, seeds collected from Sidei (Manokwari) by E.B. Hardiyanto and Seram (Maluka) by M. Subagyono were also planted in Subanjeriji. Seed from Sanga-Sanga (East Kalimantan) was also introduced into Subanjeriji, but the growth of the Sidei, Seram and Sanga-Sanga provenances was poor.

The Subanjeriji provenance seed stands were used as seed production areas managed by PT. Inhutani I. Seeds collected from these production areas were used for reforestation programs and plantation development all over Indonesia during the late 1980s. Seed production in these areas ceased in 1995 when seed of better genetic quality introduced from other provenances became available.

Figure 1. The plot of the first introduction of *Acacia mangium* into Subanjeriji, South Sumatra established in 1979. This photograph was taken in January 2006.

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Availability of genetic materials

In the early 1980s, information on the genetic variability of *A. mangium* was sparse and it was not known which of the provenances should be used for plantation development. Indeed, seeds of many provenances were not available until the CSIRO Division of Forest Research, Australia, with FAO support, made collections in the 1980s (Doran and Skelton 1980; Turnbull et al. 1983). Another collection was undertaken in 1983 within the framework of the Cooperative Seed Collection Program between the Office of Forests, Papua New Guinea and FAO's Forestry Department. Seed of *A. mangium* from these collections, together with seed collected by the Directorate-General of Forestry, Indonesia, was used for provenance trials in the early 1990s in many parts of Indonesia.

The results of provenance trials indicated that seed of the Subanjeriji local landrace and the Cairns region of Queensland, Australia had suboptimal growth, while Far North Queensland (FNQ), Papua New Guinea (PNG) and Muting Irian Jaya provenances grew better (Havmoller 1991; Hardiyanto et al. 1994; Hardiyanto et al. 1997; Leksono and Rosiawan 1997; Hardiyanto et al. 2000). The suboptimal growth of the Subanjeriji local landrace was not surprising since this seed source had originated from the Cairns region and its genetic base was known to be narrow both at growth and molecular levels (Butcher et al. 1996, 1998). Renewed efforts to broaden the genetic base of *A. mangium* were undertaken in the early 1990s by introducing seeds from a large number of provenances of PNG, FNQ and Muting. Collections were made by a number of institutions including CSIRO's Australian Tree Seed Centre (ATSC), the Department of Forestry, Faculty of Forestry, Gadjah Mada University, and several forestry companies and now form the basis of seed production areas and seed orchards. Since the mid 1990s, improved seed with a broader genetic base has become generally available for operational plantations (Hardiyanto 1998).

Plantation development

Acacia mangium has become the leading tree species in forestry plantation programs in several Asian countries (Awang and Taylor 1993; Turnbull et al. 1998). In Indonesia, the large-scale development of *A. mangium* plantations began in the early 1990s, corresponding with an ambitious program launched by the government to develop 2.3 million ha of industrial forest plantations by 2000 and 10.5 million ha by 2030 (Ginting et al. 1996). Only about 2.5 million ha had been realised as of the end 2004, with *A. mangium* the primary species planted. The properties of pulp and paper processed from *A. mangium* wood are known to be excellent and comparable with or better than those of *Eucalyptus* species used for paper making. *Acacia mangium* pulp has fairly good formation and opacity which is excellent for paper and tissue (Palokangas 1996) and the pulp has been accepted in the international pulp market and competes reasonably well with other short fibre species.

The productivity of *A. mangium* plantations varies depending upon a number of factors including seed quality, site characteristics and silvicultural practices. Current mean annual increments of total volume are in the range of 20–33 m^3/ha, but can reach more than 40 m^3/ha/year on the best sites (Hardiyanto 2005).

Research and development on *A. mangium* has been extensive across a range of fields, including tree breeding, silviculture, growth and yield, pests and diseases, and wood and non-wood products. With regard to tree breeding, second-generation improvement programs are in progress. Studies on site management and the productivity of the second rotation have also been conducted and show that the growth rates can be maintained or even increased, provided proper silvicultural practices are employed (Hardiyanto et al. 2003).

Various studies have been conducted to assess the suitability of *A. mangium* wood for solid-wood products such as furniture, flooring and plywood (Awang and Taylor 1993). A recent study on the mechanical properties of sawn timber indicated that the wood of *A. mangium* can be used for structural materials and meets the requirement of the Indonesian Standard for Wood Construction (Amalia 2003; Firmanti and Kawai 2005). The development of *A. mangium* plantations for solid-wood utilisation is in progress.

Large-scale *A. mangium* plantations have been developed mainly by forestry companies. More recently, however, smaller-scale plantations of *A. mangium* have been established in outgrower schemes by individual farmers on the farmers' land in cooperation with forestry companies which cover the costs of establishment and maintenance: the benefits are shared between the farmers and companies. Small-scale plantations may also be established by farmers using their own capital.

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As *A. mangium* is an exotic species being planted on a large scale, the potential threats to plantations have also been identified and studied. Heart rots have been reported to attack older trees, which can reduce timber volume and quality. Heart-rot surveys in Malaysia (where *A. mangium* plantations are established for the production of sawn timber) reported that heart-rot incidence varied from low to high. This caused the Malaysian Government to review its policy on the further development of *A. mangium* plantations in Peninsular Malaysia (Lee et al. 1996). A recent survey at a number of sites in Indonesia revealed that heart-rot incidence differed between regions, ranging from low to high, depending upon a combination of climate, plantation management (pruning) and age. In regions in which heart-rot incidence was low, such as in South Sumatra and East Kalimantan, the development of sawlog plantations was considered feasible (Barry et al. 2004).

Another disease that has been causing concern in *A. mangium* plantations is root-rot, associated with the fungi *Ganoderma* sp. and *Rigidoporus lignosus*. The disease often occurs in patches with a concentric pattern of spread. Trees attacked by the disease show crown thinning and subsequent death. Research on this problem is continuing in a number of projects.

Wood production

Wood production from *A. mangium* plantations was started in the early 1990s. The wood was used mainly for pulp and medium-density fibreboard. Initially, the volume of wood produced was relatively small, increasing markedly in the year 2000. Wood production in Indonesia for pulp and medium-density fibreboard harvested from *A. mangium* plantations is roughly as follows: Riau and Jambi, 5,860,000 m^3/year; South Sumatra and Lampung, 2,500,000 m^3/year; East and South Kalimantan, 750,000 m^3/year; West Kalimantan 200,000 m^3/year. The solid-wood utilisation of *A. mangium* is increasing with potential supply, which in Indonesia is currently estimated at 165,000 m^3/year (Thorp 2005).

The way forward

Over the past 25 years, *A. mangium* has gone from a virtually unknown tree in the wilds of North Queensland, Papua New Guinea and Irian Jaya to a major commercial plantation species for pulp and paper in Southeast Asia. It is only 20 years since the first introduction of this species to South Sumatra. In this short time, the natural variation within the species has been assessed, breeding programs established, molecular marker technologies applied, silvicultural studies completed, growth and yield studied, and wood and fibre properties determined. Such rapid progress in domesticating this tree species is remarkable. Nonetheless, research in these fields needs to continue in order to improve plantation productivity and sustainability. Environmental and biodiversity aspects of *A. mangium* plantations also require further study.

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Hardwood plantation development and threats to its sustainability in Indonesia

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Abstract

Sustainable management of plantation forests should embrace the interests of the *planet* — conserving biodiversity and preventing environmental degradation; the *people* — providing opportunities for social development and poverty alleviation; and *profit* — ensuring a steady supply of renewable, high quality, internationally cost-effective fibre. Options for managing plantations in this way are considered. The sustainability of plantation forests is threatened by improper management of monocultures, fire and illegal logging. This paper outlines ways of combating these threats through effective forest management and a multi-stakeholder approach to ending illegal logging. Interdisciplinary research will continue to play a role in the current and future establishment of industrial forests in Indonesia.

Indonesia is one of the major players in pulp and paper production. Strong demand for paper in Asia led to an overall increase in the global pulp market by 2.9% in 2003 (APRIL 2004). Strategic location, proximity to the fast-developing economic giants, China and India, and climatic and physiographic conditions conducive for growing short-rotation fibre plantations make Indonesia a preferred choice for pulp and paper production. In line with ever-increasing demands for wood, Indonesia has initiated a massive reforestation program. Since the mid 1980s the area of industrial forest plantations in Indonesia, particularly those of short-rotation species, has increased dramatically with a target of 10.5 million ha by 2030 (Gintings et al. 1996). The aim of the program is to provide the required supply of forest products while at the same time maintaining the remaining natural forest. It is expected that, after 2020, most of the forestry industry in Indonesia will depend more on wood from plantation forest than natural forest (Natadiwirya 1998). Dependence upon exploitation of natural forest will eventually cease: natural forests will be preserved and valued for their ecological functions and benefits. To fulfil this requirement, development of new forest plantations in balanced and responsible ways is of critical importance. This paper is intended to discuss the development of industrial plantation forests in Indonesia and constraints to their sustainability.

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Key issues

The main issue in the management of large-scale industrial plantation forests of fast-growing species is to ensure product sustainability over successive rotations. This is particularly true in areas conducive to high growth rates and thus imposing heavy demands on site resources. Nambiar and Brown (1997) have set goals for industrial plantation forestry, which include:

- *productivity* — to ensure that the trend in plantation productivity is non-declining over successive harvests
- *soil and water conservation* — to protect and if possible enhance the quality of soil and water in the plantation environment,

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- *economic viability* — to promote incentive, innovation and profit for the business of growing and utilising wood
- *socioeconomic improvement* — to improve the economic and social benefits to the community.

Sustainable management of plantation forest is not merely about maintaining tree growth, rotation after rotation. Rather, it includes a more holistic approach that fosters the long-term sustainability of the entire forest ecosystem and considers the diverse social impacts. Forest management should recognise and involve a consideration of several values, especially those of economic, environmental, and social significance. Plantation forest should be mutually acceptable to various stakeholders:

- to industries, for steady sustained wood supply
- to local people who need access to land and forest resources, minor forest products like firewood, honey, herbs etc.
- to governments and environmental groups for conservation of natural forests and the ecosystem.

The interactions between people, forests and other natural resources have to be better understood. Management of plantation forest should be considered as a business that is efficient and economically beneficial for all the above stakeholders including concessionaires and local people by providing opportunities for employment. This will also make plantation forests not only viable and sustainable but attractive to investors. Inefficient management systems may create potential social problems that lead to rapid conversion of forest resources to other land uses. Parthama and Widiarti (1998) emphasise that management options for plantation forest, low or high capital, must be carefully considered.

Ecological aspects of plantation forest establishment are critical. These should include conservation of environment and biodiversity to maintain the ecological functions of the forest landscape. Thus, changes to the environment during plantation establishment should be minimised. Strengthening of the relationship between production forests and natural landscapes includes the retention of conservation areas in such a way that losses to biodiversity are also minimised (Santosa 1998). Failure to recognise this requirement in plantation forest management may deleteriously affect the long-term sustainability of the forest landscape.

The 'mosaic' plantation concept (Figure 1) offers an important strategy in managing forest resources in a sustainable manner that balances the need for commercial, environmental and social development. Protection and conservation of *HCVF* (high conservation value forest), *riparian* areas to protect soil and maintain natural river ecosystems, and *buffer* zones to serve as habitat and corridors for wildlife, form part of this approach to maintain the balance between plantations and natural forests for sustainable outcomes.

Social aspect is another important component of plantation forest management. In the past this aspect received relatively little consideration: the main emphasis was wood production (Gintings and Suharti 1998). Social problems such as conflicts between companies and local communities over access to land, and years of experience, have indicated the importance of a participatory process in plantation forest management. The evidence suggests that such a process contributes to sustained forest productivity as well as the welfare of forest communities. Thus, active participation of local people should be continuously encouraged.

Challenges

There has been growing concern over the sustainability of wood production in industrial plantation forest management systems (Muhtaman et al. 2000). Without proper management, long-term site productivity may fall. This is particularly relevant to industrial plantation forest in Indonesia; i.e. a cultivation system involving monocultures of fast-growing species, many of them exotic. Under this system, improper management negatively affects the hydrological balance of river basins within the plantation forest areas, soil nutrients, and the food chain (Santosa 1998), leading to decreased quality of soil and water as well as higher vulnerability to potential pests and diseases. Forest fires and illegal logging also constrain the sustainable production of industrial plantation forest in Indonesia. Management options that simultaneously meet wood-production goals and avoid these threats should be developed.

Plantation forests are often established on marginal soils. One constraint to the further expansion of the plantation forest, especially of fast-growing species, is long-term site productivity. Each harvest is accompanied not only by nutrient export, but also some soil erosion, soil compaction, nutrient leaching and volatilisation. Losses of nutrients during harvest and site preparation may exceed the rate of their

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replacement by weathering of minerals in soils or by nutrient inputs through precipitation (Ruhiyat 1998; Santosa 1998). Thus, a proper fertiliser regime and effective soil and water conservation strategies are crucial to sustainable plantation forestry.

Fertiliser application can replenish the nutrient supply to maintain or even increase productivity. The results of Riaufiber research in Baserah, Riau, indicate that application of balanced NPK fertilisers and good harvest-residue management lead to increased height and diameter at breast height of 3-year-old *Acacia mangium* in the second compared with the first rotation at the same age (Siregar et al. 2004). This improved growth was in part attributable to better genetic material. Attention to detail at this level should mean that degraded forest lands can also be replanted and managed to provide wood, at the same time restoring ecosystem services such as freshwater regulation and soil retention.

Sustainable production of industrial plantation forest will also depend on the ability of managers to genetically improve planting stock. The more recent industrial plantation forests in Indonesia have been established to mainly exotic species. This has limited the availability and use of good genetic stock. Tree improvement programs are therefore crucial to the adoption of monoculture plantations. Wood volume and density are among the important characteristics of interest. Through intensive breeding programs, Wong and Wijoyo (2005) have shown that predicted genetic gain in *A. mangium* can increase to as high as 43% for wood volume and 5% for wood density, with predicted mean annual increments (m^3/ha/yr) of 66.0 (potential) and 46.2 (operational) (Table 1). In addition to wood volume and density, Riaufiber breeding programs also consider resistance to pests and diseases. Through selection, Riaufiber has demonstrated that some *Acacia crassicarpa* plus-trees have genetic resistance to *Passalora* (formerly *Pseudocercospora*) leaf and shoot blight disease (Gafur et al. 2005).

The common use of a small number of species of the same age grown in monoculture in plantation forest poses a concern for managers, as these conditions provide an opportunity for pests and diseases to multiply and reach epidemic proportions (Sitepu and Suharti 1998). An abundance of substrate over a relatively long time provides particularly suitable conditions for the development of pests and pathogens.

Figure 1. Mosaic plantation concept in Raiufiber Industrial fibre plantations (HTI)

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Table 1. Predicted gain and mean annual increment (MAI) of selected trees in some Riaufiber AMPFT trials. OP is open-pollinated; CP is close-pollinated.

Trial code	Tested		OP orchard					CP orchard					CP orchard (5 best)				
			Selection		Predicted gain (%)			Selection		Predicted gain (%)			Selection		Predicted gain (%)		
	No. of families	No. of trees	No. of families	No. of trees	Vol.	Density	Index	No. of families	No. of trees	Vol.	Density	Index	No. of families	No. of trees	Vol.	Density	Index
AMPFT01	85	4320	12	21	27.7	0.3	26.4	3	4	38.6	0.4	37.6	1	1	41.1	0.8	43.7
AMPFT02 and AMPFT03	160	13,824	37	52	29.0	4.9	14.8	4	6	37.5	5.6	23.2	3	4	42.7	5.0	27.2

Trial code	PYI Category	Predicted MAI (m^3/ha/year)				Predicted MAI (m^3/ha/year) – operational discount[a]			
		OP orchard	CP orchard	CP orchard (5 best)	CP orchard (2 best)	OP orchard	CP orchard	CP orchard (5 best)	CP orchard (2 best)
AMPFT01	Superior	57.5	62.4	63.5		40.2	43.7	44.4	
AMPFT02 and AMPFT03	Superior	58.1	61.9	64.2	66.0	40.6	43.3	45.0	46.2

[a] Operational discount rate = 30%. Test mean MAI ~ 45 m^3/ha/year

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One good example is root-rot disease. This disease, which is caused by *Ganoderma* sp., has been damaging oil-palm plantations in Southeast Asia for years. In acacia plantations, the disease is also causing high mortality (3.2–40.0%) in Southeast Asia and India (Eyles et al. 2004). Although often difficult in practice, the implementation of compatible control measures, more popularly known as integrated pest management (IPM), to maintain pest and disease losses at acceptable levels, is recommended. This will include appropriate application of chemicals, site- and species-specific silvicultural practices, biocontrol, and planting of less-susceptible species. The management of plantation forest is a key factor in pest and disease management: very often control measures fail due to improper management.

Forest fires cause devastating effects on the lands. In addition to the loss of thousands of hectares of wood, both in natural and plantation forests, fire also causes starvation or even extinction of wildlife. The smoke causes health problems in forest communities. A number of causal factors have been associated with forest fire in Indonesia, including 'manual slash and burn' land-clearing practices mainly adopted by small farmers, escaped cooking fires, careless disposal of cigarette butts and arson. Some farmers intentionally use fire to open up new land. Past logging has also left forests degraded and very susceptible to fire. Fire often gets out of control. The issue of fire can be solved only by prevention and effective management. Plantation forest management should incorporate:

- a no-burn policy for land clearing.
- plantation forest and wildfire fighting capability
- compulsory basic fire-fighting and prevention training for staff
- community awareness and participation in fire prevention and control.
- stronger regional cooperation in managing forest fires will also help.

Illegal logging has been occurring for years and is believed to have destroyed millions of hectares of forest. Wood is routinely smuggled between neighbouring islands and countries, costing the Indonesian Government millions of dollars in loss of revenues each year. Illegal loggers also cut trees in the conservation areas and in corridors left by plantation forest managers for biodiversity conservation, causing wildlife habitats to degrade. Illegal logging in Indonesia occurs in a number of ways (APRIL 2005):

- organised large-scale logging in natural forest without a permit
- smallholder farming — clearing of forest land for agriculture without permit
- excessive logging of forest concessions
- misuse of logging licenses — felling of trees in other than designated areas.

To maintain the already very limited conservation area (15–20% of concession area), forest managers must provide alternative ways to plan and manage the corridors of native forest that allow local people to collect firewood, medicinal plants and other products to support their livelihood.

Plantation forestry generates 5 direct and 30 indirect jobs for each 100 ha managed and thus offers an option for a legitimate livelihood to people currently engaged in illegal logging. A multi-stakeholder task force, involving local communities, law-enforcement authorities, environment protection agencies, non-government organisations and forest industries can be effective in combating illegal logging.

Conclusions

The development of plantation forests in Indonesia is taking place and seen as part of future progress. For sustainable production, plantation forests should be properly managed by considering several values, especially those that are of economic, environmental and social significance. There is growing demand for more-integrated approaches to natural resource management. The interactions between all the resources (soil, water, biological and atmospheric resources) within a given landscape need to be better understood. There will of course be more challenges ahead. These include quality of soil and water, plant genetic material, vulnerability to pests and diseases, forest fire, and illegal logging. Careful consideration of these aspects will help drive plantation forestry along the right track. Interdisciplinary research will continue to play an important role in the current and future establishment of industrial forests in Indonesia.

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Heart rots in plantation hardwoods: the background to this ACIAR project

Anto Rimbawanto[1]

Abstract

Heart-rot fungi are wound parasites that enter through broken branches and branch stubs after self pruning, or through singling and manual pruning. Heart rot has been identified as one of the major disease problems in *Acacia mangium*. It was therefore important to address this problem if the benefits of tree improvement programs are to be realised, particularly where trees are grown for solid-wood products. The focus of the project activities was to assess the incidence of heart rots, including links with silvicultural practice and identify any resistance to disease. DNA-based methods were to be developed and coupled with traditional taxonomic methods to identify fungi causing heart rot. Technology-transfer activities included assisting plantation managers to reduce heart rot, and staff exchange and interaction. Preliminary work was also undertaken in respect of root rot for some of the above activities.

During the past decade, Australian and Southeast Asian researchers have completed a number of collaborative forest pathology projects. Pathology and tree improvement specialists from CSIRO have collaborated with their counterparts from government research agencies and universities in Indonesia and five other Southeast Asian countries with ACIAR and Center for International Forestry Research (CIFOR) support. One of the most successful of these projects was the survey of diseases of tropical acacias carried out during 1995–96, which included a workshop in Subanjeriji. One request of the representatives at the workshop was met by the publication of the book 'A manual of diseases of tropical acacias in Australia, South East Asia and India' (Old et al. 2000). One of the major disease problems discussed at the workshop was heart rot in *Acacia mangium* Willd.

In Indonesia the demand for wood, from both domestic and international markets, continues to increase, and the Government of Indonesia has embarked on a massive afforestation and reforestation program aimed at preserving the natural forest while maintaining the supply of forest products. Since the mid-1980s, the area of forest plantations in Indonesia, particularly those grown on short rotations, has dramatically increased. The government has launched an ambitious program for rehabilitation of unproductive Imperata grassland and secondary scrubland into industrial forests in islands other than Java. The program aimed to establish 2.3 million ha of plantations by the end of 2000 and 10.5 million by 2030. The current plantation program has been planned to produce wood for pulp, paper-making or medium-density fibreboard. The Ministry of Forestry has identified the supply of genetically improved material as being critical to the success of the plantation program. The genetic material being generated is potentially significant in producing high-quality plantation for solid-wood products and for achieving sustainability by optimising yield.

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Fast-growing hardwood plantations are important to the economies of many Australasian countries, including Australia and Indonesia. The major plantation species in Indonesia is *A. mangium*, which appears to be highly susceptible to heart rot. Unless the problem of heart rot is overcome it is unlikely that the benefits of tree improvement programs will translate into increased productivity and economic gain. The financial interests of small growers and agroforestry in Indonesia must also be considered. Tree breeders work closely with their counterparts in resource protection, tree physiology, soil and silviculture to develop sustainable production systems. This type of activity best fits the role of a public research institution rather than the private sector, as such organisations have developed these interlinking contacts and are less hampered by issues of intellectual property.

Heart rot

Wood-decay fungi may be defined by the zone of the tree that the fungi invade. The outer structure of the tree consists of the sapwood, which contains functional or living cells and the heartwood, which is formed by the death of cells. The species of fungi that are able to degrade the sapwood are generally different from those that can degrade the heartwood. The decay, which establishes itself in the heartwood and becomes progressive, is defined as heart rot.

Heart rot has been reported to occur in the older trees of many species of acacias, including *Acacia auriculiformis* A. Cunn. ex Benth. and *A. mangium,* but its widespread occurrence in relatively young trees of the latter species was unexpected. Heart rot has not been reported from *Acacia aulacocarpa* A. Cunn. ex Benth. or *Acacia crassicarpa* A. Cunn. ex Benth. However, this may be because these two species have not been systematically surveyed for incidence of decay. Ito and Nanis (1994) found that *A. mangium* was more susceptible to heart rot than was *A. auriculiformis* and a hybrid between these species.

Wounds are usually the entry points for the wood-decay fungi. On *A. mangium* trees, wounds include broken branches, mechanical injuries and branch stubs after self-pruning or through singling and manual pruning operations. The incidence and extent of the decay is highly variable and depends on many factors including species, location of wound on the stem, size and season of injury, age of wounds, stand dynamics and decay organism(s).

Heart-rot fungi are generally pan-tropical or cosmopolitan saprophytic wood-decay fungi or wound parasites. A range of hymenomycetes are known to be associated with heart rot of acacias, but several fungi associated with *A. mangium* have only recently been identified.

As heart-rot fungi are wound parasites, lopping off branches with parangs or machetes should be avoided. Singling of multi-stemmed, fast-growing trees creates large stem wounds that heal slowly and are more prone to invasion by fungi. Wound protectants are commercially available but their effectiveness on *A. mangium* has not been tested. This approach is also expensive and impractical for large-scale use in forest plantations.

Quantitative information on the extent of heart rot in acacia plantations is lacking. In Peninsular Malaysia, a volume loss of up to 17.5% of the merchantable timber of *A. mangium* has been reported as a result of heart rot (Zakaria et al. 1994). Although the disease is also known to be present in *A. mangium* plantations in Indonesia, no equivalent detailed data are available.

The heart-rot project

Milestones

The project originated from within Australia but has been based on the outcomes of an industry/research provider workshop held in Indonesia (Subanjeriji in Sumatra) in April 1995 that was partly supported by ACIAR and CIFOR.

The major research on heart rot of tropical acacias has been carried out by Dr Lee Su See and her colleagues of the Forest Research Institute of Malaysia (Kuala Lumpur) and by Dr Shin-ichiro Ito of the Forest and Forest Products Research Institute (Kyoto, Japan). There is clear evidence that branch stubs and damage from singling are the main entry points of rot. Dr Caroline Mohammed and co-workers at the University of Tasmania have carried out the main body of work in Australia on entry of decay fungi via pruning wounds. This has included pruning trials and research on defence processes by Dr Karen Barry (University of Tasmania). Drs Inez Tommerup, Neale Bougher and Morag Glen (CSIRO Forestry and Forest Products, Perth) have carried out investigations in mycological taxonomy and molecular studies.

From: Potter, K., Rimbawanto, A. and Beadle, C., ed., 2006. Heart rot and root rot in tropical *Acacia* plantations. Proceedings of a workshop held in Yogyakarta, Indonesia, 7–9 February 2006. Canberra, ACIAR Proceedings No. 124.

Objectives

Factors determining the extent of decay in acacias in Southeast Asia and eucalypts in Australian hardwood have a high degree of commonality. This project sought to establish the means to minimise impacts of decay on the potential of *A. mangium* to produce high-value products. The main aims of the project activities were to:

- assess the incidence of heart rot (and root rot) in different Indonesian environments
- establish the relationship between silvicultural practices and pruning of *A. mangium* with the incidence of heart rot
- establish the relationships between trees selected for other traits such as leaf pathogen/insect resistance and form, and the incidence or risk of heart rot
- develop DNA-based methods coupled with traditional taxonomical and pathological methods to identify and characterise fungi causing rot
- assist plantation managers in Indonesia to reduce heart rot (and root rot)
- provide revised selection criteria for tree improvement projects in Indonesia to reflect outcomes of the research
- achieve technology transfer by staff exchange and interaction.

Outputs

Some of the major outputs of the project are summarised here. Other papers present the project outputs in more detail.

1. Incidence of heart rots and root-rots in *A. mangium* (see Barry et al. 2004)

Surveys were completed at four sites (Perum Perhutani Jasinga, West Java; PT Wira Karya Sakti, Jambi; PT Arara Abadi, Riau, East Kalimantan; and PT Musi Hutan Persada, South Sumatra), in which trees were assessed at the harvest age of 8 years (in addition, 3-year-old thinned trees were examined in Jasinga). A rapid method of surveying logs stacked in the plantation was developed.

The incidence of heart rot in the main stem was significantly different between some regions, ranging from 6.7% in East Kalimantan up to 46.7% in West Java (Figure 1). However, the proportion of each defect type (1–4) did not show a consistent trend across all sites. A combination of differences in plantation management (e.g. pruned or not pruned), age and climate between these five regions may explain the differences in heart-rot incidence and severity.

2. Effect of pruning on heart rot in *A. mangium* (see Beadle and Barry 2004).

This experiment in South Sumatra set out to test two hypotheses. The first was that pruning would be associated with an increase in the incidence of heart rot. Eighteen months after pruning, the incidence of heart rot was negligible or absent. As all the pruning wounds were occluded, it was likely that heart rot would not arise from pruning in this plantation in the future. Surveys of heat-rot incidence in harvested logs of trees aged 6–8 years had shown that it varied with region in Indonesia (Barry et al. 2004). While in South Sumatra the average incidence was low (11.3%), it was not absent. It was therefore dif-

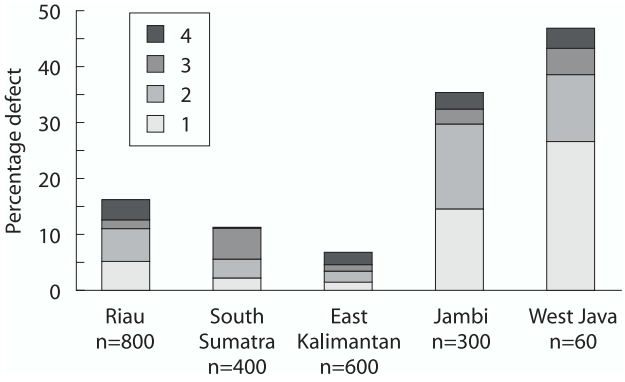

Figure 1. Percentage of logs in each defect class (1–4) for the five survey sites. The numbers correlate with increasing severity of heart rot.

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ficult to explain why heart rot was completely absent in the trees assessed from the pruning trial. The results suggested that interventions with pruning and thinning that are essential for the management of plantations for solid wood should not increase the incidence of heart rot in South Sumatra if managed carefully.

The second hypothesis tested whether form-pruning was associated with a lower incidence of heart rot and better form than was lift-pruning. The first part of the hypothesis could not be tested. However, there was strong evidence that form-pruning was associated with better form, particularly in relation to kinks, though only in the first 3 m of the stem where form-pruning had been undertaken. The results also suggested that form-pruning would have been effective in the next 3 m of the stem had it been undertaken after the end of the third growing season (March–September 2003) when the trees were 8 m high (tree height was around 12 m at harvest).

3. Surveys for root rot of *A. mangium* in Indonesia (Barry and Irianto 2004)

Of the diseases of *A. mangium* identified, root rot is the most significant in terms of loss of productivity, resulting in tree death (Old et al. 2000). Root rot of *A. mangium* plantations caused by *Ganoderma* spp. or *Phellinus* spp. has been found to occur throughout Asia, with reports from Malaysia (Lee 1997), the Philippines (Almonicar 1992), Papua New Guinea (Arentz 1996), and India (Prasad and Naik 2002). In Indonesia, the disease is also widespread (Rahayu 1999; Old et al. 2000), but there have been limited reports to date on its incidence.

This study surveyed root-rot incidence and spatial arrangement in commercial plantations and trials. In second-rotation commercial plantations in Sumatra, root-rot incidence recorded was 3–26%. The compartments surveyed in Riau province had significantly higher root rot than those in South Sumatra. Fruitbodies of *Ganoderma philippii* and other species were collected in both regions during independent collections. In a provenance/family trial of *A. mangium* in Java, root-rot incidence was surveyed at two separate times. Root rot incidence was about 7% at the time of the first survey and 13% during the second survey, 1 year later. Spatial analysis by distance indices (SADIE) revealed that infected trees were randomly distributed at the first time point but tended to become aggregated by the time of the second survey. This highlights the potential for vegetative spread of the fungus after initial introduction to the site.

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Heart rot and root rot in *Acacia mangium*: identification and assessment

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Abstract

Heart rot in *Acacia mangium* is a typical white rot caused by hymenomycetes, which attack cellulose and lignin. Its development is associated with changes in colour, texture and appearance of rotted wood. These were used as the basis for the rapid assessment of the incidence and severity of heart rot on harvested log-ends in the field. The results are compared with previous survey reports based on longitudinal sectioning of logs. An assessment of two trials, one in Riau, the other in South Sumatra, showed that provenance could influence heart-rot incidence but that this was not correlated with wood extractive content. Root rots are characterised according to the colour of the infected fungal tissues/roots and are associated with various basidiomycetes. Molecular technology has identified *Ganoderma philippii* as the causative agent of red root rot. Root-rot diseases are associated with crown dieback, reduced growth and tree death. When root rot is first detected, infected trees or disease foci tend to be randomly distributed but then enlarge and may aggregate. The rate of disease progress appears positively related to the initial incidence of root rot. Accurate surveys to investigate spread should record above-ground symptoms, inspect the extent of root infection, and observe patterns of disease infection. However, these types of surveys, especially over extensive and difficult terrain, are operationally laborious and costly. Future options for disease survey based on remote sensing are discussed.

Surveys to evaluate diseases of tropical Acacia plantations have concluded that heart rot, root rot and phyllode rust (respectively, infection of heartwood, roots and leaves by fungi) are the main threats (Old et al. 2000). Heart rot in *Acacia mangium* Willd. is a saprotrophic fungal decay of heartwood which reduces wood quality but the tree is not killed and is, in most cases, externally asymptomatic. Heart-rot fungi are wound basidiomycete parasites that enter trees through injuries and branch stubs and do not preferentially attack living tissue. Root-rot disease in *A. mangium* is a decay of roots caused by various basidiomycete pathogens, which attack living root tissue and may result in tree death or symptoms of crown decline. The disease is spread by the contact of a diseased root or infested woody debris with a healthy root. Assessments of heart-rot and root-rot diseases require different approaches. For practical management in plantation forests, methods need to be fast, accurate and easily repeatable by staff with a minimum of training.

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Heart rot

Internal symptoms of heart rot in *A. mangium*

When healthy *A. mangium* trees are dissected, sound sapwood is tan to bright yellow in colour, while sound heartwood is pale brown to grey brown. In *A. mangium* the juvenile wood is pale coloured and of low density. It is located in the centre of the log, is often soft, and can be mistaken for heart-rotted wood. Like most fast-growing trees, *A. mangium* possesses a high proportion of juvenile wood. It usually forms in the stem of the live crown and has been known to constitute up to about 10% by volume of the wood present in 8–9-year-old trees (Ivory 1993).

The first symptom indicative of fungal infection is discoloration; heartwood becomes purple/black, sapwood becomes green/brown. Incipient decay in heartwood is difficult to detect but the wood colour is intermediate between that of sound and decayed wood, i.e. it is usually of darker colour than normal heartwood. Heart rot in *A. mangium* is typical of white rot caused by hymenomycetes (a group of basidiomycetes; Hood (2006)), which attack both cellulose and lignin, finally leaving behind a yellowish-white, spongy or stringy mass. Lee and Maziah (1993) described seven types of heart rot from *A. mangium* based on differences in colour, texture and general appearance of the rotted wood. The authors attributed each type to different stages of decay as well as characteristics of the individual fungi involved. Isolated decay-columns may form in the heartwood. In extreme cases, heartwood can completely rot away leaving a hollow core.

The infection courts for heart-rot fungi in *A. mangium* are dead branch stubs, dead/broken branches and stem cankers/unhealed or slowly healing wounds such as those caused by pruning (Mahmud et al. 1993; Ito and Nanis 1994, 1997; Barry et al. 2004). Infection courts (i.e. branch stubs) are not often associated with external signs or symptoms such as fruitbodies, and heart rot can be seen only after the bolts of wood are split. Molecular techniques have greatly increased the ability to identify isolates obtained from diseased wood by matching the DNA of such isolates to that of named fruitbodies (Glen et al. 2004).

Methods of detection and assessment

Since it is not possible to assess heart rot from the external features of the living tree, most surveys of *A. mangium* heart rot have utilised destructive methods. In five major studies, the merchantable stem length was sectioned into 1 m logs that were each sliced longitudinally to allow detection and quantification of heart-rot incidence and severity (Mahmud et al. 1993; Zakaria et al. 1994; Ito and Nanis 1994; Basak 1997; Ito 2002). While destructive methods are extremely valuable for gaining exact measures of heart rot and understanding paths of fungal entry into the stem (Lee et al. 1988; Mahmud et al. 1993; Ito and Nanis 1994, 1997), they are labour intensive. This necessitates that replicate tree numbers within treatments are reasonably low; for example, 5–6 trees per 13 plantations in Bangladesh (Basak 1997) or 10 trees per 20 plots of different age in Sabah (Mahmud et al. 1993). For regular monitoring, a quicker method of heart-rot survey is desirable.

Barry et al. (2004) developed a quick and easily repeatable method of heart-rot survey to screen large numbers of logs in different regions of Indonesia. At most sites, logs were surveyed during harvest operations at the end of the rotation, generally at age 8 years, but at age 6 years in East Kalimantan. In West Java, some trees were surveyed at harvest at age 8 years (60 trees), and others at age 3 years during thinning (39 trees). The number of compartments and logs per compartment surveyed were based on availability of harvested material.

Log-ends were assessed where logs were stacked after harvest. Typically, approximately 100 logs were in each pile and 3–4 logs could have been derived from an individual tree. For example, in East Kalimantan, logs were about 3.5 m long and 3–4 logs of this length were produced from an individual tree. Therefore, logs in the piles were not uniform in size and could have come from a number of positions in the tree. The logs chosen to assess from each log pile were selected randomly by using a line transect (a piece of string placed horizontally at random), which typically selected 10–15 logs (Figure 1). Since *A. mangium* wood discolours rapidly upon cutting, logs were assessed as soon as possible after harvest. Discoloration related to heart rot can appear similar to oxidative discoloration, and this is therefore a potential source of confusion. The diameter of each log selected was measured and if a heart-rot-related defect was observed this was categorised on a 1–4 scale (Figure 1) and its maximum diameter measured. Logs with no heart rot were scored as 0. Where more than one defect type was present on one log-end, the highest rating was recorded and the widest diameter of the total defect was recorded.

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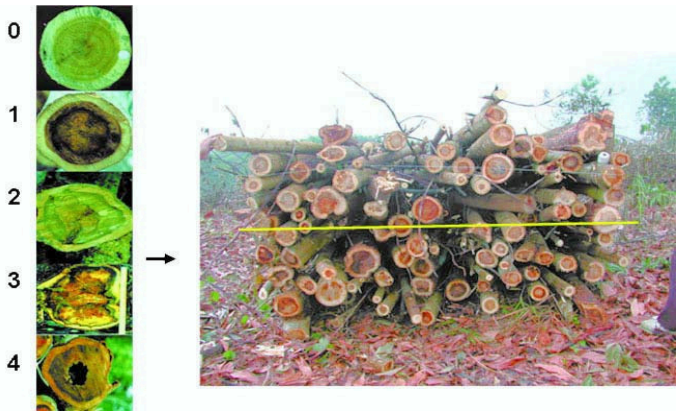

Figure 1. Heart-rot defect scale and log stack showing line transect method of Barry et al. (2004)

Comparing results of the log-stack method to other studies

In the Indonesian study by Barry et al. (2004), the East Kalimantan and South Sumatra sites were associated with the lowest heart-rot incidence (Figure 2) and the lowest average defect diameters. Lower heart-rot incidence would be expected for the East Kalimantan site, mainly because the trees were 2 years younger than at the other sites (Figure 2). This age-related trend has been evident in other studies of *A. mangium*, with positive correlations between heart-rot incidence and tree age (Zakaria et al. 1994) as well as between heart-rot diameter and tree diameter (Basak 1997). Apart from tree age, other factors such as climate and management practices may influence heart-rot incidence and spread.

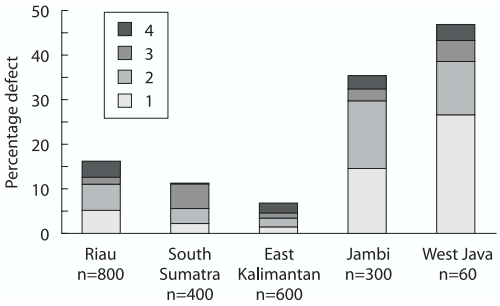

Figure 2. Percentage of logs in each defect class (1–4) for the five survey sites

Previous Malaysian surveys using destructive methods of analysis have detected higher incidences of heart rot than those found by Barry et al. (2004) in Indonesia. This difference could be due to the method used in Indonesia, which may underestimate heart-rot incidence as it examines only one cross-section of each log (although it surveys a larger number of logs). As such, it is not possible to compare the results from Barry et al. (2004) with surveys conducted by other methods. Surveys by Maurits et al. (2001) found volume losses to heart rot of 0.7–14.1% in *A. mangium* in East Kalimantan. Correlating the different methods would require a destructive analysis of a sub-sample of logs following the survey of the same logs with Barry et al.'s method. However, it is very possible that there are real differences in incidence between the various studies. For example, seed stock has improved markedly in the 15 years between the first Malaysian studies and the more recent Indonesian surveys (C. Harwood, pers. comm.) and these improvements may also reflect an associated reduction in susceptibility to heart rot; trees have become straighter, branches smaller and less numerous, thus reducing the frequency of suitable infection courts presented by broken or dead branches, especially those of large diameter (Lee et al. 1988; Mahmud et al. 1993; Ito and Nanis 1994, 1997; Lee 2002).

Assessment of intra and inter specific susceptibility to heart rot in acacia

It has been suggested that provenance affects susceptibility to heart rot (Ito and Nanis 1994). As mentioned above, provenance differences in tree architecture, especially branching, may influence heart rot. A comparison of polyphenols between

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A. mangium (heart-rot susceptible) and *A. auriculiformis* (heart-rot resistant) showed that some components are toxic to heart-rot fungi when at certain concentrations (Barry et al. 2005; Mihara et al. 2005). Wood extractive content may also vary intra-specifically with provenance and result in differing levels of heart rot.

Two trials clearly demonstrated that the effect of provenance was statistically significant for heart-rot incidence (Barry et al. 2006). The larger study in Riau assessed natural heart-rot incidence by felling trees and assessing a stem disk at 1.5 m height. In the smaller provenance trial in South Sumatra, trees were artificially inoculated. This involved creating stem wounds (drill holes), which were then inoculated with fungi capable of causing heart rot. This method offered a controlled way of testing for provenance susceptibility, as the fungal species inoculated and the size of the infection court were standardised. However, a range of other fungi infected the wounds after inoculation. While both trials revealed that provenance could influence heart-rot incidence, wood extractive content was not correlated with heart rot.

Root rot

Signs

Several types of woody root rots have been recognised in *A. mangium* and are characterised as red, brown, black or white root diseases according to the colour of the fungal tissue and/or roots that are infected. These diseases are associated with various basidiomycete fungi including *Amauroderma* cf. *parasiticum*, *Ganoderma* spp. (red rot), *Phellinus noxius* (brown rot), *Rigidoporus lignosus* (white rot) and *Tinctoporellus epimiltinus* (brown rot) (Almonicar 1992; Old et al. 2000; Lee 2002; Glen et al. 2006). Species of *Ganoderma*, however, have been reported as the most frequent root-rot causal agents in *A. mangium* in both Malaysia and Indonesia (Lee 2002). Until now, identification of the *Ganoderma* species associated with root disease has been based on morphological and taxonomic features of fruitbodies (Figure 3a). Roots affected by *Ganoderma* spp. may be covered by a reddish-brown rhizomorphic skin (Figure 3b) that is visible after the roots are washed clean of associated soil. Using molecular technology, *Ganoderma philippii* was unequivocally identified as a causative agent of red root rot (Glen et al. 2004, 2006). A white mottling pattern of mycelium is seen on the underside of the infected bark (Figure 3b) and there is a very characteristic odour. In fast-grown plantations, characteristic fruitbodies may be absent in disease centres (Lee 2000; Old et al. 2000). A species of the *G. lucidum* complex is associated with a more rubbery rhizomorphic skin than that found in red root rot, a skin which is yellow and brown on the outside with a blistered appearance and rubbery white on the inside when pulled away from the root wood (M. Glen, pers. comm.).

Symptoms

Most root-rot diseases cause similar, generally non-specific, above-ground symptoms in the host, including crown dieback and reduced growth. There is a general decline in the crown condition and the growth rate is poor. In particular, the foliage of affected trees becomes chlorotic (paling of the green

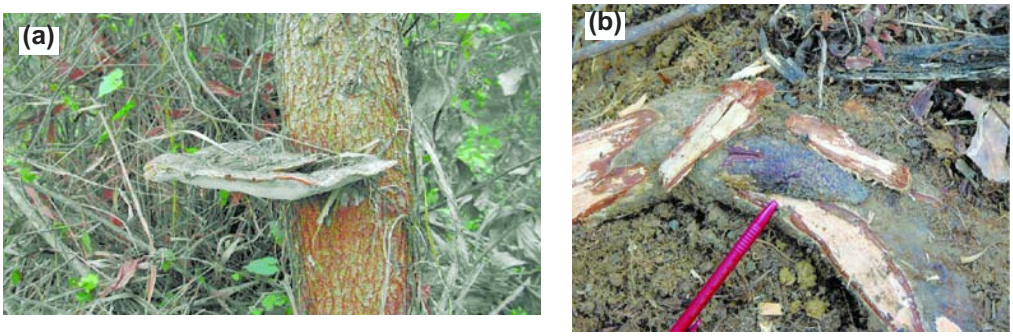

Figure 3. (a) *Ganoderma philippii* basidiocarp at the base of a dead *Acacia mangium* tree growing in East Kalimantan, Indonesia; (b) characteristics of red root rot (red bumpy skin when soil is washed away) caused by *G. philippii* on *A. mangium* with white mottling of mycelium seen on underside of bark

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coloration), much reduced in size and sparse due to reduced water and mineral uptake. Young shoots may wilt and, in trees in advanced states of root rot, foliage loss increases and trees become very susceptible to wind throw (Ariffin et al. 2000; Kile 2000; Leckie et al. 2004; Morrison et al. 2000; Old et al. 2000).

In forest stands, diseased trees tend to be clustered in patches that are roughly circular, due to the spread of root rot from around an initial inoculum source (Figure 4). Trees with advanced infections are located at the infection centre, while trees with less-advanced infections are around the periphery. These circular disease centres increase in diameter as root contact occurs between neighbouring diseased and healthy trees (Bolland 1984; Garbelotto et al. 1997; Kile 2000).

Quantitative detection using ground-based surveys

Surveys are essential as a management tool to determine if disease incidence is severe enough to warrant control practices, but also as a study tool, or for epidemiological modelling. Surveys can utilise the above-ground symptoms of root rot (e.g. crown condition, resin exudates on bark) or involve checking for signs of root infection by excavating around roots. Field-based surveys of root rot can be completed quickly for small areas or trials, but are time-consuming at the large scale of a commercial plantation. It is therefore useful to investigate efficiencies of different survey methods to save monitoring time and costs.

Some surveys have utilised a mixture of above-ground symptoms and root-infection inspections. Irianto et al. (2006) conducted a survey at different sites in Indonesia by recording the incidence of root rot in two adjacent rows of *A. mangium*, missing the next three rows and then repeating this survey pattern until a certain count was reached to represent a 40% sub-sample of a randomly selected area of the compartment. In East Kalimantan, for example, trees were assessed until a count of 200 was reached, the count therefore representing a 40% sub-sample of 500 trees. Roots were uncovered and inspected for typical symptoms of root rot (red) in trees that were standing dead, or exhibited chlorotic/yellowing foliage, loss of foliage, or fruitbodies on the stem. Trees in which root rot was confirmed by this method, plus any missing trees, were scored as a positive result. Missing trees were attributed to root rot. Results were recorded as presence/absence of root rot, then the numbers in each of the two categories were summed and expressed as a percentage. During field surveys, observations on the pattern of root-rot infections (i.e. size of root-rot disease foci) were recorded. While this survey method was time-consuming, it ensured a very accurate assessment of root-rot incidence.

Figure 4. Typical circular patch of tree mortality (3–5 trees) in a 4–5-year-old *Acacia mangium* plantation, East Kalimantan, Indonesia

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Surveys are useful not only to provide quantitative data from one time point but also as a basis for model development (Nandris et al. 1996). Barry et al. (2005) modelled six years of spatial survey data of rubber trees infected with *Rigidoporous lignosus* and *Phellinus noxius.* Using the spatial information that was collected while testing different control methods, models were determined to estimate root-rot spread and effectiveness of control measures. Successive surveys conducted in *A. mangium* plantations infected by *Ganoderma* spp. (Lee 2000) showed that the rate of disease progression in Malaysian plantations was dependent on the initial incidence of root rot. Root-rot foci enlarged over time.

Assessment of root rot in an *A. mangium* provenance/progeny trial in Indonesia (Irianto et al. 2006) revealed that infected trees were randomly distributed at a first time point but these trees or disease foci tended to become aggregated by the time of a second survey. This highlighted the high probability that exists of rapid vegetative spread by the fungus after its initial introduction to the site; the incidence of tree death doubled between 2003 and 2004. While this provenance/progeny trial is a non-commercial plantation, the results have implications for the potential rate of spread of root rot in plantations. Lee (2000) found that for first-rotation plantations grown on lowland ex-forest sites, mortality due to root rot in *A. mangium* in Malaysia could double within one year. The incidence of other root-rot fungi such as *Armillaria ostoyae* in pine forests has also been reported to double within one year (Lung-Escarmant and Guyon 2004).

Detection methods for the future

As ground surveys are time-consuming, aerial or remote methods may be suitable for detection of root rot. Remote sensing of plant health is currently based mainly on detection of chlorophyll content. Foliage thinning and chlorosis are common symptoms of root rot and, in Douglas-fir, chlorophyll *a*, nitrogen and moisture were consistently reduced in trees infected by *Phellinus weirii* (Thomson et al. 1996). In beech trees infected with the root rot caused by *Phytophthora* spp., total chlorophyll was reduced three-fold in heavily infected plants compared with controls (Fleischmann et al. 2004). The application of a high-resolution multi-spectral imagery was recently shown to be a feasible for detecting laminated root-rot centres in Douglas-fir in Canada (Leckie et al. 2004). While remote sensing methodologies require considerable development for reliable detection of specific diseases, they offer the potential for rapid and robust quantification. The symptoms of root rot in *A. mangium* are suitable for adaptation to remote sensing.

Conclusions

For both of these important diseases of *A. mangium*, assessment methods need to be rapid and accurate. Heart rot requires destructive assessment, but this can be done during thinning operations or at harvest. Root rot can be assessed non-destructively but root excavation is required for verification of the disease. Root-rot assessment methodology is therefore both laborious and costly, more suited to research investigations which do not necessarily require full spatial coverage of a forest plantation estate. Remote sensing of root-rot incidence and severity could offer 100% coverage. Knowledge of disease incidence and severity (and patterns of spread in the case for root rot) is important for developing effective disease management strategies and for calculating economic impact.

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The mycology of the Basidiomycetes

Ian A. Hood[1]

Abstract

A number of significant tree and plantation root diseases in tropical Asia are caused by certain species of basidiomycetes. This important group of fungi, which includes such familiar forms as mushrooms, toadstools, bracket, shelf, and crust fungi, puff balls, earthstars, and coral fungi, is characterised by the production of sexual spores on the outside of a microscopic structure called the basidium. Basidiomycetes occupy many niches in the environment, including decomposing litter, decaying wood and soil organic matter, in a variety of habitats such as forests and open countryside. Some species form beneficial mycorrhizal relationships with the roots of host trees, but others are important pathogens of the foliage, stems or root systems of different tree species. Basidiomycete species are traditionally identified by the form and microscopic structure of their fruitbodies, and by their appearance when isolated in laboratory culture. Important root diseases of trees in Indonesia are caused by the basidiomycete fungi *Rigidoporus microporus*, *Junghuhnia vincta*, *Phellinus noxius*, and certain species of *Ganoderma*.

Mycology is the scientific study of fungi. It is distinguished from, but related to *plant pathology*, the study of plant diseases, a large proportion of which are caused by harmful fungi (fungal pathogens).

Fungi are a very large, diverse group of living organisms found in nearly all ecosystems. Fungi are *eukaryotes*, that is, they have microscopic organelles within their cells called *nuclei* which contain genetic material in the form of thread-like *chromosomes*, enabling hereditary characters to be passed on to subsequent generations. Other eukaryotes include all members of the plant and animal kingdoms, whereas life forms such as bacteria and blue–green algae, whose genetic material is not held in a nucleus, are called *prokaryotes* (Table 1).

Recent study has shown that some of the organisms we call *fungi* actually belong to other groups. Those no longer recognised as *true fungi*, include fungus-like organisms such as the *myxomycetes* (slime moulds) and the *oomycetes* (which feature the downy mildews and microscopic root-infecting fungi such as species of *Phytophthora* and *Pythium*). Most of the true fungi exist and grow in the form of a spreading network or *mycelium* of living tube-like threads called *hyphae* (though yeasts develop as budding cells). The hyphae branch as they grow from the tip, and feed *heterotrophically*. This means that fungi cannot photosynthesise like plants and algae, which are *autotrophic*, but live either *saprobically* on dead organic matter or *symbiotically* in association with other living organisms. The latter relationship (*symbiosis*) may be harmful to the living plant or animal host (*parasitic*), mutually beneficial (as in lichens and mycorrhizas), or neutral (*commensal*, as in fungi that grow as *endophytes* within living plant leaf, root or stem tissues without causing harm).

Fungi reproduce and spread by means of sexual and asexual *spores*, both of which may be released in large numbers. In one of the two largest groups of true fungi, the *ascomycetes*, sexual ascospores are produced within a sac-like structure called the ascus. Among the larger ascomycetes are the disc and cup

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fungi and a specialised category of fungi called the lichens that form a symbiotic relationship with certain green algae. In the second large group, the *basidiomycetes*, sexual basidiospores are produced on the outside of a modified cell called the *basidium*. Ascomycetes such as *Kretzschmaria zonata* (synonym, *Ustulina zonata*; sometimes incorrectly as *U. deusta*) and *Corallomycetella repens* (synonym, *Sphaerostilbe repens*) may attack roots of hardwood crops such as rubber, tea or cacao, but basidiomycete fungi are the most common causes of root diseases and decay in natural forests and plantations.

The nature of the basidiomycetes

The basidiomycetes make up a huge variety of fungi (ca. 23,000 species are known). Determining their taxonomy has been controversial because the number of good distinguishing characters is limited, and there is uncertainty and disagreement as to which features are more important for separating the different sections and even species. The recent upsurge in DNA technology has provided a powerful addition to traditional taxonomic methods.

Most mycologists separate two major groups, the 'rusts' and the 'smuts', from among the remaining basidiomycetes (Table 2). Both contain a large number of specialised microscopic parasitic fungi whose mycelium grows within the foliage, twig or stem tissues of flowering plants, conifers or ferns. They have complicated life cycles featuring, in the case of the rusts, up to five different spore types that allow the fungus to alternate between different hosts. However, many rust species have reduced life cycles

Table 1. How the basidiomycetes relate to other living organisms

– prokaryotes (nucleus absent)	– bacteria	
	– blue–green algae	
	– other prokaryotes	
– eukaryotes (nucleus present)	– animals	
	– plants and red and green algae	
	– slime moulds (several groups)	
	– water moulds (oomycetes) and brown algae	
	– true fungi	– ascomycetes (including lichens)
		– basidiomycetes
		– other true fungi once grouped in the former 'phycomycetes' (glomeromycetes, zygomycetes, chytridiomycetes)
	– other eukaryotes	

Table 2. A simplified, artificial classification of the basidiomycetes

– rusts				
– smuts				
– others	– heterobasidiomycetes (divided basidium; e.g.wood ears, jelly fungi)			
	– homobasidiomycetes (simple, undivided basidium)			
		– 'gasteromycetes' (e.g. puff balls, earth stars, stink horns, birds-nest fungi)		
		– 'hymenomycetes'		
			– Dacrymycetales	
			– 'Agaricales' (broad sense, e.g. mushrooms, toadstools, boletes)	
			– 'Aphyllophorales' (e.g. bracket and shelf fungi, corticioid fungi, toothed and spined fungi, coral fungi)	
			– Others	

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that lack some spore states and occur on only one host. The remaining basidiomycetes, the 'others' (Table 2), include the larger forest fungi e.g. mushrooms, toadstools, jelly and bracket fungi. Many members of this group are linked in common by a unique type of septum (the cross wall that divides the hyphae into separate cells) known as the dolipore septum. These fungi fall into two sub-groups; the heterobasidiomycetes, in which the basidium initial is partitioned by walls into four cells following meiosis (Figure 1), and the homobasidiomycetes, in which it remains undivided as a single cell.

The homobasidiomycetes were once conveniently separated into the hymenomycetes, whose spores are actively ejected from the basidium in the expanded fruitbody (basidiocarp), and the gasteromycetes, whose spores are held passively. It is now generally accepted that both groups are artificial, being composed of a heterogeneous collection of different families, though for practical purposes they are still used in an unofficial way as helpful, readily understood categories. The gasteromycetes include the puffballs and earth stars, which rely on the impact of falling rain drops to puff their spores into the air; the unpleasantly smelling stinkhorn; veil and basket fungi that attract flies for spore dispersal; and the birds nest fungi that also depend on the force of falling rain to splash their 'eggs' (packets of spores) over a considerable distance. The hymenomycetes incorporate the Dacrymycetales (small, brightly coloured, jelly-like fungi that can be rewetted and rejuvenated after drying down, and which have a forked, two-spored basidium), the traditional Agaricales (also known as agarics — gilled mushrooms and toadstools, and poroid boletes), and the Aphyllophorales. Although both the Agaricales and Aphyllophorales are also heterogeneous groups containing a variety of only distantly related families, they too are still in use as convenient, all-embracing, informal categories. In the agarics, the basidia are produced on the gill surfaces. The spores, when released, fall under gravity, emerge from between the vertically orientated gills, and are blown away in the wind. The Aphyllophorales comprise many different forms including horizontal bracket and shelf fungi, sheet or crust fungi (corticioid), toothed fungi (with vertically hanging spines or teeth), and coral fungi (with erect antler- or tree-like branches). The life cycle of a typical aphyllophoroid basidiomycete, showing also a typical simple, homobasidiomycete basidium, is portrayed in Figure 2. A comparatively small number of basidiomycete species also produce asexual spores (e.g. the important Northern Hemisphere root disease fungus, *Heterobasidion annosum*).

Basidiomycetes in the environment

Like other fungi, different basidiomycetes feed saprobically or symbiotically. Saprobic basidiomycetes occur in forests (in the soil, in leaf or twig litter, or as wood colonisers in standing trees or fallen stems), in open country (also in soil or decomposing litter), and in many other places. Many basidiomycete fungi serve an important ecological role as wood decomposers (see Mohammed et al. 2006). A number of small toadstool species occupy a specialised position, along with other fungi, in colonising animal dung (coprophily), and species of *Termitomyces* (a genus of toadstool fungi) are found within termite nests.

Another important ecological niche is filled by certain agaric species (mushrooms and toadstools) and also other types of subterranean basidiomycete (e.g. *Rhizopogon* species) which beneficially infect

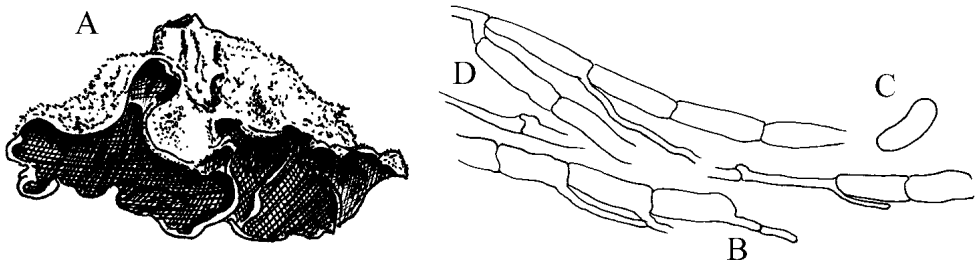

Figure 1. (A) Fruitbody, (B) septate basidia, (C) detached spore, and (D) clamped hyphae of *Auricularia* spp. (wood ear fungi), within the heterobasidiomycetes. After Hood (2003)

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the small roots of living trees forming an ectomycorrhizal association (one of several types of mycorrhiza). In this relationship, the fungus benefits by receiving carbohydrates from the host, while the mycelial network extending out into the soil from the infected root system provides the host with an enhanced supply of nitrogen and phosphorus, particularly advantageous in nutrient depleted soils.

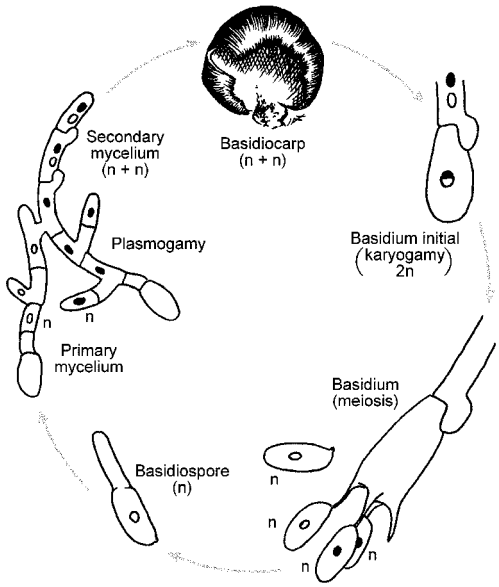

Figure 2. Lifecycle of a typical hymenomycete. Plasmogamy is the fusion of one cell from each of the paired primary mycelia without union of their nuclei; as a consequence, the new cells in the resultant secondary mycelium each contain two haploid nuclei, $n + n$. Karyogamy is nuclear union resulting in a single combined diploid, $2n$, nucleus within the cell; the haploid state is restored by nuclear division through the process of meiosis in the basidium, allowing re–assortment of the genetic material among the progeny. After Gadgil (2005)

Certain parasitic basidiomycete fungi cause important tree diseases. Examples of stem disorders include, in the tropics, pink disease (caused by *Erythricium salmonicolor*; synonyms, *Corticium salmonicolor*, *Phanerochaete salmonicolor*) and in temperate regions, silver leaf disease (caused by *Chondrostereum purpureum*). A number of agaric species belonging to *Crinipellis* and *Marasmius* are associated with leaf and twig blights in tropical forest canopies and understoreys. *Crinipellis perniciosa* and *Mycena citricola* cause witches broom and leaf spot diseases of cacao and coffee, respectively, in central and South America. Rusts and smuts cause serious diseases of major food crops. Rusts infecting *Acacia* species include *Racospermyces digitatus* (synonym, *Atelocauda digitata)*, which causes distortion and malformation of the phyllodes of species such as *A. mangium*, and *Uromycladium notabile* and *U. tepperianum*, which produce globose galls and eventually dieback on bipinnate and phyllodinous acacias.

In temperate zones, important root diseases of plantation forests are caused by species in the *Heterobasidion annosum* complex and by species of *Armillaria*. Armillaria root disease often has more impact in cooler, higher elevation forests in the tropics, while at lower altitudes, significant root diseases are caused by *Rigidoporus microporus* (synonym, *R. lignosus*), *Junghuhnia vincta* (synonym, *R. vinctus*), *Phellinus noxius*, and species of *Ganoderma*.

Identifying basidiomycetes

The body or thallus of the basidiomycete fungus (the mycelium) is normally hidden within the substrate, and it is generally only the fruitbody or basidiocarp that is visible at the surface. For this reason, and because the fruitbody tends to show the greatest morphological variation, conventional mycology relies on a number of macroscopic and microscopic features of the fruitbody to distinguish between species. A selection of the more usual of these characters is catalogued below, concentrating particularly on the Aphyllophorales (e.g. corticioid and polypore fungi), which are more likely to be associated with root disease and wood decay in hardwood plantations. The use of fruitbody morphology in distinguishing between fungi is sometimes augmented by other methods such as the appearance and form of the fungus in pure culture (see below); physiological factors, such as the degree of virulence to different hosts, environmental growth preferences (nutrients, temperature); and by relationships at the molecular level, as shown in protein profiles (e.g. immunological or isozyme analysis) and DNA sequencing.

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Macroscopic features of the basidiocarps

Longevity: annual or perennial (surviving one or more than one year).

Texture: soft and fleshy, gelatinous, cartilaginous, brittle, corky, leathery, or woody; dry, moist, or sticky; upper surface smooth, velvety, hairy or scaly.

Colour of internal tissues (context and spore-bearing component): white, pale brown, dark brown, or other (e.g. black, red).

Form: pileate (projecting out from the substrate surface), resupinate (forming a flat sheet or crust), or effused-reflexed (forming a shelf with the base extending down over the substrate as a flat sheet); if pileate: simple or compound; stalked (stipitate) or sessile; solitary or clustered (possibly imbricate, i.e. several shelves overlapping one above the other); if stalked, attached at the side or centrally.

Spore and basidia-bearing surface: smooth, folded or warty, usually forming the fruit body under surface (e.g. corticioid fungi); lining vertical gills or lamellae (e.g. agaric fungi; *Lenzites*, *Panus*, *Pleurotus* species); lining vertical, downward-directed pores (e.g. polypore fungi; boletes); on vertically hanging teeth or spines (hydnoid fungi); on erect branches (e.g. ramarioid fungi) etc.

Dimensions: size of fruitbody; width of context, pores, lamellae.

Host: the identity or type of host supporting a fructification (fruitbody) may be important.

Nature of decay associated with a basidiocarp on wood: white, fibrous or stringy (simultaneous delignification); brown, cubically fractured; pocket rot (selective delignification); appearance of any zone lines (pseudosclerotial plates).

The fruitbodies of agaric basidiomycetes (mushrooms and toadstools) are distinguished initially by their spore colour (as revealed in the spore deposit); the presence or absence of a ring around the stalk or stipe, and a cup-shaped structure called the volva at its base (the partial and universal veils); the nature of the substrate (soil or wood); surface texture (smooth, velvety, scaly; dry, sticky, with fibrils etc.); and the form of the gill attachment to the stipe.

Microscopic features of the basidiocarps

The macroscopic form of basidiomycete fruitbodies may vary considerably according to situation, and the overall appearance can sometimes be misleading without a microscopic examination to confirm the identification. Some of the microscopic characters include the following:

Hyphal composition of fruitbody tissues (Figure 3):

In simple terms there are three types of hyphae present in most corticioid and polypore fungi.

Generative hyphae: thin, or occasionally thick-walled, branched hyphae, with septa (cross walls) found in all fruitbodies. Called generative, because they give rise in some species to other types of hyphae. Septa may be simple (i.e. lack clamps) or with one, or sometimes more structures called clamp connections at each cross wall (the clamp connection is a hyphal outgrowth that bridges two adjacent cells resulting from cell division, enabling one of the daughter nuclei to pass from one cell to the other; clamps are unique to, and help to identify many basidiomycetes, but not all basidiomycetes have clamps; illustrated in Figures 1, 2 and 3).

Skeletal hyphae: long, thick-walled or solid, unbranched (or sparingly branched in some species) hyphae lacking septa, derived from generative hyphae.

Binding hyphae: much-branched, thick-walled or solid hyphae lacking septa, derived from generative hyphae. May be narrower than skeletal hyphae.

These hyphal types allow for three kinds of fruitbody composition, which are characteristic of particular genera and species:

Monomitic: composed only of generative hyphae (which in monomitic construction also provide support; characteristic, therefore of many non-woody fruitbodies).

Dimitic: composed of generative and either skeletal or binding hyphae (which provide the support).

Trimitic: composed of all three types of hyphae (support being provided by skeletal hyphae bound together by interweaving binding hyphae).

Dimitic and trimitic types of construction are characteristic of more woody, perennial fruitbodies, in which generative hyphae are more easily found at the still developing areas such as the fruitbody margin, hymenium (spore-producing layer), or pore mouth. When identifying species, it is also important to record the dimensions, colour, and colour reactions of the different types of hyphae.

Nature of the hymenium:

The hymenium is the microscopic layer containing the basidia and spores. Species can be distinguished by the form and size of the basidium (including the number of sterigmata or appendages that bear the

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spores, usually four, and whether there is a clamp present at the septum at the base), the presence and character of other structures, such as cystidia, metuloids (lamprocystidia) and cystidioles (Figure 3), and the appearance of the basidiospores, if present. Spores are distinguished by their shape and size, surface ornamentation (smooth, warted etc.), colour, and colour reaction to an iodine-based chemical preparation called Melzer's reagent (blue, red–brown, or non-reactive).

The character and appearance of the spores and various elements such as cystidia and metuloids that may be present in the hymenium layer are also important features for distinguishing agaric species, as is the internal arrangement and structure of the hyphae within the gills or lamellae.

Identification in culture

Very few basidiomycete fungi fruit in artificial culture, so although different species can be distinguished from one another by recording their vegetative appearance and structure during growth, it is frequently not possible to name them, even when comparison is made with a number of published culture descriptions currently available (see Bibliography). Under these circumstances, one way to identify an unknown culture is to match it with others isolated from the tissues of verified fruitbodies. There are also several compatibility tests that can be employed, such as the Buller test. This makes use of the observation that when an unknown field isolate is paired in culture with single-spore test isolates of known identity, a positive interaction will occur only

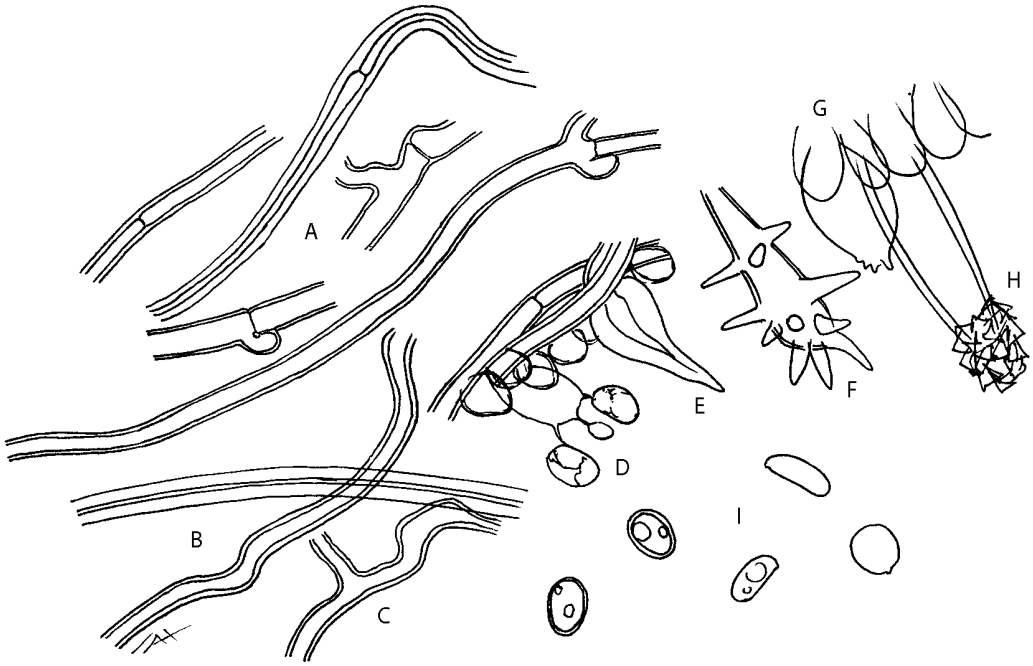

Figure 3. An assortment of microscopic elements from basidiomycete fruitbodies. The left half of the figure is composed of hyphae: (A) generative hyphae, with and without clamp connections at the septa are situated top left, and (B) two skeletal hyphae and a (C) branched binding hypha are at the lower left. At the centre and top right are sections of two hymenial (spore-bearing) surfaces with a (D) non-septate basidium (and attached spores) and a pointed (E) seta (centre), and on the right (left-to-right) are a specialised (F) setoid element with spines, a (G) cystidiole and a (H) crystal-encrusted lamprocystidium (metuloid). Various basidiospores (I) belonging to different species are present lower right.

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with isolates of the same species, thus allowing a successful identification of the field isolate. A positive interaction is indicated by a change in the nature of the test isolate from the haploid to dikaryotic condition (cells with two nuclei, $n + n$, Figure 2) as a result of the movement of nuclei from the field culture. This change can be detected by microscopic examination (e.g. by the appearance of hyphae with clamps at septa within the test isolate, which occurs only when the hyphae become dikaryotic), and sometimes by changes in the visible appearance of the test culture itself. Again, the uniform merging of two dikaryotic cultures without the formation of an incompatibility barrier zone may indicate that the cultures belong to the same genotype and therefore to the same species, which also enables identification if the identity of one of the cultures is known. However, this method has its limitations, and a control pairing using a different genotype of the same species should form part of the procedure to demonstrate the appearance of a clear zone of incompatibility.

The rapid development of molecular technology has provided another approach which is already being used to identify unknown basidiomycete isolates, by comparing DNA profiles with those of cultures from authenticated fruitbodies. As with cultural methods, which are limited by a lack of available culture descriptions, it is still necessary to build up reference libraries of DNA profiles of accurately identified species.

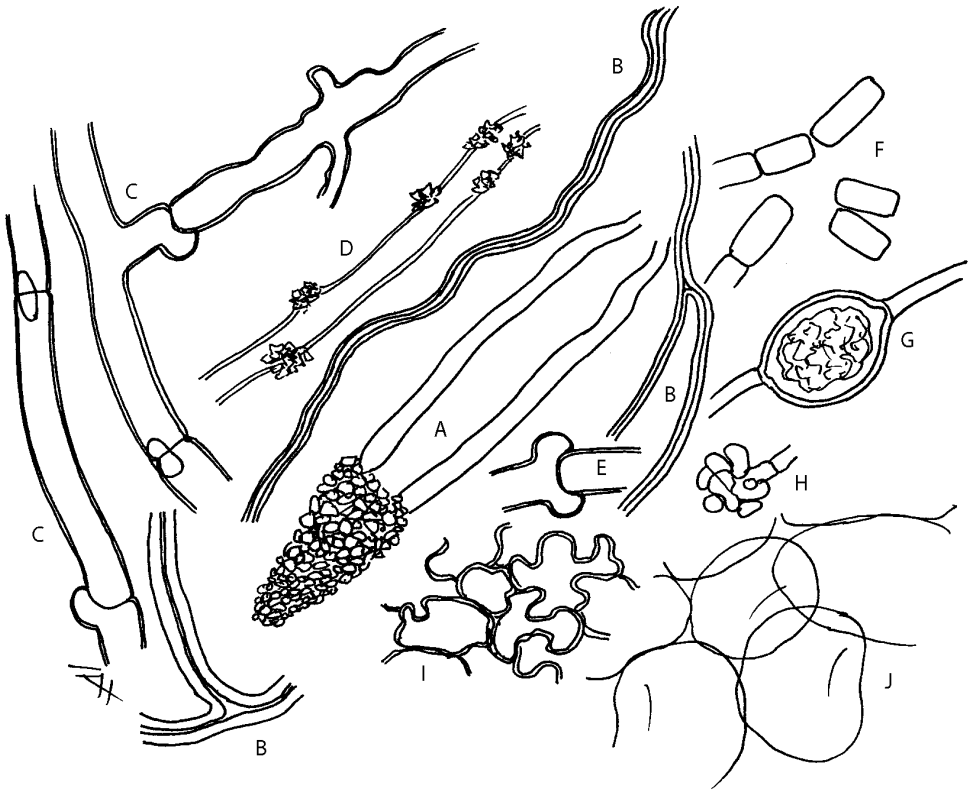

Figure 4. A collage of micro-elements from basidiomycete cultures. Straddling a crystal-encrusted lamprocystidium (A – centre) are three (B) thick-walled, non-septate skeletal hyphae (branched and unbranched; binding hyphae are not distinguished in cultural descriptions). To the left are (C) clamped generative hyphae and a hypha with (D) encrustations of crystals; (E) a hypha with two clamps at the septum is on the right. At the top right are vegetative propagatory microstructures: (F) oidia (arthrospores), above, and a (G) thick-walled, intercalary chlamydospore. At lower right is a (H) 'hyphal knot', (I) a jigsaw-like tissue of interlocking hyphae, and a cluster of (J) rounded cuticular cells.

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The procedures for describing basidiomycete species in culture rely on features such as the growth rate on standard medium, colony colour, appearance, odour, and colour reaction to a selection of reagents. Microscopic characters include the presence or absence of clamps at the colony margin and in the older culture, various types of hyphae and propagules, and a number of other distinctive microstructures (Figure 4).

Some basidiomycetes causing root disease in tropical hardwood plantations

The following basidiomycetes, all in the Aphyllophorales, cause significant root disease or heart rot in tropical forests and plantations. All are known in Indonesia. Brief descriptions are given to emphasise key macroscopic and microscopic distinguishing features. Unfortunately, fruitbodies may not appear on infected trees until disease is well advanced, so identification generally relies on field symptoms or the isolation of characteristic cultures. Many basidiomycete species have changed their names over the years as more has been learned about their relationships, so some older synonyms are also provided to help avoid confusion.

Rigidoporus microporus (synonyms, *R. lignosus*, *Fomes lignosus*)

Fruitbody a broad (to 20 cm wide), relatively thin, annual to less frequently perennial, leathery, broadly attached shelf, clustered, often imbricate; upper surface, concentrically furrowed, initially orange–red–brown, faintly velvety, later smooth, faded; lower surface bright orange–brown, eventually paling, pores fine (6–9 per mm). In section, context pale coloured. Monomitic, generative hyphae thin- or thick-walled, with cross walls (septa), without clamps, hyaline (colourless). Hymenium with cystidioles (Figure 3), basidiospores sub-globose, thin-walled, colourless, smooth, non-staining in Melzer's reagent (inamyloid), 3.5–4.5 × 3.5–4 µm. Unlike the related *R. lineatus*, has no encrusted metuloids (lamprocystidia) in either fruitbody or culture. Causes a root disease of planted trees (e.g. rubber, *Hevea*); produces white, branching mycelial cords and a white rot.

Junghuhnia vincta (synonyms, *Poria vincta*, *Rigidoporus vinctus*) (Figure 5)

Fruitbody a pinkish (variety *vincta*) or greyish-brown (variety *cinerea*) resupinate crust; pores fine (6–12 per mm). Dimitic, generative hyphae thin-walled, with cross walls (septa), without clamps, hyaline (colourless); skeletal hyphae thick-walled, slightly coloured, without septa. Hymenium with cystidioles and encrusted metuloids; basidiospores sub-globose, thin-walled, colourless, smooth, non-staining in Melzer's reagent (inamyloid), 3.5–5.5 × 3–4 µm. Occasional clamps in culture. May fruit in older culture. Causes a root disease of planted trees. Produces a white rot.

Phellinus noxius (Figure 6)

Fruitbody a cinnamon-brown, broadly attached, woody shelf, developing a blackish crust on the upper surface, or a similar coloured resupinate sheet. Lower surface, greyish to dark brown, pores fine (6–8

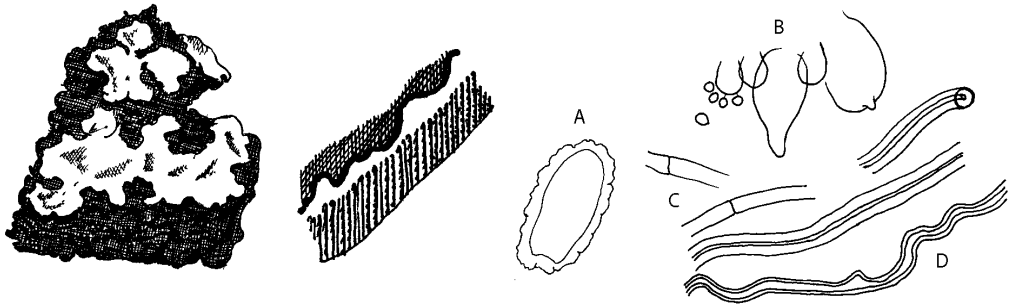

Figure 5. *Juhnghuhnia vincta*. Left-to-right, poroid, fruitbody crust; section of fruitbody showing pore zone; microscopic elements of fruitbody, including a (A) sectioned metuloid, (B) hymenium zone with basidium, spores, and two cystidioles; (C) two thin-walled hyphae with septa and (D) three thick-walled, non-septate skeletal hyphae. After Hood (2003)

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per mm). Tissues golden-brown within. Dimitic, with narrow generative hyphae having septa that lack clamps, and wider, thick-walled, non-septate, golden-yellow skeletal hyphae. No setae (Figure 3) are present in the hymenium (as in some other *Phellinus* species), but characteristic long, thick-walled, pointed, non-septate, deep red–brown setal hyphae (tramal setae) are found within the tissues. Spores smooth, ovoid, colourless, 3.5–5 × 3–4 µm. Fruitbodies are not always seen, but infected trees are recognised by a characteristic brown mycelial weft (with setal hyphae present) or a black mycelial crust that forms on infected roots and develops collar-like around the base of the stem. Decayed wood eventually becomes pale and honeycombed in sheets, with brown zone lines.

Phellinus noxius belongs to the Hymenochaetaceae, a family characterised by fungi with cinnamon-brown or golden yellow tissues, and generative hyphae without clamps. *Phellinus* species are all dimitic. Most do not cause disease, but many are responsible for heart rots in living trees on which they produce prominent shelves or hoof-like brackets.

Ganoderma philippii (synonym, *G. pseudoferreum*) (Figure 7)

Fruitbody a broadly attached, woody shelf, concentrically furrowed and warty above, smooth, semi-glossy in parts, coloured dark reddish- or purplish-brown, with a narrow, white margin; white or eventually brownish, beneath, with medium-fine pores ((4–)6–7 per mm). Tissues brown. Often grouped, imbricate. Trimitic, with narrow generative hyphae, having septa with clamps; wider, thick-walled, non-septate, brown skeletal hyphae that branch near the ends; and narrower, thick-walled, brown, branching binding hyphae. The skeletal hyphae are the most obvious within the tissues, and clamped generative hyphae are not easily seen. Spores double-walled, with fine spines between the two layers, apex truncated, ovoid, pale brownish, 6–9.5 × 4–8 µm. Causing root disease in hardwood hosts (e.g. rubber, cacao, tea). Infected roots are coated in a reddish or reddish-brown mycelial crust which has a creamy-white rhizomorph-like growing margin. Produces a white rot.

Ganoderma philippii belongs to the Ganodermataceae, a family characterised by a dimitic or trimitic hyphal system with clamped generative hyphae (often difficult to find), and especially by a characteristic spore with tiny spines or echinulae positioned between two walls. The family includes *Amauroderma*, with species that have stalked fruitbodies, and *Ganoderma*, which forms annual or perennial, corky or woody brackets. *Ganoderma* species are divided into two groups, those with a non-shiny upper fruitbody surface formed of interwoven hyphae (e.g. *G. philippii*, *G. australe*, Figure 7, upper left), and others, often stalked, with a shiny, lacquered surface composed of a palisade of hyphal ends (Figure 7, centre; e.g. *G. cupreum*, *G. steyaertanum*, Figure 7, centre, upper right). *Ganoderma* species are highly variable and difficult to separate into reliable taxa using conventional morphology. While some species in both groups are well understood, other 'species' can still not be reliably resolved, and many laccate forms will probably prove to be synonyms of other taxa. Under these circumstances, the precise identification of the species responsible for root disease in *Acacia mangium* plantations is a considerable challenge. The genus *Ganoderma* is currently 'in taxonomic chaos' (Ryvarden 1991, 1995), but it

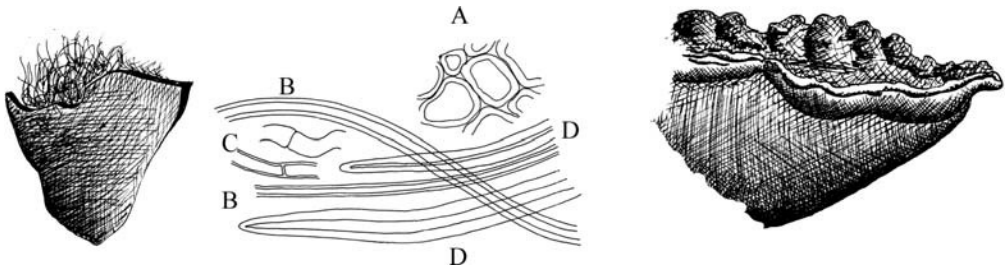

Figure 6. *Phellinus noxius*. Fruitbody (right) and segment of crust (left) taken from a diseased root. Centre, microscopic features, including (top to bottom) (A) dark-brown rounded cells from mycelial crust, (B) skeletal hyphae, (C) two thin-walled, septate, clamp-less, generative hyphae, and (D) two thick-walled, setal hyphae with pointed ends. After Hood (2003)

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appears that the use of molecular technology will soon result in a more satisfactory situation. Some species of *Ganoderma* are saprobes, causing heart rots in living trees and decaying fallen wood (e.g. *G. australe*, *G. cupreum*), while others are harmful parasites (e.g. *G. philippii*, *G. steyaertanum* and the European laccate species, *G. lucidum*).

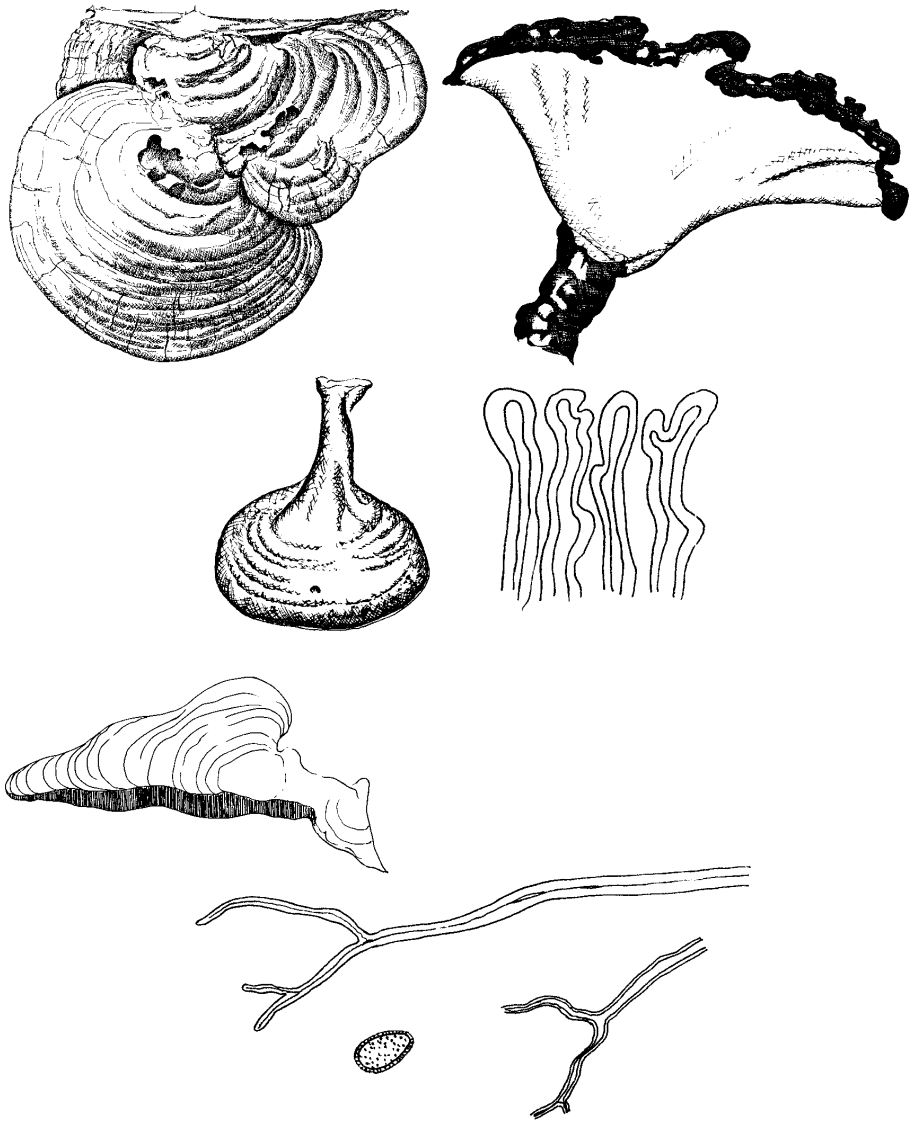

Figure 7. *Ganoderma* species. Fruitbodies of *G. australe*, with a dull upper surface (top left), and *G. steyaertanum* (top right) and *G. cupreum* (centre left), both with a shiny red laccate surface, turning into a black crust in the case of the latter. Section of *Ganoderma* sp. fruitbody (lower left). Microscopic features: section of upper surface of fruitbody with a laccate crust (centre right), and distinctive spore and branched ends of two skeletal hyphae (lower right). All three species are reported from Indonesia and elsewhere. After Hood (2003)

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The use of DNA techniques to identify fungi

Morag Glen[1]

Abstract

DNA provides an abundance of taxonomic characters for the identification of organisms that have inadequate morphological characters, or possess distinguishing features only during particular stages of their life cycle. Methods of accessing these DNA characteristics vary, though variations of PCR-based techniques are popular at present, because of their speed, sensitivity and high throughput capability. Several popular methods are described that have been applied in diverse areas including food quality, forest ecology, and human, animal and plant pathology. The method selected for a particular application will depend on several factors, including the number of samples and number of candidate species. All DNA-based techniques depend on an adequate herbarium resource of carefully preserved specimens with detailed morphological descriptions to verify the DNA-based protocol.

DNA techniques are a valuable tool in the identification of fungi. They are particularly useful as a means to determine the identity of a fungus when it is not producing fruitbodies, the morphological taxonomic characters that form the basis of the species description. While some fungi can be grown in culture with ease and produce characteristic microscopic features that assist in taxonomic identification, others are difficult to grow in culture or, when grown in culture, fail to develop key distinguishing features. The need for rapid identification, as in quarantine or biosecurity applications, may also justify the use of DNA techniques.

DNA techniques for the identification of fungi have been widely used in human and veterinary medicine, where rapid and accurate identification of fungal pathogens assists the selection of appropriate treatment. They have also been applied to food quality control for the detection of contaminating fungi in preserved and manufactured foods and species verification of high-value truffles.

DNA identification of plant-associated fungi also has many applications, including community studies of beneficial mycorrhizal fungi, monitoring the continued presence of inoculated biocontrol or symbiotic fungi, and early detection of disease-causing fungi to allow timely management.

DNA basics

DNA (deoxyribonucleic acid) is a long molecule constructed from a variable number of nucleotides, also called bases or base-pairs (bp). Each nucleotide consists of a 5-carbon sugar molecule, deoxyribose, with a phosphate group attached to carbon 5 and one of four bases attached to carbon 1 (Figure 1). The four bases are adenine, guanine, cytosine and thymine, commonly referred to as A, G, C and T, respectively. As a strand of DNA is synthesised, the phosphate group of a nucleotide is chemically bonded to the 3' hydroxyl group of the last nucleotide in the growing chain.

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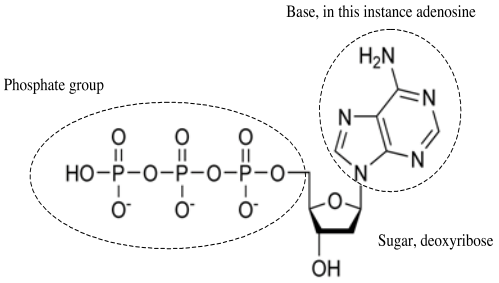

Figure 1. Deoxyadenosine triphosphate (dATP) one of the four deoxynucleotides (dNTPs) needed for synthesis of DNA

DNA occurs in cells as a double-stranded molecule, held together by electrostatic forces between the bases in strand 1 and those in strand 2. Guanine is electrostatically attracted to cytosine, and adenine to thymine, and these pairs are called 'complementary'. The double strand is thermodynamically stable under physiological conditions if the two strands consist of complementary base sequences (Figure 2). DNA polymerase is an enzyme that synthesises DNA by copying an existing strand of DNA. It does not make an exact copy, but a 'reverse complement'. The two strands of DNA are separated and a 'reverse complement' made of each strand, so that strand 1 is the template for a new strand 2, and vice versa. It is the order or sequence of the As, Cs, Gs and Ts that provides the genetic information that is passed from cell to cell and generation to generation, and it is determining this sequence of nucleotides that is referred to as 'DNA sequencing'.

Methods

There are several techniques that can be used for fungal identification. The selection of the most appropriate method depends on the application and the number of samples. However, the foundation for ***all*** methods is the use of a DNA 'signature' to link the unknown fungus to a fully described, morphologically characterised herbarium specimen. Identification of a broad range of fungi is therefore dependent on a comprehensive set of reference specimens that have been examined and identified by a mycological taxonomist with the appropriate skills for the class of fungi under consideration. Public DNA sequence databases can be used as additional sources of information, but should not be relied upon except for tentative identifications, as validating the identity of source material is rarely possible.

Methods for DNA identification of fungi include: Southern blotting; PCR (polymerase chain reaction); PCR-RFLP (PCR restriction fragment length polymorphism); T-RFLP (terminal restriction fragment length polymorphism); DNA sequencing; and microarrays. The first step for any of these methods is DNA extraction and purification, for which many protocols and manufactured kits are available.

Southern blotting

Southern blotting or dot blotting involves binding genomic DNA to a membrane, denaturation of the bound DNA, then hybridisation with a labelled probe (Goodwin et al. 1989; Figure 3). The probe consists of a short fragment (20 or more bp) of DNA that is unique to the target organism. Because of the requirement for comparatively large quantities of DNA, this method has largely been superseded by PCR-based methods.

PCR

PCR was developed in the 1980s and exploits a thermostable DNA polymerase enzyme from a thermotolerant bacterium so that the enzyme can withstand the temperature cycling required for PCR

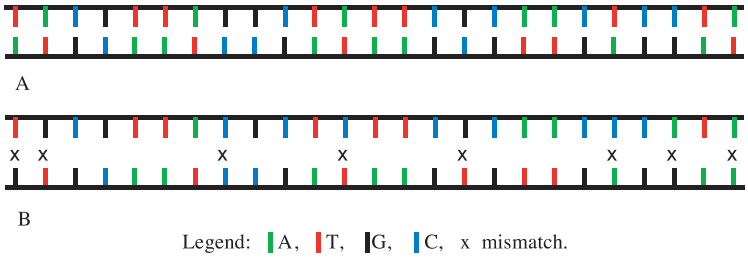

Figure 2. Two DNA strands with complementary base pairs form a stable duplex (A), but two strands with a high number of mismatches do not (B)

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(Saiki et al. 1988). PCR is used to make many copies of a small portion, usually up to 1 kbp, of genomic DNA. Two primers, oligonucleotides of approximately 20 bases, with base sequences complementary to a small fragment on either side of the target DNA are also required to initiate replication. Separation of the two template DNA strands is necessary before the primers can bind to the template, and this is achieved by heating the reaction mixture. It is then cooled to promote primer annealing and heated again to reach the optimum temperature for the DNA polymerase to extend the primers, making a reverse complement copy of the template DNA strand. This three-step heating and cooling profile is repeated for 30–40 cycles, allowing a theoretical doubling of the target sequence with each cycle (Figures 4 and 5).

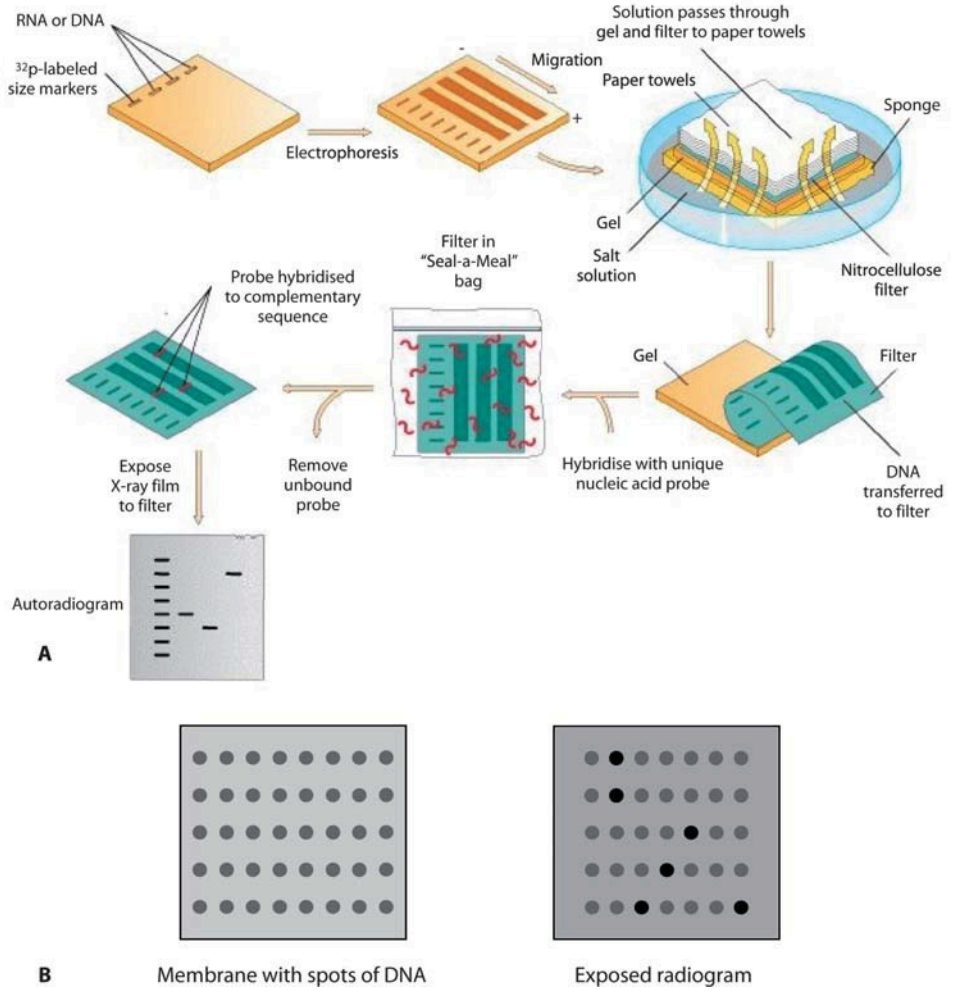

Figure 3. A. Southern blotting procedure. B. Dot blotting does not require gel electrophoresis and transfer, instead the DNA solution is spotted directly onto the membrane. DNA is denatured by heat or alkali, to separate the two strands, then applied to a nylon or nitrocellulose membrane in a regular pattern by pipette or by suction through a special device. The membrane is then incubated in a solution containing labelled probe DNA, which binds only to DNA of the target species. The label can be radioactive, fluorescent or enzymatic. After development of the radiogram (for radioactive probes), the DNA spot from the target species will produce a dark spot on the film.

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During a 30-cycle PCR, assuming 100% efficiency, 2^{30} (i.e. 10^9) copies of a target sequence can be made, sufficient for visualisation on ethidium bromide stained gels.

To use PCR as a fungal identification tool, species-specific primers are required, which will amplify a product from only the target species. Species-specific primers can be designed for well-characterised genes such as calmodulin (Mulè et al. 2004) or unknown sequences such as RAPD (random amplified polymorphic DNA) or AFLP (amplified fragment length polymorphism) products (Dobrowolski and O'Brien 1993; Vos et al. 1995; Schmidt et al. 2004). Primers can be made to target a single-copy gene such as calmodulin (Mulè et al. 2004) or multi-copy regions such as rDNA ITS (ribosomal DNA internal transcribed spacers) for greater sensitivity (Flowers et al. 2003). Extensive testing is required to validate the species-specificity of the PCR test. Internal amplification controls are also necessary to provide confidence in negative results (Hoorfar et al. 2004). Species-specific PCR can be adapted to high-throughput equipment, allowing the rapid processing of many samples, but the method is suitable for identification of only one or a few fungi, as each sample must be tested for each species of interest. Applications include early detection and identification of plant pathogens, monitoring the spread of pathogens

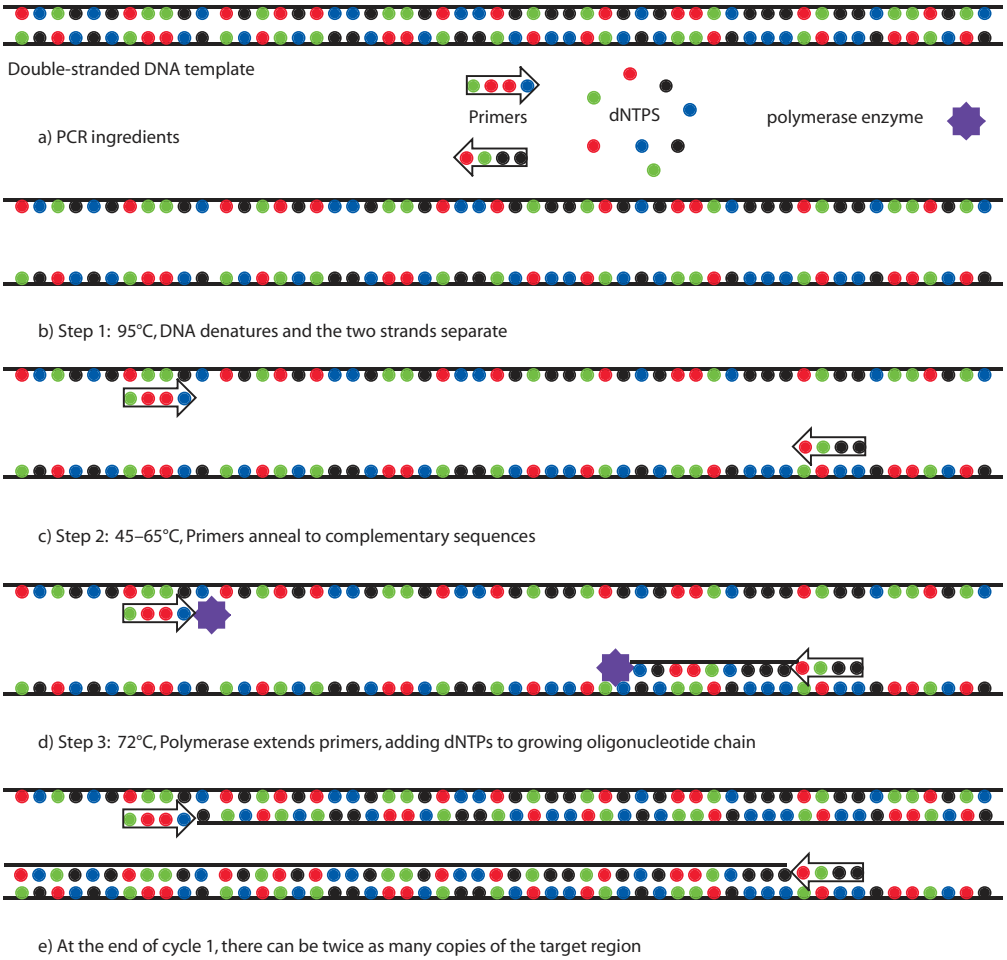

Figure 4. A schematic representation of cycle 1 of a PCR

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or biocontrol organisms and validation of disease-free status for safe germplasm movement.

PCR-RFLP

PCR-RFLP starts with a PCR, using primers with broad specificity, such as primers ITS1-F and ITS4, that will amplify a product from most if not all fungi, but do not amplify plant, animal or bacterial DNA. The PCR product is digested with a restriction enzyme that cuts the DNA strands at a particular recognition site for each enzyme. The recognition site for Hae III, for example, is GGCC. Cutting a 1 kbp PCR product with an enzyme that has a 4bp recognition site will typically cut the PCR product into 2–8 fragments of assorted sizes that will generate a particular profile when subjected to electrophoresis on an agarose or acrylamide gel (Figure 6). This method has been widely used in ectomycorrhizal research as it is suitable for samples that contain mainly one fungus that could belong to any of a large number of species (Gardes and Bruns 1993). Digestion with two or three different restriction enzymes is usually required to distinguish most species. While many species can be clearly distinguished using this

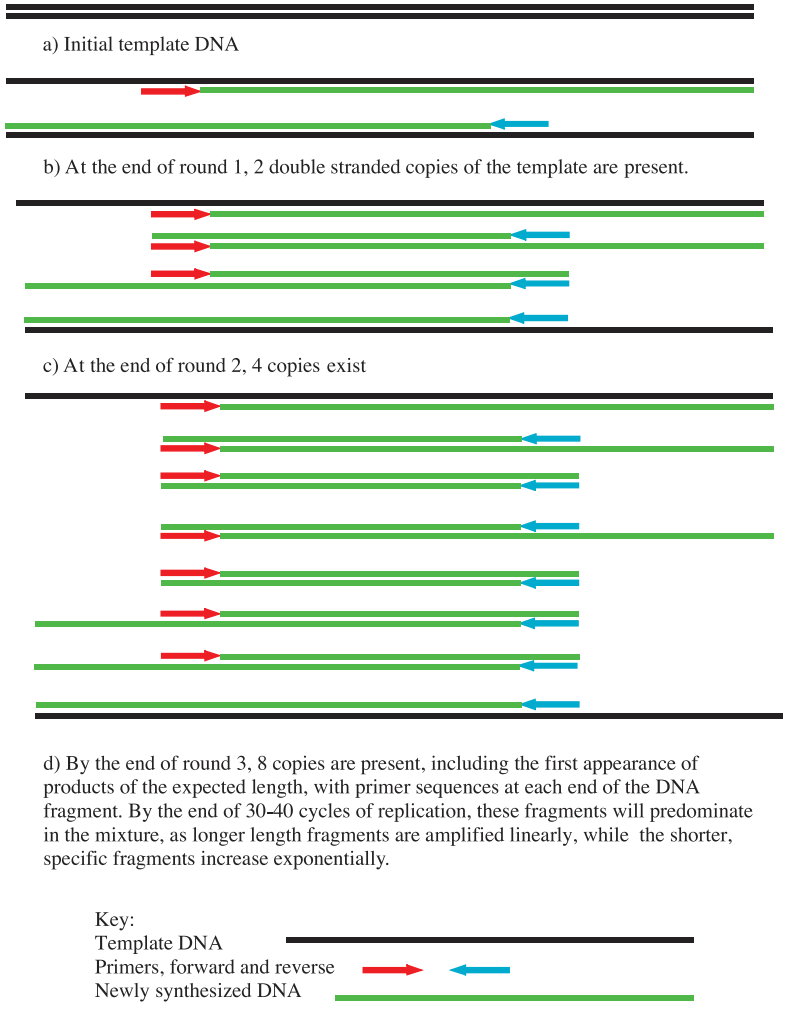

Figure 5. A representation of the first three cycles of a PCR, showing the production of specific fragments delimited by primer sequences.

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method, some genera such as *Tomentella* and *Cortinarius* have many species with very similar profiles that are difficult or impossible to distinguish.

A sizing error of up to 5% must be accommodated in profile matching, rendering highly similar profiles difficult to distinguish. In addition, in using this technique, there are often many DNA profiles that are not matched with known species, though these unmatched fungi can still be monitored for prevalence and abundance.

If warranted, further information can be sought by DNA sequencing (see below). PCR-RFLP has also been used in identification of fungal pathogens (Rolshausen et al. 2004), but the presence of multiple fungal species in one sample can render the PCR-RFLP profile very difficult to interpret.

T-RFLP

T-RFLP is a variant of PCR-RFLP that uses a fluorescent primer and a sequencing gel to obtain greater sensitivity and fragment size accuracy. However, only the fragment with the fluorescent primer registers on the sequencing gel, so the profile for each fungus/enzyme combination is a single fragment. This method has been used mainly in ectomycorrhizal research (Dickie et al. 2002). Both PCR-RFLP and T-RFLP require the establishment of a reference database of fragment sizes from identified fungi with herbarium specimens. The more extensive the reference database, the greater the likelihood of obtaining a match, and the lower the risk of applying an incorrect name. Profiles must be 100% identical — a partial match does not provide useful information for either PCR-RFLP or T-RFLP.

DNA sequencing

DNA sequencing often begins with a PCR to provide sufficient template DNA for the sequencing reaction, though this may also be achieved by cloning the desired fragment into a plasmid or bacteriophage. The sequencing reaction is very similar to a PCR, though only one primer is used per reaction and, in addition to the deoxynucleotides, di-deoxynucleotides (ddNTPs) are included in the reaction mix. These ddNTPs are labelled with one of four fluorescent dyes corresponding to each of the four bases, A, G, C and T. When a ddNTP is incorporated into the newly synthesised molecule, growth of this chain stops as the ddNTP lacks the oxygen molecule to which the next dNTP would be attached. The final product is therefore a series of fragments ranging in size from ~20 bp to *n* bp, where *n* is the length of the PCR product used for template (Figure 7), each fragment having one fluorescently labelled, terminal ddNTP. Electrophoresis on a long, high-resolution

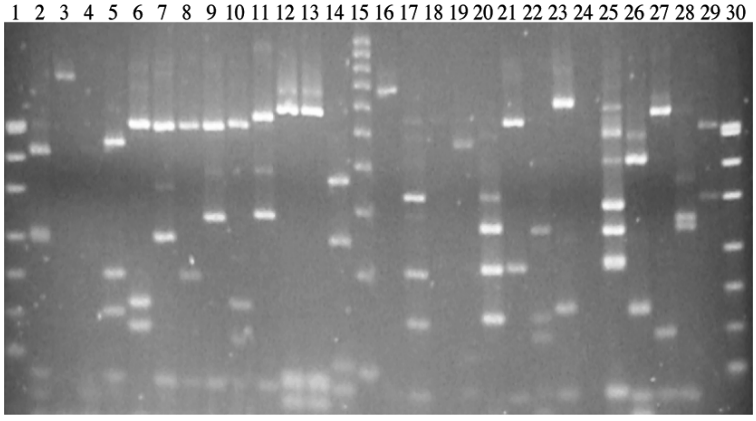

Figure 6. Agarose gel of PCR-RFLP products. Lanes 1, 15 and 30 contain DNA size markers. These can be used to estimate the sizes of the PCR-RFLP fragments. The patterns from known species can be stored as a string of fragment sizes in a searchable database. Usually digestion with two different enzymes will provide adequate discrimination among species, though certain genera with many closely related species may be more difficult.

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acrylamide gel or capillary separates DNA fragments that differ in length by as little as one nucleotide. As the fragments move past a scanner, shorter fragments followed by fragments of increasing length, the fluorescent tag on the ddNTP 'calls' the base sequence (Figure 8), which is converted by the software to a string of As, Cs, Gs and Ts.

For fungal identification, a database of sequences from identified fungi is searched with the sequence from the unknown fungus. If a 100% match is not found, information can be derived from the best percentage match and a likely genus may be inferred, sometimes with the assistance of phylogenetic analysis (Bruns et al. 1998; Glen et al. 2002).

This would identify target genera for expansion of the reference database. This method of identification is applicable to many areas of research, and is particularly suitable where there is a large number of candidate species and it is known that not all species of interest are included in the reference database. The occurrence of more than one fungal species in a sample, such as may occur in a well-rotted root, can cause problems, though, if warranted, cloning of the PCR product before sequencing can accommodate these difficulties.

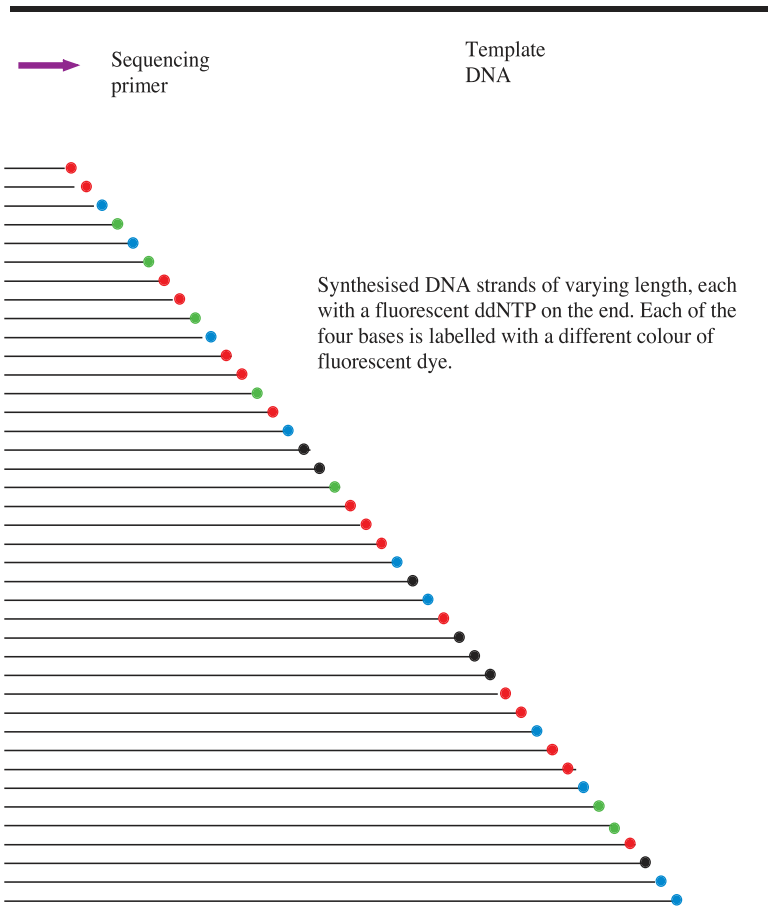

Figure 7. During a dye terminator sequencing reaction, a single primer is used to initiate copies of one of the two strands of DNA template. Each copy is terminated at a random point by the incorporation of a fluorescently labelled dideoxy nucleotide (ddNTP). The products therefore consist of single stranded DNA of varying length.

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Micro-arrays

A promising new approach is the use of DNA micro-arrays for fungal identification. DNA micro-arrays exploit probe/target binding in a similar manner to Southern or dot blotting, though the format is much smaller and the support consists of a glass slide rather than a nylon or nitrocellulose membrane (Southern 1996). Many probes can be crammed into a very small area, making this method ideal for high throughput or simultaneous analysis of many genes — hence the popularity of the micro-array in gene expression studies examining a large number of genes (Ramsay 1998). This technique has been used for identification of medically significant fungi (Leinberger et al. 2005) and work is proceeding on developing the method for high throughput detection and identification of plant pathogenic fungi (Anderson et al. 2005). This technique is expensive to develop but will be well suited to analyses requiring very high throughput.

Application to heart and root rot in *Acacia mangium*

Many fungi are implicated in heart and root rots in *A. mangium*. Currently, the most suitable method of detecting/identifying these fungi involves the use of PCR and DNA sequencing, as species-specific primers have been developed for only a few of the many root or butt rot species (Lim et al. 2005; Suhara et al. 2005). While many sequences of fungi of interest, such as those of *Ganoderma* spp. and *Phellinus* spp., are available on public DNA databases, not many of them are linked to herbarium specimens that

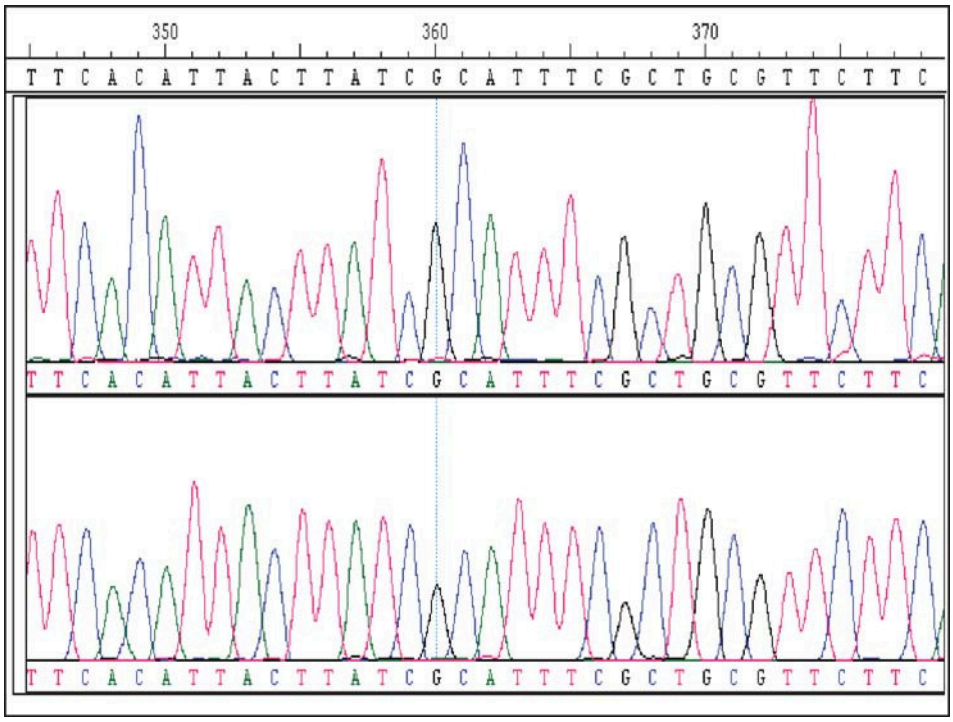

Figure 8. The products of the dye terminator sequencing reaction are subjected to electrophoresis on a high-resolution gel or capillary that passes a scanner attached to a computer. The shorter fragments migrate faster through the gel and therefore reach the scanner first, followed by the longer fragments. The scanner picks up the signal from each fluorescent nucleotide as it passes, and this is converted by the computer to an electropherogram, above, and a DNA base sequence. The electropherogram provides visual confirmation of data quality, allowing manual editing of the derived sequence.

can be readily accessed. Accumulating a comprehensive database of fungal sequences linked to herbarium vouchers with detailed macroscopic and microscopic descriptions is therefore a high priority. The taxonomy of the polypore species associated with heart, butt or root rot is, in many cases, in need of clarification and DNA sequencing can help resolve some species delimitations.

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Molecular identification of organisms associated with root and heart rot in *Acacia mangium*

Morag Glen[1], Karina Potter[1,2] and Purnamila Sulistyawati[3]

Abstract

This paper discusses the application of molecular identification of fungi to research into heart and root rots of *Acacia mangium*, using four different examples. These examples illustrate the utility of DNA sequence information in clarification of the ecology, biology and pathology of fungal species

An overview of different techniques for molecular identification of fungi is given by another paper in these proceedings (Glen 2006). All of these techniques are based on linking an unknown organism to a well-characterised herbarium specimen by a similar DNA profile. As a large number of fungal species are implicated in heart and root rots, a technique that can simultaneously identify many species is more efficient than species-specific probes or primers. In addition, as no comprehensive database of root and heart rot fungi from *Acacia mangium* Willd. exists, techniques that require an exact match, such as PCR-RFLP and T-RFLP, will provide only limited information.

The method that is most likely to efficiently provide a useful level of information is to sequence a well-characterised region of DNA and compare this to sequences of known fungi from public and private databases. This may give identification only to genus or family level in some cases, but this level of information allows the selection of specific groups of fungi for further analysis.

The ribosomal DNA internal transcribed spacers (rDNA ITS) is a genomic region that is widely used in fungal systematics and identification (Figure 1). Its advantages lie in the highly conserved nature of the ribosomal DNA genes, which means that primers can be designed to amplify DNA from a broad range of species and the high degree of variation in the non-coding region. As the spacers region does not code for a functional gene, there is little, if any, constraint on mutations in this region. There is, therefore, a useful degree of inter-specific variation that facilitates species discrimination and identification. In addition, the ribosomal DNA genes are contained in tandem repeats, with up to 50 copies per genome. This makes amplification by PCR much easier than for a single-copy gene.

DNA sequencing has been used to identify fungi causing disease in *A. mangium* in four different experiments, as part of the ACIAR 'Heartrots in plantation hardwoods' project (FST/2000/123):

1. Detection of *Ganoderma* and *Amauroderma* mycelium in rotten root and butt samples from trees with and without associated fruitbodies.
2. Fungi isolated from a provenance wounding trial.

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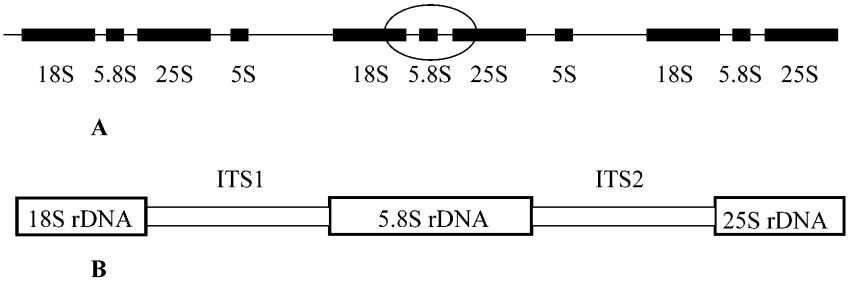

Figure 1. A schematic representation of the organisation of the rRNA gene repeat indicating the location of the internal transcribed spacers (ITS). B gives an enlarged view of the circled section in A. Primer sites for DNA amplification from a broad range of fungi are located in the 18S and 25S rRNA genes.

3. Direct detection of fungi in woodblocks from the same wounding trial.
4. Identification of *Ganoderma* spp. isolates recovered from roots used in somatic incompatibility tests.

In the first experiment, samples of rotten roots and butts of *A. mangium* trees that showed symptoms of 'red root rot' were collected, along with fruitbodies of *Ganoderma philippii, Amauroderma rugosum* and other fungal species from several *A. mangium* plantations throughout Indonesia (Glen et al. 2006). *Ganoderma philippii* was suspected to be the causal agent of this red root-rot disease, based on similarities to disease caused by this species in other woody crops and the prevalence of *G. philippii* fruitbodies in affected plantations. The rDNA ITS was amplified and sequenced from the fruitbodies. Fungal rDNA was also amplified from the root and butt samples using fungi-specific primers to avoid the amplification of DNA from bacteria, insects or plants that may have been present in the sample. The PCR products amplified from the rot samples were clean enough to sequence directly, indicating that the samples were colonised by one predominant fungus. The sequences matched those from the *G. philippii* fruitbodies (Figure 2). This indicated the presence of the fungus in the rotten wood, though was insufficient evidence to prove that the rot was caused by *G. philippii*. The roles of other *Ganoderma* and *Amauroderma* species also need further investigation.

In the second experiment, fungi were isolated as part of a provenance wounding trial that took place in Sumatra in 2002–2003 (Barry et al. 2006). Trees were inspected for signs of heart rot before holes were drilled at three heights and inoculum plugs inserted into two of the holes, which were then sealed. The two isolates of fungi used for inoculating the wounds were a *Pycnoporus* sp. and an unidentified fungus, heart-rot isolate 3-2-A1. The third wound was left uninoculated. The 72 trees were felled 11 months after inoculation and the wood was assessed for fungal decay and discoloration. An attempt was made to isolate fungi from three regions around each of the three wounds per tree (Figure 3). Some 172 fungal isolates were obtained, but none of the isolates recovered from the experiment corresponded morphologically to the isolates used in the wound inoculation.

The recovered isolates were sorted into 15 groups based on gross morphological characteristics. Enzyme production from these isolates was tested using spot tests. Only one of the morphological groups appeared to produce easily detectable quantities of laccase, indicating white-rot capabilities (group 7). Low levels of tyrosinase were also detected in this group. The ITS of a sub-set of isolates (n = 20) were sequenced, focusing primarily on group 7. Results of BLAST searches of public databases are given (Table 1). A sequence has not yet been successfully obtained from either of the original fungal isolations used to inoculate the wounds.

The first three fungi in Table 1 (*Oudemansiella*, *Pycnoporus* and *Trametes*) are likely to have a role in heart rot, as enzyme tests demonstrated the presence of laccase and tyrosinase. While all these isolates were placed in group 7 on the basis of their gross culture morphology and enzyme test results, they were classified as three different basidiomycete fungi based on their ITS sequences. The *Pycnoporus* isolate recovered from the wounding trial wood block is likely to be the same fungus that was inoculated, as its sequence is a very high match to the fruitbody from which the original isolate was obtained.

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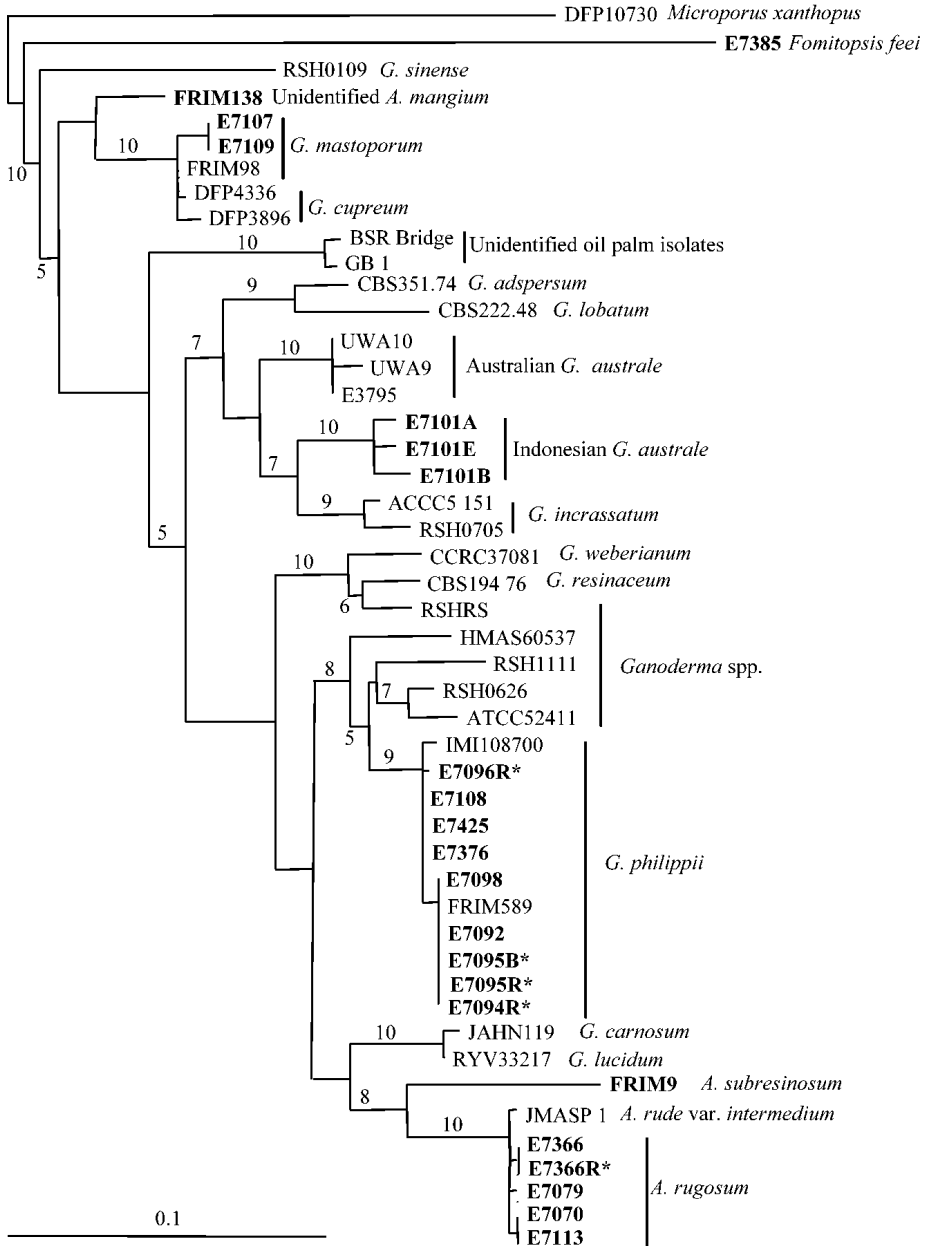

Figure 2. Maximum parsimony tree of ITS1 and ITS2 sequences from *Ganoderma* and *Amauroderma* species and sequences derived directly from wood. Bootstrap values over 50% are given. Scale bar represents 0.1 expected nucleotide substitutions. Collections from *Acacia mangium* plantations are in bold; sequences amplified from root or butt wood are marked with an asterisk. From Glen et al. (2006)

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The *Pycnoporus* was re-isolated from a wood block sampled from within the rotten area, while the *Oudemansiella* was isolated from a wood block taken from adjacent to the rotten area in a different tree that had also been inoculated with the *Pycnoporus* isolate. The *Trametes* sp. isolate is also a recognised wood rotter and was recovered from within the rotten area of a control wound. It is likely that the *Oudemansiella* and *Trametes* isolates either invaded via the treatment wound or were already present before the inoculation. The remaining fungi are all ascomycetes, and while some may be important in other diseases, they do not have wood-rotting capabilities.

The woodblocks from the wounding trial were stored to attempt direct detection of fungi. DNA was extracted from a small number of these woodblocks, but the PCR product was not clean enough to sequence. The PCR products are currently being cloned to obtain clean DNA sequences.

The final example involves the identification of fungi isolated from roots and fruitbodies from an *A. mangium* provenance/family trial that has suffered severe root rot at Wonogiri. The purpose of the experiment was to determine the main mode of dispersal of the predominant fungus. Many *Ganoderma* fruitbodies occur on this site and have been identified as species from the *Ganoderma lucidum* complex (Irianto et al. 2006). *Ganoderma lucidum* consists of a complex of at least three and possibly six species (Moncalvo et al. 1995a; Hseu et al. 1996).

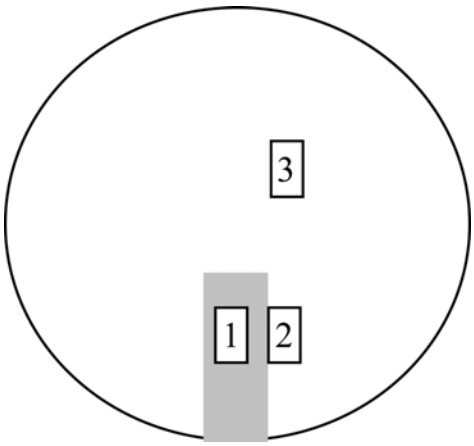

Figure 3. A diagram of the wood discs after harvesting, the grey area represents the rotten wood surrounding the wound. Wood blocks were removed from three areas of each disc — (1) from within the rotten wood, (2) adjacent to the rotten wood, and (3) away from the rotten section.

Table 1. Tentative identification of fungi isolated from the wounding trial

Isolate[a]	Group	Results of sequence database search
13A2-Y	7	*Oudemansiella aff. canariensis*
70A1	7	*Pycnoporus aff. sanguineus*/*cinnabarensis*
49C1	7	*Trametes* sp.
53A1	1	*Phaeoacremonium aff. rubrigenum*
58A1	2	*Articulospora* sp.
16C1-Y	4	Ascomycete, unknown species
69B2	6	*Pestalotiopsis* spp.
63C1	8	*Phaeoacremonium aff. rubrigenum*
69C1-X	8	*Phaeoacremonium aff. rubrigenum*
57B1-Y	8	*Bionectria* spp.
37C1	8	*Clonostachys*/*Nectria gliocladioides*
26A1-X	8	*Clonostachys*/*Nectria gliocladioides*
1C1-X	13	*Aspergillus* sp.
63A1-X	Un-grouped	*Pestalotiopsis* spp.
69C1-Y	Un-grouped	*Nectria gliocladioides/Clonostachys* sp.
67C1	Un-grouped	*Clonostachys* sp./*Nectria gliocladioides*

[a] The number before the letter in the isolate number indicates the tree number. The letter within the isolate number indicates the wound inoculum (A = *Pycnoporus* sp., B = heart-rot isolate 3-2-A1 and C = control, sterile inoculum) and the number after the letter indicates the location of the wood block sample (see Figure 3). The letter after the hyphen distinguishes individual isolates recovered from the same wood block.

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Fungi were isolated from fruitbodies and from the roots of diseased trees. These isolates were then used for somatic incompatibility tests to determine the number and extent of genetic individuals. In order to validate the test results, it was necessary to demonstrate that the isolates belong to the same species. ITS sequences of the isolates confirmed the morphological identification (Irianto et al. 2006) as *Ganoderma aff. lucidum*. Phylogenetic analysis may allow us to determine in which species group of the *Ganoderma lucidum* complex this species belongs.

Identification of *Ganoderma* species can be a challenge for even an experienced mycological taxonomist (Moncalvo et al. 1995a,b; Gottlieb and Wright 1999; Gottlieb et al. 2000; Smith and Sivasithamparam 2000, 2003). DNA sequences can assist taxonomists to infer species boundaries and inter-specific relationships, and can also be used as a 'fingerprint' or 'barcode' to identify fungal species in life stages that lack the characters on which traditional classification is based. This fingerprint can also be used by the non-specialist to assist in classification, but close co-operation with an experienced fungal taxonomist, and retention of reference specimens, are still vital.

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Root rot in tree species other than *Acacia*

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Abstract

Surveys of root disease were conducted in forest plantations of *Azadirachta excelsa, Tectona grandis* and *Khaya ivorensis* throughout Peninsular Malaysia. Two major root diseases were found, namely white root disease and brown root disease caused by *Rigidoporus lignosus* and *Phellinus noxius*, respectively. A destructive root disease of *K. ivorensis* caused by an unidentified fungus was found in the state of Negeri Sembilan. These diseases were observed to be closely associated with poor land preparation and areas with a previous history of root disease. Based on experience gained from the management of root-rot disease in rubber plantations, good land management, the construction of isolation trenches and the application of fungicides are suggested as valuable tools in the control of root-rot disease in forest tree plantations.

Root disease causes significant mortality in many forest plantations and is a common explanation for failure in the early phase of plantation development (Wingfield 1999). In particular, root disease is a major threat to plantation monocultures that have been established on land converted from natural forest with poor land-clearing techniques (Lee 1993). Furthermore, the low species and genetic diversity and uniform age of plantations create conditions favourable for the development and spread of root-disease pathogens.

In India, Indonesia, Malaysia and Thailand, root rot has been identified as the most serious disease in plantations of tropical acacias (Old et al. 1997). Root rot is also the most destructive disease of rubber trees and can kill the tree irrespective of age or health status, causing economic losses to the latex industry in many countries (Nandris et al. 1987a; Liyanage 1997; Semangun 2000: Guyot and Flari 2002). The disease has also been reported to aggressively kill fruit trees (Singh 1973; Wood and Lass 1985; Ann et al. 2002).

In Malaysia, interest in forest plantations boomed in the 1990s, and several fast-growing species were introduced. Among these were sentang (*Azadirachta excelsa* (Jack) Jacobs), teak (*Tectona grandis* L.) and khaya (*Khaya ivorensis* A. Chev.). These species were planted with little knowledge of potential pest and disease threats. In 1997, the Forest Research Institute Malaysia (FRIM) began disease surveys throughout Peninsular Malaysia to determine the health status of the most common forest plantation species. Root-disease incidence was the main thrust of the surveys as root diseases have the potential to cause high levels of tree mortality.

Materials and methods

Disease surveys

Root-disease surveys were conducted randomly on 34 forest plantations throughout Peninsular Malaysia from 1997 (Mohd Farid et al. 2005). The surveys focused on plantations of sentang, teak and khaya. Complete and random censuses were conducted in

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small (<0.4 ha) and large-scale plantations (>0.4 ha), respectively. In large-scale plantations, plots of 20 × 20 trees were randomly established and surveyed. In both small and large-scale plantations, trees selected for sampling were inspected individually. Background information on the establishment of the plantations was documented, including planting techniques, land clearing, site history, source of planting materials and silvicultural treatments. Trees suffering from root disease were identified based on observation of both above- and below-ground symptoms. The root system of suspected diseased trees was excavated to examine for potential pathogens.

Pathogens were isolated from root samples in an attempt to identify the organisms causing the root-rot disease. Morphological studies were conducted both macroscopically and microscopically and fungal features were described according to Stalpers (1978). Pathogenicity tests were conducted on 6-month-old seedlings using the method described by Mohd Farid et al. (2001). These tests were conducted under natural field conditions using selected isolates of *Rigidoporus lignosus* (Batu Anam, Johor), *Rigidoporus vinctus* (Pelong, Terengganu) and *Phellinus noxius* (Lendu, Malacca and Tebuk Pulai, Selangor).

Results and discussion

Disease survey

The surveys revealed that disease incidence was variable depending on tree species, land-management practices and location. Root disease was found in only 10 of 34 plantations surveyed (Table 1). Two main diseases were found: white root and brown root disease. White root rot was more dominant on sentang, while brown root disease was present in both teak and sentang plantations. White root disease was recorded in monocultures of sentang at Sik, Kedah and in sentang inter-planted with rubber trees in Batu Anam, Segamat, Ca'ah and Labis in Johor. Disease infection on sentang was recorded from as early as 1 year after planting. Similar results were gained by Maziah et al. (2001) with root-rot disease identified as a major threat to sentang plantations in the early stages of establishment (1–3 years old) and to rubber trees 1–4 years old (Tan and Ismail 1991).

Brown root disease was observed in teak plantations in Sabak Bernam, Selangor and Kuala Kangsar in Perak as well as on sentang in Sik, Kedah and Lendu, Malacca. Maziah and Lee (1999) have previously reported that teak trees 2 years old and above were frequently infected by root disease in Malaysia, and Browne (1968) notes that brown root disease was destructive on teak of 2–12 years old in forest plantations in some countries. In this study, infection of teak trees was recorded from as early as 1 year after planting.

Root disease was found mostly in plantations with poor land preparation, where stumps and wood debris had been left on the ground to decay. The disease was also frequently observed in plantations with a previous history of root disease. Usually, untreated disease centres were also associated with disease incidence and it was considered likely that the pathogen in the new plantations originated from the diseased roots of previous crops. Root disease was low or absent in plantations that has received good land preparation by removing most of the stumps and wood debris (Table 1). Similar observations have been made by Van der Pas and Hood (1983) in *Pinus radiata* plantations in New Zealand.

Overall, the majority of the plantations surveyed were free from root disease. This appeared to be associated with good land preparation and no previous history of root disease. Stumps and wood debris were mechanically removed and burned in the majority of these plantations. However, while root disease was not evident, symptoms of poor tree health, such as stunted growth, small canopy size, small stems and leaves, multiple branching, and sparse and pale foliage, were common. The majority of teak and sentang growers considered poor tree health to have a significant impact on production, particularly during the early phase of plantation establishment (Mohd Farid et al. 2005). The condition is thought to be due to lateritic and/or compacted soil, which impedes the growth of trees. Below ground, cracking of the root surface and a lack of feeder roots were common external signs of affected trees. The main anchoring root, however, was consistently found to be alive and strong enough to support the tree.

Root disease

Macroscopic and microscopic identification of fruitbodies, infected roots and isolates, as well as pathogenicity tests, led to the identification of white root disease and brown root disease caused by *R. lignosus* and *P. noxius,* respectively. The fungi were the causal organisms as proven in the pathogenicity tests.

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Table 1. Host, location, pathogen and land management of forest plantations in Peninsular Malaysia

Host	Site and state	Pathogen	Land management			Age
			Planting technique	Previous history of root disease (Yes/No)	Land preparation	
Teak	Sabak Bernam, Selangor[a]	*Phellinus noxius*[d]	Monoculture	Yes	Poor	10
Teak	Sabak Bernam, Selangor[b]	*P. noxius*[d]	Monoculture	Yes	Poor	10
Teak	Perak River Estate, Kuala Kangsar, Perak	*P. noxius*[d]	Monoculture	Yes	Good	1
Teak	Kaki Bukit, Perlis[a]	Nil	Monoculture	No	Good	8
Teak	Kaki Bukit, Perlis[b]	Nil	Monoculture	No	Good	10
Teak	Kaki Bukit, Perlis[c]	Nil	Monoculture	No	Good	9
Teak	Timah Tasuh, Perlis	Nil	Monoculture	No	Good	8
Teak	Chuping, Perlis	Nil	Monoculture	No	Good	8
Teak	Kompt. 1A/ 99 & 1B/ 99. Bukit Bintang Forest Reserve, Perlis	Nil	Monoculture	No	Good	4
Teak	Bukit Bintang Forest Reserve, Perlis	Nil	Monoculture	No	Good	16
Teak	Kompt. 22 & 23. Bukit Perangin Forest Reserve, Changlon, Kedah	Nil	Monoculture	No	Good	18
Teak	Kompt. 1, 2, 3 & 5 Bukit Perangin Forest Reserve, Changlon, Kedah	Nil	Monoculture	No	Good	18
Teak	Bukit Enggang Forest Reserve, Changlon, Perlis	Nil	Monoculture	No	Good	21
Teak	Tampin, Negeri Sembilan.	Nil	Monoculture	No	Good	7
Sentang	Batu Anam, Segamat, Johor.	*Rigidoporus lignosus*[e]	Mix (sentang × rubber)	Yes	Poor	6
Sentang	Km5. Labis Muar, Johor	Nil	Mix (sentang × rubber)	No	Good	7
Sentang	Pelong, Terengganu	*R. vinctus*	Monoculture	Yes	Poor	3
Sentang	Lendu, Malacca	*P. noxius*[d]	Monoculture	No	Good	1
Sentang	Labis – Muar, Johor	Nil	Monoculture	No	Good	5
Sentang	Ca'ah- Muar, Johor	Nil	Monoculture	No	Good	5

Table 1. (cont'd) Host, location, pathogen and land management of forest plantations in Peninsular Malaysia

Host	Site and state	Pathogen	Land management			Age
			Planting technique	Previous history of root disease (Yes/No)	Land preparation	
Sentang	KM 37.5, Labis- Yong Peng, Johor	Nil	Monoculture	No	Good	8
Sentang	Km 0.5, Segamat-Muar, Johor	Nil	Monoculture	No	Good	7
Sentang	Bukit Hari, FRIM	Nil	Monoculture	No	Good	5
Sentang	Sik, Kedah	*R. lignosus* [e] *P. noxius* [d]	Monoculture	Yes	Poor	7
Sentang	Kaki bukit, Perlis	Nil	Monoculture	No	Good	8
Sentang	Jementah, Johor	Nil	Monoculture	No	Good	8
Sentang	Ulu Tiram, Johor	Nil	Monoculture	No	Good	6
Sentang	Teluk Intan, Perak	Nil	Monoculture	No	Good	6
Sentang	Bt 9, Jeniang, Kedah	Nil	Monoculture	No	Poor	6
Sentang	Sg. Chinoh Estate, Trolak Perak.	Nil	Monoculture	No	Good	5
Sentang	Labis, Johor	*R. lignosus* [e]	Mix (sentang × rubber tree)	Yes	Poor	7
Sentang	Ca'ah, Johor	*R. lignosus* [e]	Mix (sentang × rubber tree)	Yes	Poor	5
Khaya ivorensis	Felda Titi, Negeri Sembilan	Unidentified	Monoculture	Yes	Poor	3
Khaya ivorensis	Felda Jengka, Pahang	Nil	Monoculture	No	Good	1

[a,b,c] Distinguish between plantations located in the same district
[d] Brown root disease
[e] White root disease

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These pathogens have wide host ranges and are responsible for most root and butt rot diseases in tropical rainforests (Lee 1997). Both *R. lignosus* and *P. noxius* are economically important pathogens as they are responsible for causing more losses than all other pests and diseases combined, especially in rubber plantations (Fox 1977a; Johnston 1989). *Rigidoporus lignosus* is economically important on rubber and timber, especially in Indonesia, Malaysia, Sri Lanka and the Ivory Coast (Liyanage 1997a; Semangun 2000). It is the most destructive disease of rubber (Fox 1977b; Nandris et al. 1987a; Guyot and Flori 2002) and the only disease that directly kills the tree irrespective of age and vigour (Tan and Ismail 1991). *Phellinus noxius* is also a destructive fungal pathogen. It has been recorded on rubber, cocoa, tea, fruit trees and forest trees (Pegler and Waterston 1968; Singh 1973; Wood and Lass 1985; Nandris et al. 1987a,b; Chang 1995; Ann et al. 2002).

In the field, the above-ground symptoms associated with each disease were similar; below-ground symptoms varied. Above ground, the symptoms were wilting, yellowing of leaves, loss of leaf lustre, bark shrinkage, large canopy gaps, defoliation and dieback. The presence of these symptoms usually indicated that the trees were beyond the point of recovery, as the fast progress of infection leads to rapid death (Ismail and Azaldin 1985). Spread of both diseases to adjacent healthy trees was primarily through root contact. This is the most common mode of disease-spread in plantation-grown rubber (Anon. 1974; Holliday 1980; Nandris et al. 1983, 1987a; Rajalakshmy and Jayarathnam 2000), teak (Tewari 1992), *Acacia mangium* (Lee 1997; Maziah 2002; Ito, unpublished data) and Douglas-fir (Wallis and Reynolds 1965). Frequently, the source was infected old stumps or wood debris remaining in the soil or standing diseased trees. In rubber plantations, the source of inoculum for root disease infection is mainly from infected rubber trees, stumps or forest trees (Rajalakshmy and Jayarathnam 2000). This is similar to root rot observed on *A. mangium* by Old et al. (2000).

White rhizomorphs on the root surface were the main indicator in identifying *R. lignosus* in the field. Their presence means that the whole root system has already been exposed to the disease (Wheeler 1974). The disease survey showed that white root disease occurred in both sentang monocultures and sentang inter-planted with rubber. In monocultures, disease incidence was relatively low and diseased trees were scattered or solitary. In mixed plantations, dead trees were more clustered.

Brown root disease was found in both teak and sentang plantations. On teak, the disease caused basal root rot (BRR) at Sabak Bernam, Selangor and root rot at Kuala Kangsar, Perak. Its diagnosis was based on rotting symptoms advancing up the root collar. At present, BRR has been found only on teak at or above 10 years of age where it is planted on marine clay soil known locally as Bernam series. Patches of dead trees were also an indicator of BRR. Below ground, BRR was identified by the presence of a rough sheet of brown mycelial crust on the root surface. Soil particles, mainly of sand, frequently adhered to the crust. In more advanced stages, a honeycomb structure of golden brown hyphae was formed in the rotted wood. These features have been observed and described in detail by various other investigators (Anon. 1974; Nandris et al. 1983, 1987b; Ann et al. 1999). Two plantations of sentang located in Sik, Kedah and Lendu, Malacca were infected by brown root disease that killed both solitary trees and patches of trees irrespective of their health status.

One incidence of root disease was observed in *K. ivorensis* at Felda Titi, Negeri Sembilan caused by an unidentified fungus. Both below-ground and above-ground observations revealed that the disease symptoms were similar to brown root disease caused by *P. noxius*, with some small variations. The surface of the infected tree root was often covered with a brown mycelial crust with adhering soil particles. The crust was usually whitish in colour when young, becoming brownish over time. Further study is needed to identify the unknown pathogen.

Control and management of root disease

At present, the incidence of root disease in forest plantations in Peninsular Malaysia is relatively low compared with that in rubber tree and oil-palm plantations. This destructive disease has the potential, however, to be a major threat to the timber plantation industry in the future. Experience gained in the containment and control of root disease in rubber plantations could provide useful strategies to prevent the spread of the disease in forest plantations.

In rubber plantations, root diseases are managed by cultural practices, especially through land clearing (Old et al. 2000). Therefore, during the conversion of

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land for the establishment of timber tree plantations, stumps and woody debris should be removed and destroyed. The aim of this activity is to reduce the source of potential inoculum in the soil. This approach could minimise the incidence of the disease in the plantations (Fox 1977b; Liyanage and Peries 1982). In rubber plantations, the construction of isolation trenches around trees identified as diseased is another recommended control strategy. This is the most common and popular method of root disease control to prevent disease spread to adjacent healthy trees through root contact (Maziah and Lee 1999). However, the method is costly and labour intensive, especially when large areas or many patches of disease occur in the plantation.

The most common practice of rubber growers to combat white root disease is the application of fungicides by means of soil drenching. Several fungicides, such as hexaconazole, tridemorph, propiconazole, tridemefon, cyproconazole and penconazole, have shown promise in the control of disease caused by *R. lignosus*. The efficacy of the fungicide treatment, however, reduces with increasing levels of infection (Ismail and Shamsuri 1998). Fungicides should therefore be applied only on newly infected trees or trees at mild infection levels.

Conclusion

Two major root diseases of forest plantation species, white root (*R. lignosus*) and brown root (*P. noxius*), were found during the surveys. Sentang was susceptible to white root disease, especially when interplanted with rubber trees. Brown root disease, in comparison, was found in both teak and sentang plantations. A root disease caused by an unidentified fungus was found on khaya at Felda Titi in Negeri Sembilan. This pathogen killed its host aggressively without showing early symptoms. Trees infected by this disease were almost indistinguishable from healthy trees, with symptoms discernible only during the advanced stages of infection.

The survey also revealed that infection started mainly from infected roots of trees, stumps or root remnants remaining in the soil, and that spread to adjacent trees was through root contact. Good land preparation by removing all woody debris and stumps in the soil before plantation establishment was therefore considered vital to reduce disease incidence.

Based on the experience of rubber-tree growers in the control of white root rot disease caused by *R. lignosus*, good land clearing during site preparation, the construction of isolation trenches and the application of fungicides are recommended as valuable tools to help manage root rot disease in forest tree plantations.

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The biological control of Ganoderma root rot by *Trichoderma*

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Abstract

This paper describes the ability of *Trichoderma* spp. to act as agents for biological of *Ganoderma*. Certain species of *Ganoderma* are potential root-rot pathogens and are capable of causing serious damage to many types of plantation-tree species in Indonesia. Biological control of plant pathogens aims to decrease dependence on chemical treatments which may cause environmental pollution and the development of resistant strains. Filamentous fungi such *Trichoderma* that are mycoparasites of plant pathogens have potential for the biocontrol of plant disease. Species of *Trichoderma* are one of the most widely tested agents. Although the mechanism of mycoparasitism is not fully understood, expression of extracellular cell-wall degrading enzymes is assumed to be involved in this process, including the action of chitinolytic and glucanolytic enzymes. As reported for other chitinolytic systems, the endochitinase (EC 3.2.1.14) is among the most effective for both antifungal and lytic activities in comparison with other types of chitinolytic enzymes. Recently, 32-kDa endochitinolytic enzymes have been purified from *Trichoderma reesei* and characterised. We have tested the antagonistic ability of *Trichoderma* isolates against some plant pathogenic fungi, such as *Ganoderma* spp., *Rigidoporus microporus*, *Rhizoctonia* spp., *Fusarium* sp., and *Sclerotium rolfsii*. Results show that *Trichoderma* spp. can suppress the development of fungal pathogens in vitro and in glasshouse experiments.

Intensive forestry is based on growing one or few tree species over large areas. These plantation ecosystems are ecologically unbalanced and favour epidemics of pathogens or pests that interfere with the production of a healthy, valuable tree crop. Prevention of such epidemics in forest plantations cannot be achieved through the use of chemical fungicides since it would not be cost-effective or environmentally sustainable. Consumers are increasingly concerned about the chemical pollution of the environment, and pathogens could become resistant to available chemicals if these are used indiscriminately. The options currently available to manage soil-borne disease are limited, and measures must be developed to avoid the start of an epidemic by preventing inoculum build-up.

In agriculture, and to some extent forestry, the chemical treatment of pests has been replaced or reduced through the use of biologically based fungicides. A broad definition of biological control is the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than humans (Cook and Baker 1983). This definition includes the use of less-virulent variants of the pathogen, more resistant cultivars of the host, and microbial antagonists that interfere with the survival or disease-producing activities of the pathogen. This paper discusses the development of a biological control for Ganoderma rot root disease using antagonistic fungi of the genus *Trichoderma.*

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What are *Ganoderma*?

The Ganodermataceae are cosmopolitan basidiomycetes which cause root rot of many temperate and tropical hardwoods by decomposing lignin as well as cellulose and related polysaccharides (Hepting 1971; Blanchette 1984; Adaskaveg and Ogawa 1990; Adaskaveg et al. 1991, 1993). Successive replanting of monocultures such as *Acacia mangium* Willd. in Southeast Asia can be rapidly exploited by soil-borne fungi such as *Ganoderma*, and this particular problem will become more serious as more areas move into second or even third-rotation planting. Environmental considerations also mean that native forest areas can no longer be exploited, making further replanting of these plantation forests inevitable. It is thus essential to develop appropriate, integrated management systems for root rot diseases.

Ganoderma spp. have been found worldwide on a range of broad-leaved hosts (Phillips and Burdekin 1989). Butt rot and root rot symptoms of *Ganoderma* spp. have been recognised on planted *Acacia* in northern Australia (C. Mohammed, pers. comm.), in a provenance trial in Peninsular Malaysia, in northern Sumatra (Lee 1996) and elsewhere in Indonesia (Irianto et al. 2006). Root-rot disease caused by *Ganoderma* spp. has been reported as the most serious disease of *A. mangium* in West Bengal, India (Sharma and Florence 1996).

What are *Trichoderma*?

Trichoderma spp. are fungi that are often dominant components of the soil microflora in widely varying habitats. This may be attributable to their diverse metabolic capability and their aggressively competitive nature. Strains of *Trichoderma* are rarely associated with disease in living plants.

The high degree of ecological adaptability shown by strains within the genus, coupled with their amenability to cultivation on inexpensive substrates, make *Trichoderma* isolates attractive candidates for a variety of biological control applications (Hjeljord and Tronsmo 1998). As fast-growing saprophytes, *Trichoderma* can compete ecologically over the long term as well as at the time of application and are able to colonise potential infection courts, such as growing roots, wounds, or senescent tissue, as they become available. As living organisms, biological control agents can act as aggressive mycoparasites and adapt to changes in their habitat in a manner not possible by chemical fungicides. *Trichoderma* can be used to attack established pathogens as well as preventing the establishment of disease.

Antagonistic mechanisms of *Trichoderma*

In addition to colonising roots, *Trichoderma* spp. attack, parasitise and otherwise obtain nutrition from other fungi. Since *Trichoderma* spp. grow and proliferate when there are abundant healthy roots, they have evolved numerous mechanisms to enable them to attack other fungi and to enhance plant and root growth.

A list of recently described mechanisms follows:

- mycoparasitism — in which one fungus derives its nutrition from another without any benefit in return. The interaction can be where the parasite is biotrophic or necrotrophic as is the case for *Trichoderma*.
- antibiosis — an association between two organisms that is detrimental to the vital activities of one of them and, in fungi, is usually mediated by toxic metabolites produced by one organism
- competition for nutrients or space — the active requirement for resources in excess of those immediately available to two or more organisms. The production of toxic metabolites is known to be affected by the nutrient status of the growth medium (Ghisalberti and Sivasithamparan 1991; Howell and Stipanovic 1995).
- tolerance to stress through enhanced root and plant development
- solubilisation and sequestration of inorganic nutrients
- induced resistance
- inactivation of the pathogen's enzymes.

Trichoderma strains produce a variety of volatile and non-volatile toxic metabolites. Of these, some are considered to be antibiotics as they can inhibit the growth of other microorganisms without physical contact between the fungi. The best known of the antifungal metabolites produced by isolates of this genus is the coconut-scented 6-n-pentyl-2H-pyran-2-one (PPT) (Claydon et al. 1987). In addition, many strains of *Trichoderma* are able to produce extracellular cell-wall-degrading enzymes which are also capable of killing at a distance. These, however, are traditionally included in the concept of mycopara-

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sitism, due to their integral role in direct physical interactions.

Trichoderma as biological control agents for agents of root-rot disease including *Ganoderma*

In order to test the potential antagonistic effects of *Trichoderma* spp. against various root-rot pathogens causing problems in Indonesia, we prepared cultures by placing agar plugs containing the mycelia of the two antagonists (*Trichoderma* and root-rot pathogen) on opposite sides of the agar plate. The plant pathogenic fungi tested against *Trichoderma* spp. isolates were *Ganoderma* spp. (Widyastuti and Sumardi 1998; Widyastuti et al. 1998a, 1999), *Rigidoporus microporus* (Widyastuti et al. 1998b), *Rhizoctonia* spp. (S.M. Widyastuti, Harjono, Sumardi and N.S. Lestari, unpublished data; S.M. Widyastuti, Sumardi, Harjono and E. Windyarini, unpublished data), *Fusarium* sp. (S.M. Widyastuti, Sumardi and Y. Mitikauji, unpublished data) and *Sclerotium rolfsii* (Widyastuti et al. 2003). Control plates were inoculated with only one of the antagonists but on both sides of each individual plate in order to simulate growth conditions as comparable as possible to those in the test plates. Replicates comprised of a minimum of three plates per combination of each pair of fungi. Interaction zones, i.e. the areas of contact and subsequent overlap of hyphae of *Trichoderm*a spp. and pathogenic fungi, were observed at various magnifications in situ by light microscopy.

Among the 120 isolates of *Trichoderma* spp. tested against the pathogenic fungi we recorded examples of the basic types of antagonistic behaviour — antibiosis, mycoparasitism, and competition (Figure 1). The type of antagonism that appeared the most effective in inhibiting the root-rot pathogens tested was mycoparasitism (when the *Trichoderma* grew within the pathogen's colony (Figure 1a)). Antagonism by competition occurred when both the pathogen and *Trichoderma* grew but the growth of *Trichoderma* limits the full access of the pathogen to the substrate (Figure 1b). No one colony was able to dominate the substrate once hyphal contact was established (Figure 1b). Interactions typical of antibiosis were observed, i.e the *Trichoderma* isolate is producing toxic compounds and the colony of the root-rot pathogen is severely restricted and clearly contained by the *Trichoderma* (see Figure 1c in comparison to 1b).

In another experiment, three *Trichoderma* isolates, previously shown to have high antagonistic ability (*T. koningii* isolate T1, *T. reesei* isolate T13 and *T. harzianum* isolate T27; Figure 2) were tested against isolates of *Ganoderma* collected from different tree species. It was significant, however, that these *Trichoderma* isolates of known high antagonistic ability actually varied greatly in their level of antagonism towards the different *Ganoderma* isolates. Widyastuti et al. (1999) showed in paired cultures that a *Trichoderma* isolate which was highly effective against one isolate of a pathogen could have minimal effect on other isolates of this pathogen (Table 1). This might be related to the high pathogen–*Trichoderma* specificity of antagonistic mechanisms due to antibiosis (Howell and Stipanovic 1995) and cell-wall-degrading enzymes (Haran et al. 1996). This specificity theory is most probable since the *Ganoderma* isolates could have been different isolates of the same *Ganoderma* species or isolates of different *Ganoderma* species and their identity will be tested in future work.

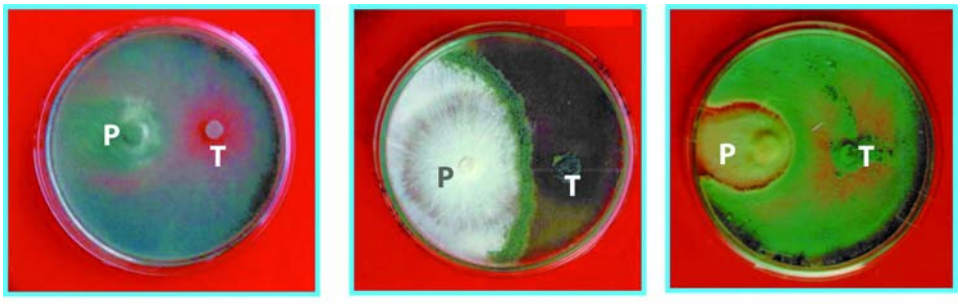

Figure 1. Three basic interaction mechanisms between *Trichoderma* and pathogenic fungal isolates: (a) mycoparasitism, (b) competition, (c) antibiosis. Key**: T =** *Trichoderma,* **P =** Pathogen.

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Microscopic observation of antagonistic interactions between *Ganoderma philippi* and *Trichoderma*

Hyphal interactions were observed under a light microscope with hyphae that had been stained in 10% (v/v) lactophenol blue for 5 minutes. Observations of the zone of the confrontation between *Trichoderma* and *Ganoderma philippi* isolates indicated a range in the types of hyphal interactions. The diameter and the intensity of staining of *Ganoderma* and *Trichoderma* fungal hyphae were different, so they were easily distinguished from each other (Figure 3a–c). All *Trichoderma* isolates were frequently observed to grow parallel to the pathogen. Hyphae of *T. reesei* and *T. harzianum* often coiled around the pathogen (Figure 3a,b), but no coiling was shown by *T. koningii* (Figure 3c). *Trichoderma reesei* and *T. harzianum* both produced appresoria at the tips of short branches (Figure 3d) or formed a hook-like structure (Figure 3e). No such structures were produced by *T. koningii*. The above responses observed (growing parallel to the pathogen, coiling, appresoria, hooks) are considered to be examples of mycoparasitic activity and are similar to those described by Elad et al. (1983), who observed the mycoparasitic activity of *T. harzianum* against *S. rolfsii* and *R. solani* using scanning electron and fluorescence microscopy.

Trichoderma reesei was the most effective mycoparasite in interactions with *Ganoderma* isolates, followed by *T. koningii* and *T. harzianum* (Widyastuti et al. 2003; Figures 2–3 in this paper). In interactions with pathogenic fungi other than *Ganoderma*, *T. koningii* has displayed a typical antibiosis inhibition pattern of confinement (e.g. Figure 1c), whereas in interactions with *Ganoderma* the *T. koningii* isolate T1 demonstrated limited mycoparasite properties (with hyphae growing parallel to the pathogen). This isolate T_1 can most probably exhibit both main types of fungal inhibition, mycoparasitism and antibiosis depending on the particular species of fungal pathogens with which it is interacting (Widyastuti et al. 2003).

Table 1. Growth inhibition of *Ganoderma* spp. from eight different tree species in co-culture with three *Trichoderma* spp. isolates

Isolate number	Tree species	Mean growth inhibition (%)		
		T. koningii (T_1)	*T. reesei* (T_{27})	*T. harzianum* (T_{13})
13	*Paraserianthes falcataria*	62.8	100.0	56.4
24	*Leucaena leucochepala*	99.5	100.0	89.4
2	*Acacia mangium*	94.3	96.5	79.8
9	*A. auriculiformis*	96.4	98.4	86.6
19	*Cassia siamea*	98.6	98.3	90.6
10	*Delonix regia*	97.0	98.2	83.0
4	*A. mangium*	98.8	98.2	84.6
17	*Dalbergia latifolia*	98.5	95.7	71.6

(a) (b) (c)

Figure 2. Antagonistic ability of three *Trichoderma* isolates varied towards pathogenic fungi (*Ganoderma* sp.) grown in paired cultures on agar: (a) *Ganoderma* sp. vs *T. Reesei*; (b) *Ganoderma* sp. vs *T. Koningii*; (c) *Ganoderma* sp. vs *T. harzianum*. Key: **P** = Pathogen, **T** = *Trichoderma*

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Extra-cellular enzyme production in *Trichoderma–Ganoderma philippi* interactions

Extracellular cell-wall-degrading enzymes such as chitinolytic and glucanolytic enzymes are believed to be involved in mycoparasitism. Recently, 32-kDa endochitinolytic enzyme was purified and characterised from *T. reesei* (Harjono et al. 2001; Harjono and Widyastuti 2001a) and tested for its ability to inhibit colony and hyphal growth of *G. philippii* isolate T_{13} (Harjono and Widyastuti 2001b).

The colony development of *G. philippii* is clearly inhibited at concentrations of 90–200 mg/mL (Figure 4a). A concentration of 80 µg/mL shows only an obscure inhibition zone. No inhibition zone is observed at concentrations of 40–70 µg/mL.

Microscopic observations revealed that morphological changes in *G. philippii* hyphae were induced by *T. reesei* 32-kDa endochitinase (Table 2). Branching and segmentation of hyphal tips occurred at 60 µg/mL of endochitinase application (Table 2 and Figure 4b,c). At this concentration, the enzyme also caused swollen hyphal tips (Figure 4d).

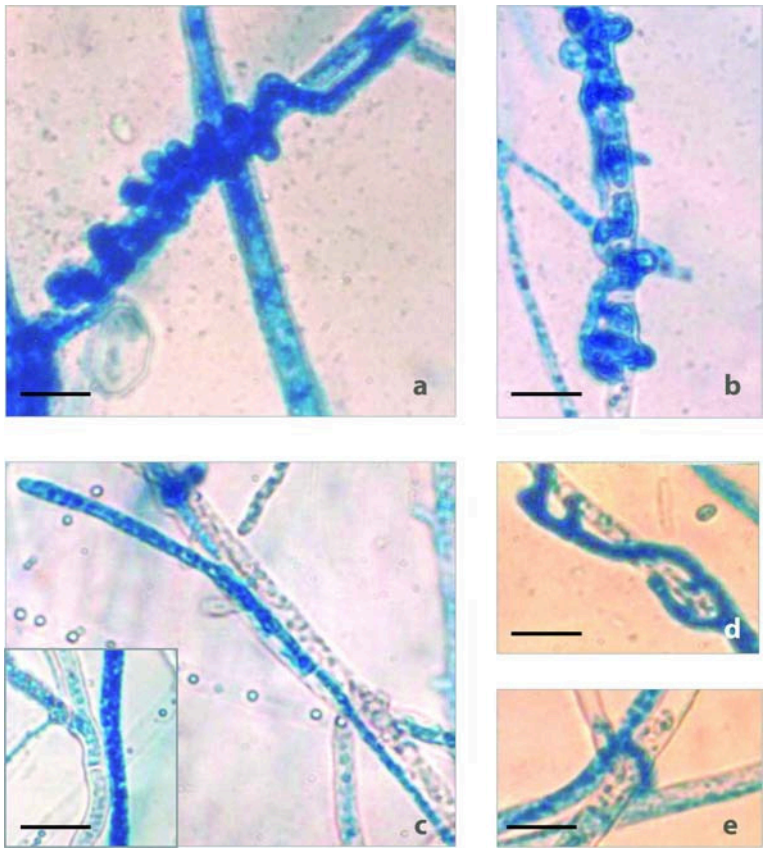

Figure 3. Light microscopy of *Trichoderma* hyphae interacting with that of *Ganoderma*: (a and b**)** Condensed coiling of *T. reesei* and *T. harzianum*, respectively, around a hypha of *Ganoderma* sp. (c**)** *T. koningii* could grow parallel to hypha of *Ganoderma* sp. but no coiling was observed. (d) Appresorium-like structures, formed by *T. reesei,* also found in *T. harzianum* but not in *T. koningii*. (e) Hook-like structure found in *T. reesei* and *T. harzianum* which serve as an attachment to the host mycelium. (Bar represents 10 µm).

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Hyphal necrotic lesions were observed at concentrations 80, 90, 100 and 200 µg/mL (Table 2). The latter 32-kDa endochitinase concentrations caused permanent hyphal necrosis of *G. philippii* (Figure 4e). Hyphal lysis by the enzyme was observed at a concentration of 90 µg/mL and the mean percentage increased dramatically at a concentration of 200 µg/mL (Table 2). At this high concentration, cell bursting and protoplast release was observed not only on hyphal tips (Figure 4g), but also on mature hyphae (Figure 4h). Enzyme application at low concentrations (80 µg/mL) failed to suppress the growth of hyphae (Figure 4f).

Efficacy of formulated *Trichoderma* as a biological control agent tested against *Sclerotium rolfsii*

Greenhouse experiments were used to evaluate the efficacy of isolates T_{13} (*T. reesei*), T_{27} (*T. harzianum*) and T_1 (*T. koningii*) as biological control agents in a formulation of alginate beads (Widyastuti et al. 2003, 2006a,b,c), against a common damping-off pathogen, *Sclerotium rolfsii*. The levels of biological control achieved for the damping-off by *S. rolfsii* on pine seedlings by applying *Trichoderma* spp. to soil are recorded in Table 3. The application of formulated *Trichoderma* into potting soil 4 days before the pathogen *S. rolfsii* was inoculated gave the best disease suppression, with the *Trichoderma* delaying the initiation of symptoms and reducing disease incidence. *Trichoderma reesei*, *T. harzianum* and *T. koningii* delayed the initiation of symptoms and decreased disease incidence by 73%, 67% and 47%, respectively, when applied 4 days before disease inoculation. Disease reduction by *Trichoderma* declined drastically when *Trichoderma* was applied 4 days after inoculation with *S. rolfsii*. Adaptation and establishment of *Trichoderma* before pathogen inoculation probably increases antagonistic capability. Our results agree with findings of Widyastuti et al. (2002) that formulated *Trichoderma* needs a period of adaptation in order to actively inhibit the growth of pathogenic fungi.

Table 3. Control by three isolates of *Trichoderma* formulated in alginate beads of damping-off caused by *Sclerotium rolfsii* in greenhouse-grown pine seedlings

Trichoderma isolates	Disease reduction according to *S. rolfsii* inoculation[a]		
	4 days before	At the same time	4 days after
T. koningii isolate T_1	46.7*b*	43.3*ab*	3.3*b*
T. reesei isolate T_{13}	73.3*a*	50.0*a*	23.3*a*
T. harzianum isolate T_{27}	66.7*a*	56.7*a*	6.7*b*

[a] Data in each column which are followed by a common letter are not statistically different (p=0.05). Percentage disease reduction was calculated by counting the number of dead or dying plants in 15-day-old pine seedlings.

Table 2. Microscopic observations of the morphological anomalies in *G. philippii* hyphae caused by 32-kDa *Trichoderma reesei* endochitinase[a]

Endochitinase concentration (µg/mL)	Appearance of abnormal hyphae[b]				
	Segmented	Branched	Swollen	Necrotic	Lysed
0	–	–	–	–	–
40	–	–	–	–	–
50	–	–	–	–	–
60	1.7	0.8	1.8	–	–
70	1.7	2.5	0.8	–	–
80	4.2	2.5	2.5	5.0	–
90	6.7	5.0	2.5	8.3	1.7
100	10.8	5.8	7.5	11.7	9.2
200	7.5	8.3	6.7	15.8	14.2

[a] Data obtained 12 hours after enzyme application.
[b] Percentage of 120 hyphae observed under microscope (mean of two replications).
– Indicates that at these concentrations the endochitinase did not induce a morphological response in *Ganoderma* hyphae.

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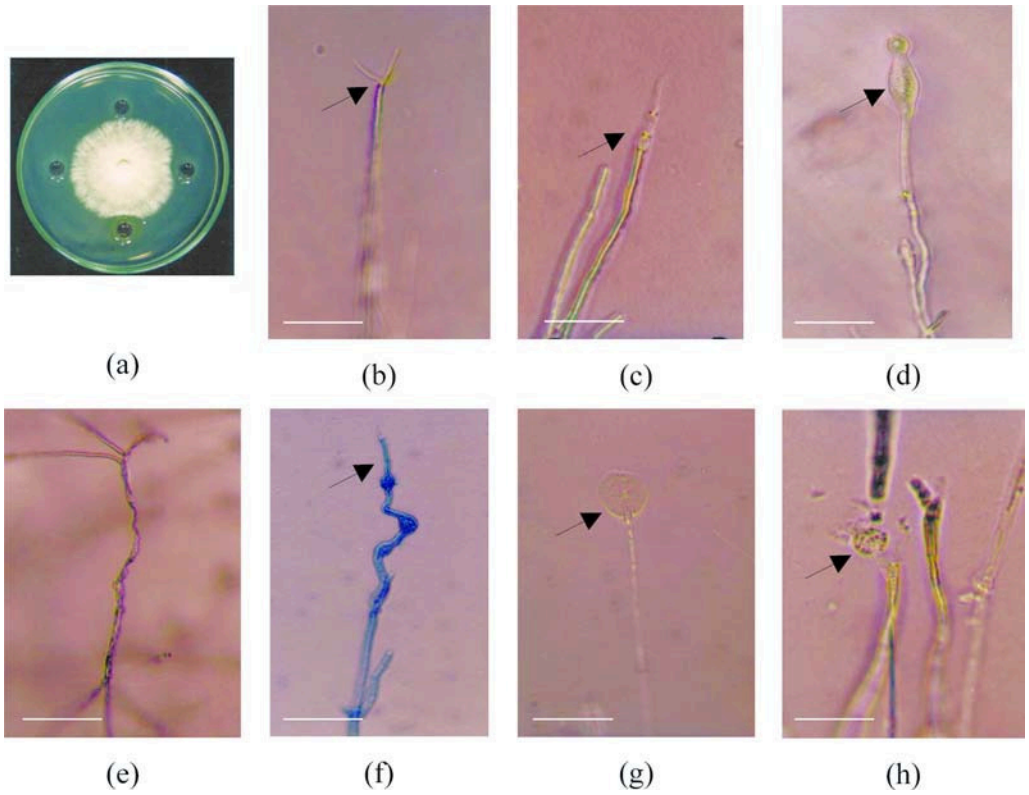

Figure 4. Effects of the 32 k-Da *T. reesei* endochitinase on hyphae of *G. philippii*: (a) Growth inhibition of *G. philippii* in response to various endochitinase concentrations (from top, in clockwise direction, 50, 100, 200, 0 μg/mL); (b, c, d) Branching, segmentation and swollen hyphal tip of *G. philippii* in the presence of endochitinase at 60 μg/mL and above; (e) *G. philippii* hyphae necrotic at hyphal tip grown in the presence of endochitinase higher than 70 μg/mL; (f) Hyphae of *G. philippii* after enzyme exposure of 80 μg/mL showing recovery after swelling and continued normal hyphal growth; (g) Cell bursting and protoplast release of hyphal tips in the presence of endochitinase at 90 to 200 μg/mL; (h) Cell bursting and protoplast release of mature hyphae. (Bars = 30 μm)

Conclusion

This paper provides an overview of various aspects of the antagonism of *Trichoderma* isolates that are relevant to their use as biological control agents. We are now able to screen *Trichoderma* isolates for traits relevant to antagonism, and select isolates according to their potential for biological control. Applied in appropriate biologically based formulations and delivery systems and at the right stage of the disease cycle, specific *Trichoderma* isolates selected for high levels of *Ganoderma*-suppressive activity warrant further investigation. Such *Trichoderma* isolates offer the potential for consistent and effective control of *Ganoderma* root rot without using chemical fungicides and the ensuing dangers of environmental pollution and the development of fungicide resistance.

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Minimising disease incidence in *Acacia* in the nursery

Budi Tjahjono[1]

Abstract

Disease management in the nursery requires detailed knowledge of the potential pathogens and their interactions with the seedlings and their environment. The behaviour of the pathogen must also be considered in the wider context of the nursery operation. The conditions that favour a range of foliar, stem and root diseases of *Acacia* seedlings in the nursery are described. Integral control strategies are discussed in general, and in detail for *Xanthomonas* sp., the common bacterial leaf blight of *Acacia crassicarpa*.

Reforestation of the industrial plantation forest (or HTI) in Indonesia should be undertaken by planting seedlings immediately after an area has been cut over. Implementing this recommendation, however, requires the timely supply of seedlings and often creates a demand for high rates of production from plantation nurseries. To attain this goal, uniform, vigorous, high-quality seedlings must be produced and disease incidence and losses kept to a minimum. Seedling losses in the nursery directly affect rates of plantation establishment, as well as increasing maintenance costs during the rotation.

Nursery management

It is important to identify the actual and potential disease problems in the nursery in order to implement an effective disease-management strategy. Detailed knowledge of the pathogen and its interaction with the seedling and environment is vital. Information on the origin of the pathogen, how it is spread, the severity of its impact, what environmental and host factors favour its development, and its response to fungicides, all contribute to the development of control strategies.

Disease management must be considered in the context of the wider nursery operation: container management, shading, irrigation, fertiliser application, pest control and sanitation. Most diseases in nurseries can be adequately managed by the application of good cultural practices. Poor practices, such as incorrect sowing depth, high seedling density and inappropriate levels of shade, can increase the incidence and severity of diseases.

Crowding of seedlings encourages the development of some diseases that are much reduced or disappear after the stock has been transplanted in the field. Whether the seedlings are grown under shade net, plastic protection or the open sky also affects the occurrence of nursery diseases. Excessive shade, resulting in low light intensity, tends to lower soil temperature and raise the water potential, favouring the development of damping-off disease. Conversely, seedbeds under high light intensity tend to have higher soil temperature and lower soil-water potential, conditions that favour the development of seedling blight and shoot blight.

The selection of growing medium is also important in nursery disease management. Seedbed soils and container mixes for acacia seedlings need to have good

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structure, be free-draining and slightly acid (pH5–5.5). Poor drainage causes waterlogging damage and can lead to the development of soil-borne diseases. An ideal medium for container production of acacia seedlings has an organic base to support the nursery production phase (about 10–12 weeks), with sufficient added fertiliser to reduce follow-up fertiliser application to a minimum. Organic-based media also provide a receptive base should inoculations of mycorrhizae or biological control agents be required. Acacia seedling growth is generally restricted in the nursery after 10 weeks through reduced irrigation and fertiliser. If seedlings are retained in the nursery for more than 12 weeks, there is potential for severe levels of diseases to develop.

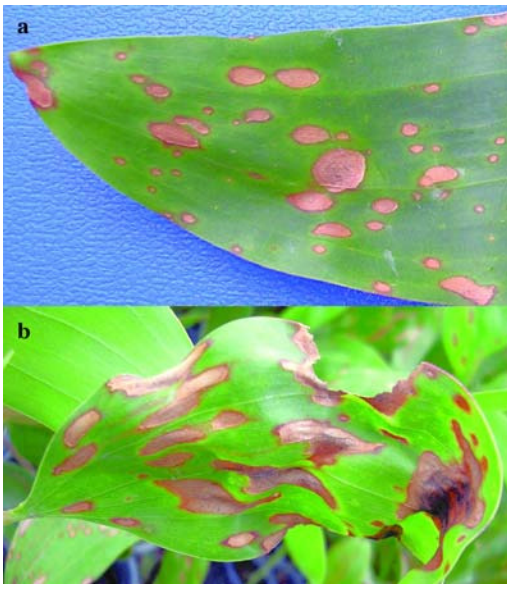

Figure 1. Common leaf diseases in *Acacia crassicarpa*: (a) *Pestalotiopsis* leaf spot; (b) *Phaeotrichoconis* leaf spot

Foliage diseases

For an infection to be established, fungi and bacteria that cause foliage diseases usually require conditions of high moisture and free water in and around stems and foliage for long periods. Foliage diseases are therefore most prevalent in locations where frequent rain or the continuous use of overhead irrigation allows these conditions to develop.

Common leaf diseases in *Acacia crassicarpa* A. Cunn. ex Benth. seedlings are *Pestalotiopsis* leaf spot (Figure 1a), *Phaeotrichoconis* leaf spot (Figure 1b) and bacterial leaf blight caused by *Xanthomonas* sp. Important leaf diseases on *Acacia mangium* Willd. seedlings are phyllode rust disease caused by *Atelocauda digitata* (Figure 2), anthracnose disease and tip necrosis caused by *Colletotrichum* sp.

Stem and root diseases

From germination through the first few weeks after emergence, the succulent radicle and hypocotyl tissues of *Acacia* seedlings are extremely susceptible to attack by damping-off fungi. *Pythium, Rhizoctonia* and *Fusarium* are fungi that commonly cause damping-off. The exact cause of many root diseases is often difficult to determine without laboratory analysis. Pre-emergence damping-off is caused by fungi that rot seedlings before they emerge. Post-emergence damping-off is caused by fungi that infect and kill stem tissues at ground level after seedling emergence, resulting in seedling collapse. Because damping-off is significantly influenced by environmental conditions, the severity of this disease fluctuates from year to year. In general, conditions that reduce seedling growth and vigour predispose nursery stock to increased infection from damping-off fungi. Nitrogen fertilisers applied before or during the first few weeks after seedling emergence may also increase infection. Other diseases of seedlings include canker and dieback pathogens. These attack the stems and branches of nursery seedlings, cause sunken lesions and malformations, and often lead to seedling death.

Integrated disease management

The basic approach to disease management in the nursery should be to avoid disease rather than have to apply controls after a disease outbreak. Common ways to minimise disease in nurseries that raise *Acacia* seedlings include:

- use of high-quality seeds from genetic material that has disease resistance
- cultural control through growing conditions, media and cultural practices
- proper irrigation and drainage
- good hygiene and quarantine
- the application of chemical or biological control, including selective pesticides that kill or inactivate the pathogen but cause relatively no harm to the seedlings.

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Figure 2. Phyllode rust caused by *Atelocauda digitata* on *Acacia mangium* seedlings

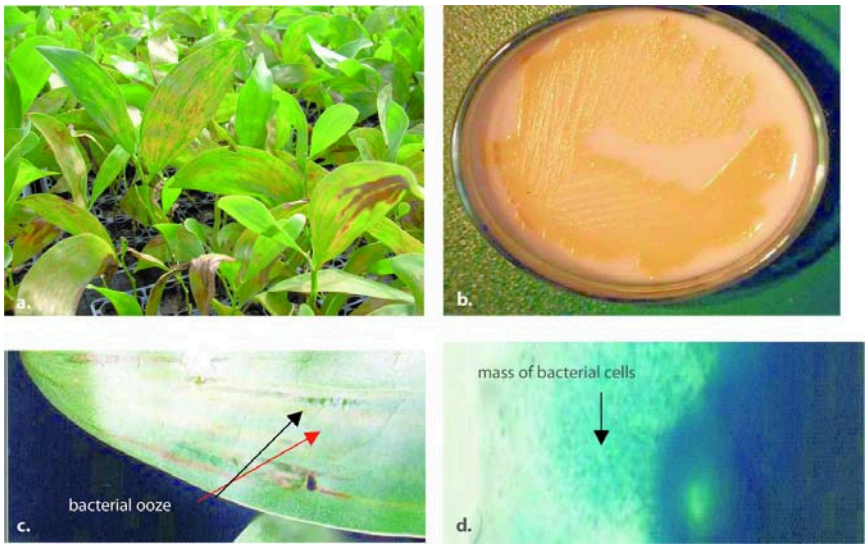

Figure 3. Common bacterial leaf blight in *A. crassicarpa* seedlings: (a) symptoms; (b) yellow colony of pathogenic bacteria *Xanthomonas* sp.; (c) leaf with bacterial ooze; (d) cross-section of infected leaf (400× magnification)

Several cultural practices can reduce or control nursery diseases. Good-quality growing media and full containers encourage the growth of vigorous seedlings that are better able to resist stress and disease. Good hygiene and housekeeping, based on the nursery area being kept well-drained, clean and orderly, reduce disease infection and loss of seedlings. Watering should be done only as needed and the irrigation systems should be regularly inspected for leaks or blockages. Foliage diseases in particular can be inhibited by reducing seedbed density in order to lower humidity and increase air circulation around the leaves. The removal and controlled burning of heavily infected and dead seedlings from seedbeds will

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reduce the source of inoculum, as well the likelihood of subsequent infection of other nursery seedlings.

Chemical or biological pesticides may be used to protect seedlings from infection or eradicate a pathogen already present in the nursery. Spraying with a fungicide, for instance, protects the seedlings by coating the foliage with a chemical barrier that is toxic to foliar pathogens. Such chemical control requires proper timing to coincide with periods of potential infection. Soil fumigation before planting can control root-disease pathogens, but seldom will it completely eradicate them.

The concept of integrated disease management in the nursery can be represented by the palm of the hand and its five fingers. Each finger represents one of the five components of disease control (see dot points above); the palm is the nursery manager. The palm is central to coordinating the five fingers. A single approach to disease management in the nursery seldom leads to disease control.

The integrated management of common bacterial leaf blight — a case study

Bacterial leaf blight of *A. crassicarpa* caused by *Xanthomonas* sp. is a common problem in the nursery (Figure 3). *Acacia mangium* and *Acacia auriculiformis* A. Cunn. ex Benth. appear immune to this disease. Conditions that predispose seedlings to the development of bacterial leaf blight include:

- stress due to excess or lack of water, lack or imbalance in nutrition, unsuitable growing media and/or excess nitrogen fertiliser application
- poor sanitation of media, water supplies and tube sterilisation
- intense rainfall or strong splash water from irrigation
- leaf scars/lesions as entry points for the bacteria.

The recommended steps for integrated control are:

- use suitable growing media with adequate porosity but high water retention
- water only when required and ensure fertiliser applied is balanced
- maintain good nursery hygiene with proper drainage
- remove and burn dead and severely diseased plants
- handle seedlings gently to reduce scars and lesions
- sterilise (using steam if possible) equipment, seedbed, containers
- apply bactericide: Agrept 20 WP and Kibox (copper oxychloride)
- plant non-host plant material, i.e. species other than *A. crassicarpa.*

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Developing a strategy for pruning and thinning *Acacia mangium* to increase wood value

Chris Beadle[1]

Abstract

Timber production from *Acacia mangium* plantations in Indonesia can potentially supply both domestic and export markets for furniture and other functional and appearance products. In plantations, there is potential for large and persistent branches to develop, and the threat of persistent dead branches and of heart rot. This has resulted in the need for pruning systems based on the removal of green branches from below, and thinning systems that ensure final-crop trees retain green branches until pruning is completed, as well as maintaining acceptable rates of growth of the retained trees. This paper outlines a pruning and thinning strategy for *A. mangium* that attempts to meet the criteria required for growing high-quality, clear-wood plantations. Form-pruning to encourage the development of good form ahead of lift-pruning is used in a system that results in about 300 stems/ha of trees pruned to 4.5 m in the final crop. It is concluded that heart rot is made worse by pruning when the plant material is susceptible and a sufficient source of fungi is present to invade pruning wounds.

Acacia mangium Willd. plantations represent a significant proportion of the wood supply to the pulp and paper industry in Indonesia (Rimbawanto 2002). The species is fast growing and of medium density. When free from defects, the wood looks similar to teak (Gales 2002). The timber is also used in the domestic market in Indonesia for furniture and other functional and appearance products, and exported for finger-jointing in a developing overseas market (Hardiyanto 2006). With relatively short rotations of no more than 20 years to produce saw logs (Srivastava 1993), *A. mangium* timber production could supply a substantial part of the solid-wood market in the medium term. Knots and heart rot are undesirable characteristics that reduce the strength, appearance and value of the timber. Variable numbers of trees are also multi-stemmed at the base, the proportion probably related to genotype and site conditions (Srivastava 1993). Single stems are required for plantation silviculture and are essential for solid-wood production. A pruning strategy therefore becomes necessary.

When managed for pulpwood, *A. mangium* plantations are established at around 1000 stems/ha. Such planting densities are too high for all the trees to be managed for solid wood. A lower stocking at planting is one option but experience from growing other species suggests that starting with higher stockings ensures that there are sufficient potential final-crop trees in the stand that meet criteria for pruning (Beadle et al. 1994). Higher stockings also impose some control on average branch size. Large branches are more difficult to prune and potentially more susceptible to decay entry. A thinning strategy therefore becomes an inevitable part of the silviculture of *A. mangium* plantations for solid-wood production.

Pruning

Acacia mangium has persistent branches. This has led to the development of lift-pruning regimes where

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branches are removed from the base of the tree upwards to convert the bottom log to clear or knot-free wood (Mead and Speechly 1991; Weinland and Zuhaidi 1991). The results of studies of a number of species indicate that the preferred practice is green pruning that removes live rather than dead branches. Dead branches are associated with a high percentage of discoloration and decay in unpruned *A. mangium* (Ito and Nanis 1994). Pruning live branches also prevents the development of loose knots and decreases the size of the knotty core.

The removal of live branches inevitably reduces the productive capacity of an individual tree because of the loss of green leaf area. This is of particular concern in systems where only a proportion of the planted trees are selected for pruning, potentially putting them at a competitive disadvantage against the unpruned trees. Prescriptions must therefore optimise clear-wood production in such a way that there is no significant effect on tree growth after pruning.

Large branches are generally associated with poor stem form and with a high risk of decay entry after pruning. In a study of fungal infection two years after pruning *A. mangium*, Weinland and Zuhaidi (unpublished data; see Srivastava (1993)) noted that wounds of diameter >20 mm were always infected whether the branches had been removed when green or dead. In *Eucalyptus nitens* (Maiden), the length of decay columns increased exponentially when the diameter of pruned branches was >20 mm. Branches at a narrow angle to the stem were also associated with a greater risk of decay (Mohammed et al. 2000). Thus, control of branch size is of vital importance in the silviculture of plantations managed for solid wood. Stocking and form-pruning provide two options for such control. Increasing the initial stocking density will reduce the incidence of large branches (Neilsen and Gerrand 1999) but more intense inter-tree competition will then reduce the average growth of individual trees. Form-pruning selectively removes branches throughout the crown and can be used to reduce average branch size before subsequent lift-pruning (Pinkard 2002).

The proportion of branches that can be removed at any one pruning before growth is affected is a function of species and site (Table 1). Slower-growing species, for example, are more affected by pruning than faster-growing species. In one experiment in Malaysia, *A. mangium* tolerated the removal of 40% of its crown length by lift-pruning before growth was significantly affected (Majid and Paudyal 1992). Growth is less affected by pruning on a high than a low quality site (Pinkard and Beadle 1998a), so pruning severity must be reduced on sites associated with slower rates of growth. In general, pruning affects height growth less than it does diameter growth. In a stand planted at 1100 stems per hectare in South Sumatra (C.L. Beadle, K. Barry, E. Hardiyanto, R. Irianto, Junarto, C. Mohammed and A. Rimbawanto, unpublished data), height and diameter growth following removal of 25% of crown length using lift-pruning was significantly greater 18 months after pruning than in an unpruned control (Table 2). However, the pruning treatment had been singled while the controls had not, and it is likely that competition between multiple stems slowed individual stem growth of the controls.

In the same stand in South Sumatra, a form-pruning treatment removed selected branches up to 3 m height that were either large or competing with the leader and until 25% leaf area had been removed from the tree. The effects on tree growth were not significantly different from those observed with lift-pruning (Table 2). However, form-pruning was associated with improved stem straightness expressed as reduced kink: more than 80% of the trees were assessed as having slight or no kinks in the first 3 m of the stem, the part that had been form-pruned (Table 3). Although current tree spacings at planting and existing planting stock are inevitably associated with the development of some large branches (>30 mm), pruning appeared to offer some level of control of their number in this experiment.

Table 1. The percentage of crown length removed above which growth is reduced in a range of tree species pruned in plantations

Species	%
Acacia mangium[a]	40
A. melanoxylon[b]	25
Eucalyptus grandis[c]	40
E. nitens[d]	50
Pinus patula[e]	25
P. radiata[f]	35
P. sylvestris[g]	40
Cryptomeria japonica[h]	30

[a]Majid and Paudyal (1992); [b]Medhurst et al. (2003); [c]Bredenkamp et al. (1980); [d]Pinkard and Beadle (1998); [e]Karani (1978); [f]Sutton and Crowe (1975); [g]Långström and Hellqvist (1991); [h]Fujimori and Waseda (1972)

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Table 2. Mean stem diameter, height increment and green crown lift of *Acacia mangium* 18 months after pruning at a plantation in South Sumatra. Means sharing the same letters are not significantly different at $p<0.05$. At age 18 months, when the trees were pruned (see text), their average height and diameter were 4.3 m and 4.1 cm, respectively, and there were no significant differences between treatments.

Pruning treatment	Diameter increment (cm)	Height increment (m)	Green crown lift (m)
Control	6.83 *a*	7.44 *a*	2.49 *a*
Form	8.28 *b*	8.35 *b*	3.07 *a*
Lift	8.06 *b*	8.20 *b*	2.74 *a*

Green pruning triggers physiological responses that increase biomass production to a level that is significantly greater than in a similar unpruned tree growing under the same environmental conditions. These physiological changes can be detected within a few days to weeks after pruning in eucalypts and acacias, and may be sustained for several months (Pinkard and Beadle 2000; Medhurst et al. 2006). These changes in physiological activity are observed as an increased rate of crown development (Pinkard and Beadle 1998b) expressed through higher rates of leaf expansion, greater leaf development in the upper crown, greater leaf area to branch basal area ratio and reduced leaf senescence. Significant increases in light-saturated rates of single-leaf net photosynthesis are also observed to occur throughout the crown, though decrease in magnitude with depth in the canopy (Pinkard et al. 1998; Medhurst et al. 2006).

Table 3. Percentage of assessed trees by kink class between 0–3 m height, 18 months after pruning at a plantation in South Sumatra. The class codes for kink were: (1) no kinks, (2) slight kinks, (3) stem deviation stays within the centre line, (4) stem deviates outside the centre line. The trees had been form-pruned to 3 m height at pruning (see text).

Pruning treatment	Percentage of trees by class code			
	1	2	3	4
Control	25.0	36.2	19.4	19.4
Form	55.6	27.8	8.3	8.3
Lift	25.0	36.1	13.9	25.0

The implication of these findings is that pruning severity should be linked to the capacity of these compensatory responses to result in no significant change in the growth of the pruned trees compared with the unpruned trees in the stand. As pruning is normally undertaken to a height of at least 4.5 m, two or more lifts are required to ensure that crown removal in any one lift does not exceed the level that triggers a significant reduction in growth rate. While the compensatory responses just referred to have been demonstrated to work adequately for first-lift pruning in *E. nitens* and *A. melanoxylon* growing in a temperate climate (Pinkard and Beadle 2000; Medhurst et al. 2006), the physiological responses to subsequent lifts have not been as thoroughly investigated. The much higher rates of growth observed in a tropical species mean that the elapsed time between each lift-pruning will be a few months only. In the experiment in South Sumatra referred to above, the base of the green crown was, on average, between 2.5 and 3.1 m above ground level 18 months after pruning (Table 2), and substantially beyond the height (≤1.5 m) to which branches had been pruned in the lift-pruning treatment.

Thinning

Thinning serves to maximise the diameter growth of trees by reducing intra-specific competition. In the present context this is achieved by the harvesting or removal of unpruned trees. In *A. mangium* plantations managed for solid wood, the numbers of pruned trees in the final crop and the thinning strategy adopted will depend on the target tree-size and rotation length. The commercial value of the thinned trees may also dictate the timing of the thinning operation. There are, however, some useful principles that have emerged from thinning trials with other species that can help develop a workable strategy for thinning *A. mangium*.

Thinning intensity affects stand growth. In a *E. nitens* stand established at 1143 stems/ha (3.5 m × 2.5 m spacings), cumulative basal area growth per hectare was unaffected by thinning to 300 stems/ha seven years after thinning, while the removal of greater than 66% of standing basal area at thinning

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(that resulted in a stand density of 100 stems/ha) was associated with a significant reduction in cumulative basal area growth per hectare (Medhurst et al. 2001). Individual tree growth was improved with thinning intensity and, in general, the dominants and co-dominants were the trees in the stand that produced a significant basal area response to thinning.

The growth response occurs because of changes in the distribution of light and canopy photosynthesis in the stand following thinning (Medhurst and Beadle 2005). Significantly higher fractions of incident light are found in the middle and lower crowns of the trees and significantly greater light-saturated rates of photosynthesis are observed in the leaves of the lower crown, compared with unthinned stands. In the results of a study by Medhurst and Beadle (2005), these changes were expressed through changes in the distribution of foliar nitrogen (N) content that result in positive relationships between N content and the fraction of incident light or light-saturated photosynthetic rate. The increases in N content are related to a significant decrease in specific leaf area (leaf area per unit leaf weight; Medhurst and Beadle (2005)). Changes in leaf area per tree are associated with an increase in crown length in thinned stands (Medhurst 2000).

These observations suggest that thinning at or soon after canopy closure will maximise the growth response: conversely, delaying thinning until after there has been appreciable crown lift will reduce the magnitude of the response. Thinning intensity should be commensurate with the maximisation of light interception, and therefore of growth, by individual trees. However, as thinning regimes aim to maximise stand production as well as optimise tree size, the ideal residual stand density after thinning will be that which will lead to maximum leaf area index (leaf area per unit ground area) towards the end of the rotation, or just ahead of the next thinning. This will depend, of course, also on the tree sizes that are being sought at harvest. Medhurst et al. (2001) found that, in general, the lower the quality of the site, the lower was the ability of the stand to respond to thinning. Thinning intensity therefore needs to increase with increasing site quality: smaller individual tree sizes are a given on lower-quality sites. As trees have a capacity to develop longer tree crowns on high-quality sites, delaying thinning will not, up to a point, preclude a growth response from thinning.

A pruning and thinning strategy

The pruning and thinning strategy presented here uses current establishment practices for *A. mangium*. The pruning strategy is in part based on current practice used for *A. mangium* in Indonesia and *Eucalyptus globulus* and *E. nitens* in Australia (Gerrand et al. 1997), with the addition of form-pruning. As form-pruning to date has been used only as a research tool, its benefits require further testing. The thinning strategy is linked to observations of current growth rates of *A. mangium* in Australian and Indonesia. The timing of each operation attempts to meet the criteria described above for optimising the quantity and quality of clear wood produced.

Acacia mangium plantations are established at 3 × 3 m spacings (1111 stems/ha). The pruning and thinning strategy is then based on the following requirements:

- Form and lift-pruning to 4.5 m tree height, and thinning to reduce the stocking from 1111 to 296 stems/ha.
- Four silvicultural interventions after planting, excluding the need for any fertiliser application and weed control.
- Every fifth row is an outrow removed at first thinning. These rows, equivalent to 222 stems/ha, are not used to select trees for pruning.
- The maximum number of trees available to select those for pruning is therefore 889 stems/ha (1111 – 222 = 889 stems/ha), equivalent to a selection ratio based on 296 stems/ha at final stocking of about 1:3.

Implementing the strategy is a four-step process (see Figure 1).

Step 1

Stage of growth: age 4–6 months

Operations

- Single trees

Step 2 (Figure 1a)

Stage of growth: Mean total height @ 4 m

Operations

- Form-prune two of every three trees of 889 trees, i.e. 593 stems/ha, to 2.5 m (removes large branches >15–20 mm diameter and high angle branches). The trees selected for form-pruning should be those that meet criteria for good form (Table 4).

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Step 3 (Figure 1b,c)

Stage of growth: Mean total height @ 8 m

Operations

- Form-prune the best one of two trees pruned to 2.5 m (i.e. 296 of 593 stems/ha) to 4.5 m.
- Lift-prune these trees to 2.5 m.
- Thin all outrow trees (222 stems/ha) and the trees that were not pruned at Step 2 (296 stems/ha). This reduces the stocking from 1111 to 593 stems/ha).

Step 4 (Fig. 1d)

Stage of growth: Mean total height @ 12 m

Operations

- Lift-prune to 4.5 m trees that were form-pruned to 4.5 m.
- Thin those trees that were form-pruned to 2.5 m only (295 stems/ha). This will create the final stocking of 296 stems/ha.

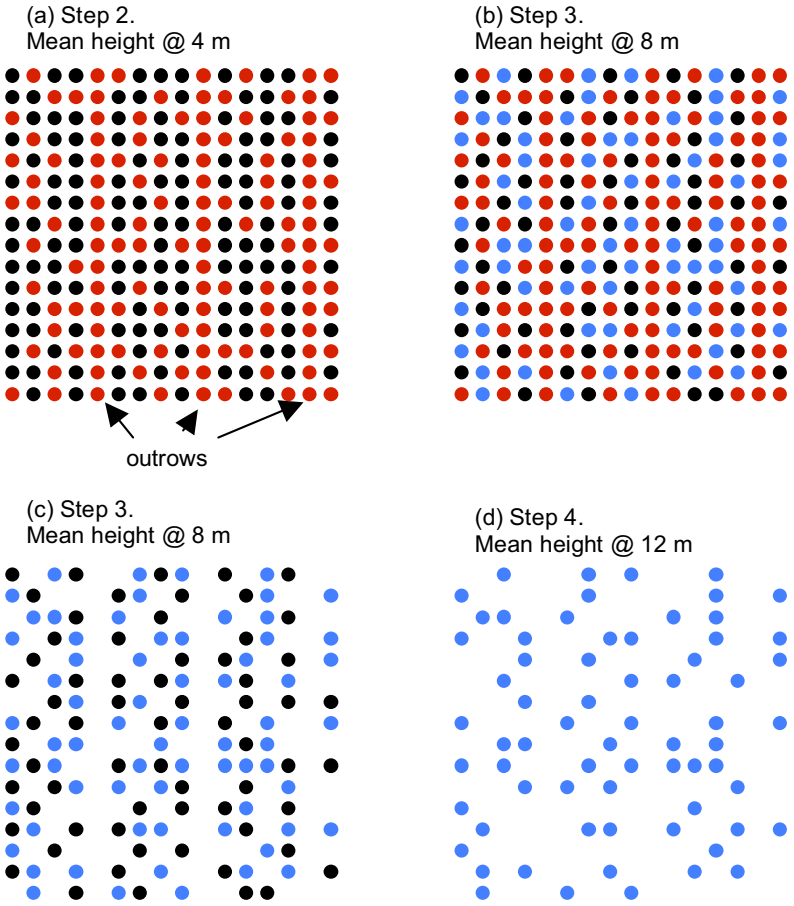

Figure 1. A representation of a pruning and thinning strategy for *Acacia mangium*. The trees are singled at age 4–6 months (see *Step 1* in text). (a) In *Step 2,* two (black) out of every three trees are form-pruned to 2.5 m. (b) In *Step 3,* the better (blue) of each two trees form-pruned in *Step 2* is now form-pruned to 4.5 m. The same trees are lift-pruned to 2.5 m. (c) Also in *Step 3,* the outrows (red) and the trees not pruned (also red) in *Step 2* are thinned (harvested). (d) In *Step 4,* the trees form-pruned to 4.5 m are now lift-pruned to 4.5 m. The trees form-pruned only to 2.5 m in *Step 2* are harvested. The final stocking rate is 296 stems/ha.

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This strategy is based on form and lift-pruning to 4.5 m in height. Both are done in two sections (to 2.5 m and then to 4.5 m), first the form-pruning then, after the tree has grown another 4 m in height, the lift-pruning is undertaken in the section that was previously form-pruned. The removal of branches >15–20 mm diameter during form-pruning anticipates that these branches would have been >30 mm diameter at lift-pruning. Care should be taken not to remove more than 25–30% of the total leaf area during pruning at any of the above stages.

Table 4. Criteria that together define acceptable form for pruning an individual tree (adapted from Beadle et al. (1994)). These are used to ensure that the better trees are selected for pruning. If insufficient trees required for clear wood meet these criteria, it may be necessary to select some trees that do not meet all the criteria. Form-pruning is a mechanism for increasing the number of trees that will eventually meet the criteria.

1	Single-stemmed and free of secondary leaders[a]
2	Straight stem with minimal stem deformations from the vertical
3	Stems free from wounds and disease
4	No branches already >30 mm in diameter
5	Butt sweep limited to the bottom 0.3 m

[a] Secondary leaders that form above the final pruning height can be acceptable

Two thinning operations are also required, one when the average tree height is about 8 m, the second when tree height is about 12 m. The size of the thinned trees may be suitable for pulpwood but care should be taken not to damage retained trees during the thinning operations. This is assisted by the creation of an outrow at the first thinning to help access to the other rows for individual tree removal. In one sense the strategy is conservative in that it may not be necessary in *Step 2* to form-prune twice the number of trees that are going to be retained in the final crop: the additional form-pruning is a cost but it allows greater choice of the final-crop trees at *Step 3*. However, the timing of the thinning operation is to some extent speculative and assumes that each of the two operations will occur before canopy lift commences in the pruned trees. The required size of the final-crop trees will determine the time of harvest. Given the types of growth rates experienced in *A. mangium* plantations, rotation lengths of no longer than about 10–12 years are assumed or inter-tree competition will probably start to compromise individual tree-growth rates. Thinning regimes that reduce final-crop stocking to lower levels will then be required (Mead and Speechly 1991; Srivastava 1993) and will be essential anyway if tree sizes >30 cm diameter breast height over bark are required.

Heart rot

Cut surfaces from pruning and singling are potential infection courts for the entry of decay-causing fungi (Gales 2002; Lee 2002). Infection of heartwood by a range of white-rot fungi leads to heart rot, in which the decayed wood appears fibrous and stringy as well as a pale yellowish-white (Lee et al. 1988). Whereas heart rot can be tolerated in wood destined for pulp, it is not acceptable in solid-wood products.

Heart rot has been recorded in pruned plantations of *A. mangium* during a number of studies in Malaysia (Lee et al. 1988; Ito 2002). Unhealed pruning wounds have been recorded as contributing to up to 62.5% of heart rot infections (Lee et al. 1988). During a survey of heart rot in *A. mangium* plantations in Indonesia, Barry et al. (2004) found the highest incidence of heart rot was associated with a region where pruning occurred. In contrast, Zakaria et al. (1994) found no relationship between the incidence of heart rot and pruning of *A. mangium* in Malaysia. In a study in South Sumatra, no heart rot was detected 18 months after pruning (Beadle et al., unpublished data). In an *A. mangium* provenance trial, also conducted in South Sumatra, Suberanjeriji-plus (seed stock similar to that used by Beadle et al.) trees were found to have the second lowest incidence of heart-rot infection associated with drill wounds of a total of six provenances (Barry et al. 2006). Thus, while pruning has been linked to increased heart rot in some regions in Indonesia (Barry et al. 2004) and Malaysia (Lee et al. 1988) it appears to be of importance only when the plant material is susceptible and a sufficient source of heart rot fungi is present to invade the wounds.

Synthesis

While the fundamental requirements of green pruning and thinning in plantations managed for solid wood are well established, the development of pruning and thinning schedules for *Acacia mangium*

remain in its infancy. Form-pruning appears to offer an approach for improving stem straightness. However, its coordination with subsequent lift-pruning requires further investigation. How to schedule the timing of thinning operations is a complex issue. The first requirement is that their timing should ensure that branches remain green but at the same time do not become large. The second requirement is that the timing of thinning should aim maximise tree growth rates, stand production and the value of the thinned as well as the final-crop trees. Just how to manage what can be difficult compromises should also be a focus for new research.

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Options for solid-wood products from *Acacia mangium* plantations

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Abstract

Acacia mangium wood has proved to be not only suitable for producing high-quality pulp and paper, but also an excellent material for solid-wood products. There has been growing interest in utilising wood of *A. mangium* for solid wood, corresponding with the declining availability of logs from native forests. The currently available sawlogs of *A. mangium* are harvested from unpruned and unthinned plantations, and consequently are of low quality (many knots and poor stem form) with poor recovery of sawn timber. Proper silvicultural techniques that incorporate pruning and thinning are of crucial importance in growing plantations for solid wood. *Acacia mangium* can be grown for solid-wood products with a rotation of around 10 years that is expected to produce a total stem volume more than 200 m^3 per ha: about 30% of it will be for solid wood. Minimum tree diameter at breast height will be 30 cm. The current price of sawlogs of *A. mangium* (from unpruned and unthinned plantations) is considered low, but it is expected to increase with reducing availability of logs from natural forest, increased familiarity of its users with the wood, and better log quality from plantations managed specifically for solid-wood products.

Since the early 1990s, industrial forest plantations have been developed in Indonesia. As of 2004, a total of 2,500,966 ha of pulp plantation and 865,256 ha of sawlog plantation had been established (Dirjen BPK 2005). Acacia plantations have been established primarily to produce wood for the pulp and paper industries. *Acacia mangium* Willd. is the main plantation species planted on mineral soils, while *Acacia crassicarpa* A. Cunn. ex Benth. is the only species grown on peat land. The pulp properties of *A. mangium* wood are comparable to those of many *Eucalyptus* species. A number of studies on the utilisation of *A. mangium* show that its wood is not only excellent for pulp and paper, but also good for wood products such as plywood, furniture, flooring and light construction. Lately there has been growing interest in utilising wood of *A. mangium* for solid-wood products. This corresponds with the reduced availability of logs from native forests. The continuing pressure to protect native forest will further enhance the opportunity to develop sawlog plantations of *A. mangium*.

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Solid-wood utilisation

Various studies have been conducted to examine the suitability of *A. mangium* for products other than pulp and paper. The density of *A. mangium* wood varies from 420 to 483 kg/m^3. It is considered to be stable, with shrinkage from fresh to air dry of around 6.4% tangentially and 2.7% radially (Abdul-Kader and Sahri 1993). Its fibre is relatively straight and only in certain cases is found to have interlocked grain (Yamamoto 1998).

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The timber of *A. mangium* dries well, fairly rapidly and without serious defects when a suitable kiln schedule is used (Abdul-Kader and Sahri 1993). However, the wood is prone to initial checking and can also collapse and shrink during drying, resulting in substantial losses (Yamamoto 1998), hence the need for a proper kiln schedule to obtain good-quality timber.

The wood of *A. mangium* is classified as light hardwood with low to moderate strength. It has good machining properties and is suitable for making furniture, cabinets, mouldings, and door and window components (Abdul-Kader and Sahri 1993). The wood is also suitable for light structural works, agricultural implements, boxes and crates (Abdul-Kader and Sahri 1993; Yamamoto 1998). A recent study on the mechanical properties of sawn timber indicated that the wood of *A. mangium* can be used for construction and meets the requirements of the Indonesian Standard for Wood Construction (Amalia 2003; Firmanti and Kawai 2005). The wood of *A. mangium* can also be easily processed into veneer and plywood. The peeling process is considered easy and the green veneers produced are tight, smooth and of acceptable quality (Abdul-Kader and Sahri 1993; Yamamoto 1998).

Heart-rot disease in *A. mangium* has been reported in a number of studies and can reduce the utilisation of solid wood since heart rots reduce timber volume and quality (Lee et al. 1988; Lee 2002; Ito 2002). A recent survey conducted at a number of sites in Indonesia revealed that the incidence of heart rot varied according to site, from low (6.7% East Kalimantan, 11.3% South Sumatra) to reasonably high (35.3% Jambi, 46.7% West Java). A combination of differing plantation management strategies—for example, pruning, age and local conditions—explained the differences in heart-rot incidence (Barry et al. 2004). Pruning was reported to be linked to increased incidence (Barry et al. 2004), but may be of importance only when the plant material is susceptible and a sufficient source of heart-rot fungi is present in the environment to invade the wounds caused by pruning. In addition, while wounds caused by singling are typically assumed to have a large impact on the incidence of heart rot, an influence of singling on heart-rot incidence in a provenance trial in the Riau province of Sumatra was not apparent (Barry et al. 2006). A pruning study in South Sumatra also detected no heart rot 18 months after singling and pruning (C. Beadle, K. Barry, E.B. Hardiyanto, R. Irianto, Junarto, C. Mohammed and A. Rimbawanto, unpublished data). In areas where the incidence of heart rot is low, the development of sawlog plantations is considered to be possible.

Knots are the major factor limiting the utilisation of solid wood harvested from unpruned stands of *A. mangium*. Consequently, unless silvicultural techniques are used to control branching and hence the occurrence of knots, the quality of wood from this species may limit its utilisation. The recovery rate of sawn timber board from sawlogs harvested from unpruned and unthinned *A. mangium* stands is low; about 38% at PT. Musi Hutan Persada (Supriyadi, pers. comm. 2005) and 35% in S. Kalimantan (Thorp 2005), for example. By applying specific silvicultural treatments, there is the potential to substantially increase the recovery rate of *A. mangium* sawlogs.

Another defect that may reduce the general quality of the end product and limit the usefulness of the *A. mangium* sawlogs is end-splitting, particularly of trees harvested from stands under 10 years old. End-splitting is a phenomenon manifested during felling and cross-cutting of logs; it is caused by high growth stress which normally occurs in fast-growing tree species, particularly hardwoods. Growth stress is generated within woody tissue as a result of the tendency of differentiating cells to contract during cell maturation in the longitudinal direction and expand in the transverse direction (Malan 1995). In *Eucalyptus grandis,* growth stress is reported to be highly heritable, and has no relationship with other characteristics such as diameter, tree height and wood density (Malan 1995). Observations of logs harvested from *A. mangium* trees of more than 10 years of age revealed that the proportion of logs showing end-splitting was small.

Silviculture of plantation for solid-wood products

Establishment

The method of plantation establishment of *A. mangium* developed for solid-wood products is similar to that for plantations grown for pulp. The site is generally prepared manually by blanket spraying existing unwanted vegetation with herbicide. Seedlings raised in the nursery are planted manually at an initial stocking rate of 1100/ha. Genetically improved seeds should be used for growing sawlog plantations. Trees should be fertilised using a basal fertiliser of phosphorus at planting time, usually in the planting hole. Nitrogen fertiliser may not be required in the

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second rotation as a lack of a positive response is likely due to high nitrogen content available in the soil as a result of the fixing of atmospheric nitrogen by the previous plantation (Hardiyanto et al. 2004). Reducing weed competition is of crucial importance in the early phase of plantation establishment so that trees can grow optimally and close their canopy in the first year. Weeds are normally controlled by hand-weeding at age 3–4 months followed by a second hand-weeding and herbicide application at about 6 months of age. Depending on the weed growth, the hand-weeding may be reapplied at age 12 months.

Singling and pruning

In the establishment of plantations for solid wood it is of paramount importance to have early silvicultural intervention that utilises pruning and thinning. The timing and method of pruning and thinning is crucial. As *A. mangium* has weak apical dominance and is inherently multi-stemmed, singling is necessary. Singling to cull and leave one of the best stems is carried out when trees are around 3–4 months old.

Unlike plantations grown for pulp, plantations to be utilised for solid-wood have to be pruned to produce high-quality logs. This is important, as *A. mangium* does not shed dead lower branches naturally and often the dead branches persist for a long time and are associated with deterioration in wood quality (Malan 1995; Waugh 1996). Green and dead knots caused by branching reduce sawn timber yields. Dead branches result in loose knots that fall out to leave holes in the sawn timber. Dead branches are also associated with a high percentage of discoloration and decay (Ito and Nanis 1994). The objective of pruning is therefore to maximise the amount of clear wood produced by a tree. If branches are properly pruned while green, then there is a high probability that new wood will grow over the pruned branch stubs and that knot-free clear wood will be grown on the stem. Green pruning, that is, removal of leaf area, has the potential to decrease tree growth rates, particularly stem diameter. A study on pruning of *A. mangium* revealed that removing the crown below 50% of tree height depressed growth significantly, especially stem diameter (Majid and Paudyal 1992). The first lift-prune, to 40% of the tree height, should therefore be carried out when trees are about 6 months old and about 2.5–3.0 m tall. This should avoid a significant reduction in stem diameter growth. The second pruning, to a height of 4 m, should be conducted at 1.5–2.0 years old, at the same time as the first thinning when trees are around 8–9 m tall. The last pruning should be carried out at 4–5 years of age to a pruning height of 6 m. The minimum length of sawlog is 4 m (Hardiyanto 2004).

As mentioned previously, *A. mangium* has poor apical dominance and tends to have large branches. In a study of fungal infection 2 years after pruning, Weinland and Zuhaidi (1992) noted that wounds of diameter >20 mm were always infected whether the branches had been removed when green or dead. Therefore, control of branch size using form-pruning is vital in the silviculture of plantations managed for solid wood. Unlike lift-pruning, form-pruning selectively removes branches throughout the crown and can be used to reduce average branch size before a subsequent lift-pruning (Pinkard 2002) or to correct potential deviation of stems from a pathway of vertical growth (Medhurst et al. 2003). A pruning trial conducted at an 18-month-old stand of *A. mangium* at PT. Musi Hutan Persada's plantation found that form-pruning reduced the number of trees with large branches and increased the number of trees with better stem form (reduced the number of stem kinks) (C. Beadle et al., unpublished data). In the same trial, lift and form-pruning were found to have no association with heart rot (C. Beadle et al., unpublished data).

Thinning

Thinning involves the removal of part of the stand in order to concentrate future volume growth on fewer and better-quality stems. The advantage of thinning is that trees left behind have greater resources (water, light, space to grow) and therefore increase in size more rapidly. Thus, by selecting the best trees for retention and enabling those to grow faster, the rate of increase of stand value increases.

Thinning improves stand quality through the removal of deformed trees and a reduction in the time taken for trees to reach valuable sawlog size. Thinning is the most powerful tool to manipulate the development of the plantation and the quality of the final stand. Thinning increases timber revenue by increasing the volume of sawlog produced. This is because larger trees attract significantly higher prices, as they are less expensive to harvest (on a Rp/m^3 basis) and yield more-valuable end products.

Acacia mangium is very competitive for light, moisture and nutrients and it is important that it maintains active growth. Early thinning allows the best

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trees in the stand to take advantage of any improved growing conditions. To ensure this, the first thinning should be conducted when the majority of trees are around 8–9 m in height; this should occur at 1.5–2.0 years of age, with overall stocking reduced to 500–550 trees/ha. The remaining trees can then maintain good growth until about age 4–5 years when inter-tree competition resumes. The second thinning is then carried out to leave 200–250 trees/ha. The remaining trees are grown on until the final harvest at around age 10 years. The growth data reported from *A. mangium* stands previously thinned show that at about 10 years of age the average diameter at breast height is more than 30 cm and the average height was more than 26 m. About 30% of the total stem volume can be utilised for sawlog while the remaining wood can be used for pulp (Figure 1) (Hardiyanto and Supriyadi 2005).

Economic prospects

At present it is difficult to estimate the future demand for *A. mangium* timber, as its milling, kiln drying and related characteristics are still being assessed. However, demand for *A. mangium* timber has increased steadily in recent years as the industry gains more confidence in its availability and experience in how to use it, and wood supplies from natural forest continue to decline.

The increasing demand for wood from plantations, including that of *A. mangium,* will likely cause a rise in log prices and the investment in plantations for solid-wood utilisation will become economically viable. Current prices of harvested sawlog from unpruned and unthinned *A. mangium* stands are considered low due to low log quality (many knots and poor stem form). Logs harvested from managed sawlog plantations are expected to have better sawlog yield, wood quality and product value and command higher prices. Nevertheless, the economic aspects of growing plantations for solid wood (growing cost and value of logs) need to be assessed and compared with those of growing pulp plantations

Future research and development

As mentioned in the preceding section, the development of *A. mangium* plantations for solid wood is still in its infancy and therefore much research work has

Figure 1. A 10-year-old, thinned stand of *Acacia mangium* in Merbau, South Sumatra having average stem diameter, height and total stem volume of 36.6 cm, 26 m and 228.7 m^3/ha, respectively.

yet to be done. The following areas need attention in the future research and development of *A. mangium* plantations for solid-wood products:

- genetic improvement — to improve growth, minimise the size and severity of the juvenile core, reduce shrinkage properties and reduce growth stress
- clonal propagation — to reduce variation between trees in a stand and increase the potential for processing more uniform logs
- silviculture — to manipulate tree spacing, thinning, and fertiliser application after thinning to reduce growth stress, tension wood and end-splitting
- harvesting and log grading — to develop techniques in felling, log-making and log handling to reduce end-splitting and improve log sorting.

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